

# First trimester serum tests for Down's syndrome screening (Review)

Allred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, Alfirevic Z



**THE COCHRANE  
COLLABORATION®**

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2015, Issue 11

<http://www.thecochranelibrary.com>

**WILEY**

## TABLE OF CONTENTS

HEADER . . . . .	1
ABSTRACT . . . . .	1
PLAIN LANGUAGE SUMMARY . . . . .	2
BACKGROUND . . . . .	3
OBJECTIVES . . . . .	5
METHODS . . . . .	6
RESULTS . . . . .	9
Figure 1. . . . .	10
Figure 2. . . . .	12
DISCUSSION . . . . .	21
AUTHORS' CONCLUSIONS . . . . .	22
ACKNOWLEDGEMENTS . . . . .	22
REFERENCES . . . . .	23
CHARACTERISTICS OF STUDIES . . . . .	56
DATA . . . . .	161
Test 1. 1T PAPP-A, 5% FPR. . . . .	166
Test 2. 1T PAPP-A, ≤5th percentile. . . . .	166
Test 3. 1T PAPP-A, mixed cut-points. . . . .	167
Test 4. 1T free βhCG, 5% FPR. . . . .	167
Test 5. 1T total hCG, 5FPR. . . . .	168
Test 6. 1T AFP, 5% FPR. . . . .	168
Test 10. 1T AFP, mixed cut-points. . . . .	169
Test 11. 1T Inhibin, 5FPR. . . . .	169
Test 12. 1T ADAM 12, 5FPR. . . . .	170
Test 13. 1T SP1, 5% FPR. . . . .	170
Test 17. ba_hcg_ratio, 0.25MoM. . . . .	171
Test 18. 1T uE3, 5% FPR. . . . .	171
Test 19. 1T PIGF, 5FPR. . . . .	171
Test 20. 1T PAPP-A and 1T free βhCG, 5% FPR. . . . .	172
Test 21. 1T PAPP-A and 1T free βhCG, mixed cut-points. . . . .	172
Test 22. 1T PAPP-A and 1T AFP, 5% FPR. . . . .	173
Test 23. 1T PAPP-A and 1T ITA, 3% FPR. . . . .	173
Test 24. 1T PAPP-A and 1T ITA, 5% FPR. . . . .	173
Test 25. 1T free βhCG and 1T Inhibin, 5% FPR. . . . .	174
Test 26. 1T free βhCG and 1T AFP, 5% FPR. . . . .	174
Test 27. 1T PAPP-A and 1T ITA, 10% FPR. . . . .	174
Test 28. 1T PAPP-A, 1T free βhCG and 1T ITA, 5% FPR. . . . .	175
Test 29. 1T PAPP-A, 1T free βhCG and 1T ITA,3% FPR. . . . .	175
Test 30. 1T PAPP-A, 1T free βhCG and 1T ITA, 10% FPR. . . . .	175
Test 31. 1T total hCG, 1T free αhCG and 1T progesterone, 0.34 MoM. . . . .	176
Test 32. Age, 1T Inhibin, risk 1:100. . . . .	176
Test 33. Age, 1T Inhibin, risk 1:250. . . . .	176
Test 34. Age, 1T Inhibin, risk 1:400. . . . .	177
Test 35. Age, 1T Inhibin, 5FPR. . . . .	177
Test 36. Age, 1T Inhibin, mixed cut-points. . . . .	178
Test 37. Age, 1T PAPP-A, 5FPR. . . . .	178
Test 38. Age, 1T PAPP-A, mixed cut-points. . . . .	179
Test 39. Age, 1T free βhCG, 5FPR. . . . .	179
Test 40. Age, 1T free βhCG, risk 1:384. . . . .	180
Test 41. Age, 1T free βhCG, mixed cut-points. . . . .	180
Test 42. Age, 1T total hCG,risk 1:384. . . . .	181

Test 43. Age, 1T total hCG, mixed cut-points. . . . .	181
Test 44. Age, 1T AFP, 5FPR. . . . .	182
Test 45. Age, 1T AFP, risk1:384. . . . .	182
Test 46. Age, 1T AFP,mixed cut-points. . . . .	183
Test 47. Age, 1T uE3, risk 1:384. . . . .	183
Test 48. Age, 1T uE3, mixed cut-points. . . . .	184
Test 49. Age, 1T free $\alpha$ hCG, risk 1:384. . . . .	184
Test 50. Age, 1T SP1, 5FPR. . . . .	185
Test 51. Age, 1T ProMBP, risk 1:250. . . . .	185
Test 52. Age, 1T ITA, 5FPR. . . . .	185
Test 53. Age, 1T ADAM 12, risk 1:400. . . . .	186
Test 54. Age, 1T PAPP-A and 1T free $\beta$ hCG, risk 1:250. . . . .	186
Test 55. Age, 1T PAPP-A and 1T free $\beta$ hCG, 5FPR. . . . .	187
Test 56. Age, 1T PAPP-A and 1T free $\beta$ hCG, mixed cut-points. . . . .	188
Test 57. Age, 1T PAPP-A and 1T free $\beta$ hCG, mixed cut-points without 5FPR. . . . .	189
Test 58. Age, 1T total hCG and 1T PAPP-A, 5FPR. . . . .	190
Test 59. Age, 1T PAPP-A and 1T Inhibin, risk 1:100. . . . .	191
Test 60. Age, 1T PAPP-A and 1T Inhibin, risk 1:250. . . . .	191
Test 61. Age, 1T PAPP-A and 1T Inhibin, risk 1:400. . . . .	191
Test 62. Age, 1T PAPP-A and 1T Inhibin, 5FPR. . . . .	192
Test 63. Age, 1T PAPP-A and 1T Inhibin, mixed cut-points. . . . .	192
Test 64. Age, 1T PAPP-A and 1T ITA, 5FPR. . . . .	193
Test 65. Age, 1T PAPP-A and 1T AFP, 5FPR. . . . .	193
Test 66. Age, 1T free $\beta$ hCG and 1T Inhibin, risk 1:100. . . . .	194
Test 67. Age, 1T free $\beta$ hCG and 1T Inhibin, risk 1:250. . . . .	194
Test 68. Age, 1T free $\beta$ hCG and 1T Inhibin, risk 1:400. . . . .	194
Test 69. Age, 1T free $\beta$ hCG and 1T Inhibin, 5FPR. . . . .	195
Test 70. Age, 1T free $\beta$ hCG and 1T Inhibin, mixed cut-points. . . . .	195
Test 71. Age, 1T free $\beta$ hCG and 1T AFP, 5FPR. . . . .	196
Test 72. Age, 1T free $\beta$ hCG and 1T AFP, risk 1:250. . . . .	196
Test 73. Age, 1T free $\beta$ hCG and 1T AFP, risk 1:384. . . . .	197
Test 74. Age, 1T free $\beta$ hCG and 1T AFP, mixed cut-points. . . . .	197
Test 75. Age, 1T AFP and 1T uE3, risk 1:384. . . . .	198
Test 76. Age, 1T AFP and 1T free $\alpha$ hCG, risk 1:384. . . . .	198
Test 77. Age, 1T free $\beta$ hCG and 1T total hCG, risk 1:384. . . . .	198
Test 78. Age, 1T free $\beta$ hCG and 1T uE3, risk 1:384. . . . .	199
Test 79. Age, 1T free $\beta$ hCG and 1T uE3, 5FPR. . . . .	199
Test 80. Age, 1T free $\beta$ hCG and 1T uE3, mixed cut-points. . . . .	200
Test 81. Age, 1T free $\beta$ hCG and 1T SP1, 5FPR. . . . .	200
Test 82. Age, 1T free $\beta$ hCG and 1T SP1 risk 1:250. . . . .	200
Test 83. Age, 1T AFP and 1T total hCG, 1:384. . . . .	201
Test 84. Age, 1T free $\beta$ hCG and 1T free $\alpha$ hCG, risk 1:384. . . . .	201
Test 85. Age, 1T total hCG and 1T uE3, risk 1:384. . . . .	202
Test 86. Age, 1T total hCG and 1T Inhibin, 5FPR. . . . .	202
Test 87. Age, 1T total hCG and 1T free $\alpha$ hCG, risk 1:384. . . . .	202
Test 88. Age, 1T uE3 and 1T free $\alpha$ hCG, risk 1:384. . . . .	203
Test 89. Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T AFP, 5FPR. . . . .	203
Test 90. Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T AFP, mixed cut-points. . . . .	204
Test 91. Age, 1T free $\beta$ hCG, 1T AFP and 1T uE3, 5FPR. . . . .	204
Test 92. Age, 1T free $\beta$ hCG, 1T AFP and 1T uE3, risk 1:384. . . . .	205
Test 93. Age, 1T free $\beta$ hCG, 1T AFP and 1T uE3, mixed cut-points. . . . .	205
Test 94. Age, 1T total hCG, 1T AFP and 1T uE3, risk 1:384. . . . .	205
Test 95. Age, 1T total hCG, 1T AFP and 1T uE3, mixed cut-points. . . . .	206

Test 96. Age, 1T AFP, free $\alpha$ hCG and 1T uE3, risk 1:384. . . . .	206
Test 97. Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T Inhibin, 5FPR. . . . .	207
Test 98. Age, 1T PAPP-A, 1T total hCG and 1T Inhibin, 5FPR. . . . .	207
Test 99. Age, 1T PAPP-A, sp1 and 1T ProMBP, 5FPR. . . . .	207
Test 100. Age, 1T PAPP-A, sp1 and 1T ProMBP, risk 1:250. . . . .	208
Test 101. Age, 1T free $\beta$ hCG, 1T total hCG, 1T AFP and 1T uE3, risk 1:384. . . . .	208
Test 102. Age, 1T total hCG, 1T AFP, 1T uE3 and 1T free $\alpha$ hCG, risk 1:384. . . . .	209
Test 103. Age, 1T PAPP-A, 1T free $\beta$ hCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR. . . . .	209
Test 104. Age, 1T PAPP-A, 1T total hCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR. . . . .	209
Test 105. Age, 1T free $\beta$ hCG, 1T total hCG, 1T AFP, 1T uE3 and 1T free $\alpha$ hCG, risk 1:384. . . . .	210
Test 106. Age, 1T hPL, risk 1:250. . . . .	210
Test 107. Age, 1T hPL, 1T PAPP-A, risk 1:250. . . . .	211
Test 108. Age, 1T hPL, 1T free $\beta$ hCG, risk 1:250. . . . .	211
Test 109. Age, 1T hPL, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250. . . . .	211
Test 110. Age, 1T PGH, risk 1:250. . . . .	212
Test 111. Age, 1T PGH, 1T PAPP-A, risk 1:250. . . . .	212
Test 112. Age, 1T PGH, 1T free $\beta$ hCG, risk 1:250. . . . .	213
Test 113. Age, 1T PGH, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250. . . . .	213
Test 114. Age, 1T GHBP, risk 1:250. . . . .	213
Test 115. Age, 1T GHBP, 1T PAPP-A, risk 1:250. . . . .	214
Test 116. Age, 1T GHBP, 1T free $\beta$ hCG, risk 1:250. . . . .	214
Test 117. Age, 1T GHBP, 1T PGH, risk 1:250. . . . .	215
Test 118. Age, 1T GHBP, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250. . . . .	215
Test 119. Age, 1T GHBP, 1T PGH, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250. . . . .	215
Test 120. Age, 1T ADAM 12, risk 1:250. . . . .	216
Test 121. Age, 1T ADAM 12, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250. . . . .	216
Test 122. Age, PIGF, 1T PAPP-A, 1T free $\beta$ hCG, 5FPR. . . . .	217
Test 123. Age, 1T PAPP-A and 1T free $\beta$ hCG, risk 1:300. . . . .	217
Test 124. Age, 1T PAPP-A, 1T Hyperglycosylated hCG, 5FPR. . . . .	218
Test 128. Age, ADAM 12, 1T PAPP-A, 5FPR. . . . .	218
Test 129. Age, ADAM 12, 1T PAPP-A, 1T free $\beta$ hCG, 5FPR. . . . .	218
Test 130. Age, 1T PIGF, 5FPR. . . . .	219
Test 131. 1T PIGF, 1T PAPP-A, 1T free $\beta$ hCG, 5FPR. . . . .	219
Test 132. Age, 1T ADAM 12, 1T PAPP-A, 1T free $\beta$ hCG, mixed cut-points. . . . .	220
Test 133. Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T Inhibin, risk 1:250. . . . .	220
Test 134. Age, 1T PAPP-A, 1T free $\beta$ hCG, and 1T Inhibin, mixed cut-points. . . . .	221
ADDITIONAL TABLES . . . . .	221
APPENDICES . . . . .	229
CONTRIBUTIONS OF AUTHORS . . . . .	237
DECLARATIONS OF INTEREST . . . . .	237
SOURCES OF SUPPORT . . . . .	237
DIFFERENCES BETWEEN PROTOCOL AND REVIEW . . . . .	238
NOTES . . . . .	238

[Diagnostic Test Accuracy Review]

# First trimester serum tests for Down's syndrome screening

S Kate Alldred<sup>1</sup>, Yemisi Takwoingi<sup>2</sup>, Boliang Guo<sup>3</sup>, Mary Pennant<sup>4</sup>, Jonathan J Deeks<sup>2</sup>, James P Neilson<sup>1</sup>, Zarko Alfirevic<sup>1</sup>

<sup>1</sup>Department of Women's and Children's Health, The University of Liverpool, Liverpool, UK. <sup>2</sup>Public Health, Epidemiology and Biostatistics, University of Birmingham, Birmingham, UK. <sup>3</sup>School of Medicine, University of Nottingham, Nottingham, UK. <sup>4</sup>Public Health Directorate, Cambridgeshire County Council, Cambridge, UK

Contact address: S Kate Alldred, Department of Women's and Children's Health, The University of Liverpool, First Floor, Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool, L8 7SS, UK. [katealldred@gmail.com](mailto:katealldred@gmail.com).

**Editorial group:** Cochrane Pregnancy and Childbirth Group.

**Publication status and date:** New, published in Issue 11, 2015.

**Review content assessed as up-to-date:** 17 December 2013.

**Citation:** Alldred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, Alfirevic Z. First trimester serum tests for Down's syndrome screening. *Cochrane Database of Systematic Reviews* 2015, Issue 11. Art. No.: CD011975. DOI: 10.1002/14651858.CD011975.

Copyright © 2015 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

## ABSTRACT

### Background

Down's syndrome occurs when a person has three, rather than two copies of chromosome 21; or the specific area of chromosome 21 implicated in causing Down's syndrome. It is the commonest congenital cause of mental disability and also leads to numerous metabolic and structural problems. It can be life-threatening, or lead to considerable ill health, although some individuals have only mild problems and can lead relatively normal lives. Having a baby with Down's syndrome is likely to have a significant impact on family life.

Noninvasive screening based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allows estimates of the risk of a pregnancy being affected and provides information to guide decisions about definitive testing. However, no test can predict the severity of problems a person with Down's syndrome will have.

### Objectives

The aim of this review was to estimate and compare the accuracy of first trimester serum markers for the detection of Down's syndrome in the antenatal period, both as individual markers and as combinations of markers. Accuracy is described by the proportion of fetuses with Down's syndrome detected by screening before birth (sensitivity or detection rate) and the proportion of women with a low risk (normal) screening test result who subsequently had a baby unaffected by Down's syndrome (specificity).

### Search methods

We conducted a sensitive and comprehensive literature search of MEDLINE (1980 to 25 August 2011), Embase (1980 to 25 August 2011), BIOSIS via EDINA (1985 to 25 August 2011), CINAHL via OVID (1982 to 25 August 2011), The Database of Abstracts of Reviews of Effectiveness (*The Cochrane Library* 25 August 2011), MEDION (25 August 2011), The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (25 August 2011), The National Research Register (Archived 2007), Health Services Research Projects in Progress database (25 August 2011). We did forward citation searching ISI citation indices, Google Scholar and PubMed 'related articles'. We did not apply a diagnostic test search filter. We also searched reference lists and published review articles.

### Selection criteria

We included studies in which all women from a given population had one or more index test(s) compared to a reference standard (either chromosomal verification or macroscopic postnatal inspection). Both consecutive series and diagnostic case-control study designs were included. Randomised trials where individuals were randomised to different screening strategies and all verified using a reference

---

First trimester serum tests for Down's syndrome screening (Review)

Copyright © 2015 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

1

standard were also eligible for inclusion. Studies in which test strategies were compared head-to-head either in the same women, or between randomised groups were identified for inclusion in separate comparisons of test strategies. We excluded studies if they included less than five Down's syndrome cases, or more than 20% of participants were not followed up.

### **Data collection and analysis**

We extracted data as test positive or test negative results for Down's and non-Down's pregnancies allowing estimation of detection rates (sensitivity) and false positive rates (1-specificity). We performed quality assessment according to QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria. We used hierarchical summary ROC meta-analytical methods or random-effects logistic regression methods to analyse test performance and compare test accuracy as appropriate. Analyses of studies allowing direct and indirect comparisons between tests were undertaken.

### **Main results**

We included 56 studies (reported in 68 publications) involving 204,759 pregnancies (including 2113 with Down's syndrome). Studies were generally of good quality, although differential verification was common with invasive testing of only high-risk pregnancies. We evaluated 78 test combinations formed from combinations of 18 different tests, with or without maternal age; ADAM12 (a disintegrin and metalloprotease), AFP (alpha-fetoprotein), inhibin, PAPP-A (pregnancy-associated plasma protein A, ITA (invasive trophoblast antigen), free  $\beta$ hCG (beta human chorionic gonadotrophin), PlGF (placental growth factor), SP1 (Schwangerschafts protein 1), total hCG, progesterone, uE3 (unconjugated oestriol), GHBP (growth hormone binding protein), PGH (placental growth hormone), hyperglycosylated hCG, ProMBP (proform of eosinophil major basic protein), hPL (human placental lactogen), (free  $\alpha$ hCG, and free  $\beta$ hCG to AFP ratio. Direct comparisons between two or more tests were made in 27 studies.

Meta-analysis of the nine best performing or frequently evaluated test combinations showed that a test strategy involving maternal age and a double marker combination of PAPP-A and free  $\beta$ hCG significantly outperformed the individual markers (with or without maternal age) detecting about seven out of every 10 Down's syndrome pregnancies at a 5% false positive rate (FPR). Limited evidence suggested that marker combinations involving PAPP-A may be more sensitive than those without PAPP-A.

### **Authors' conclusions**

Tests involving two markers in combination with maternal age, specifically PAPP-A, free  $\beta$ hCG and maternal age are significantly better than those involving single markers with and without age. They detect seven out of 10 Down's affected pregnancies for a fixed 5% FPR. The addition of further markers (triple tests) has not been shown to be statistically superior; the studies included are small with limited power to detect a difference.

The screening blood tests themselves have no adverse effects for the woman, over and above the risks of a routine blood test. However some women who have a 'high risk' screening test result, and are given amniocentesis or chorionic villus sampling (CVS) have a risk of miscarrying a baby unaffected by Down's. Parents will need to weigh up this risk when deciding whether or not to have an amniocentesis or CVS following a 'high risk' screening test result.

## **PLAIN LANGUAGE SUMMARY**

### **Screening tests for Down's syndrome in first three months of pregnancy**

#### **Background**

Down's syndrome (also known as Down's or Trisomy 21) is an incurable genetic disorder that causes significant physical and mental health problems, and disabilities. However, there is wide variation in how Down's affects people. Some individuals are severely affected whilst others have mild problems and are able to lead relatively normal lives. There is no way of predicting how badly a baby might be affected.

Expectant parents are given the choice to be tested for Down's during pregnancy to assist them in making decisions. If a mother is carrying a baby with Down's, then there is the decision about whether to terminate or continue with the pregnancy. The information offers parents the opportunity to plan for life with a Down's child.

The most accurate tests for Down's involve testing fluid from around the baby (amniocentesis) or tissue from the placenta (chorionic villus sampling (CVS)) for the abnormal chromosomes associated with Down's. Both these tests involve inserting needles through the mother's abdomen and are known to increase the risk of miscarriage. Thus, the tests are not suitable for offering to all pregnant women. Rather, tests that measure markers in the mother's blood, urine or on ultrasound scans of the baby are used for screening. These screening tests are not perfect, they can miss cases of Down's and also give a 'high risk' test result to a number of women whose babies are not affected by Down's. Thus, pregnancies identified as 'high risk' using these screening tests require further testing using amniocentesis or CVS to confirm a diagnosis of Down's.

### **What we did**

The aim of this review was to find out which of the blood screening tests done during the first three months of pregnancy are the most accurate at predicting the risk of a pregnancy being affected by Down's. We looked at 18 different blood markers that can be used alone or in combination, taken before 14 weeks gestation, thus creating 78 screening tests for Down's. We found 56 studies, involving 204,759 pregnancies of which 2113 had pregnancies affected by Down's.

### **What we found**

For the first 14 weeks of pregnancy, the evidence supports the use of the double test of two blood markers; pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotrophin ( $\beta$ hCG), in combination with the mother's age. This test detects around seven out of every 10 (68%) pregnancies affected by Down's. It is common practice to offer amniocentesis or CVS to women with a high risk test result. About one in 20 women (5%) having this test will have a 'high risk' result but most of these women will not be carrying a baby with Down's. We found for tests in the first 14 weeks of pregnancy, there is little evidence to support the use of serum tests made up of more than two blood markers.

### **Other important information to consider**

The blood tests themselves have no adverse effects for the woman, over and above the risks of a routine blood test. However some women who have a 'high risk' screening test result, and are given amniocentesis or CVS have a risk of miscarrying a baby unaffected by Down's. Parents will need to weigh up this risk when deciding whether or not to have an amniocentesis or CVS following a 'high risk' screening test result.

## **BACKGROUND**

This is one of a series of reviews on antenatal screening for Down's syndrome following a generic protocol (Allred 2010) - see [Published notes](#) for more details.

### **Target condition being diagnosed**

#### **Down's syndrome**

Down's syndrome affects approximately one in 800 live-born babies (Cuckle 1987a). It results from a person having three, rather than two, copies of chromosome 21, or the specific area of chromosome 21 implicated in causing Down's syndrome, as a result of trisomy or translocation. If not all cells are affected, the pattern is described as 'mosaic'. Down's syndrome can cause a wide range of

physical and mental problems. It is the commonest cause of mental disability, and is also associated with a number of congenital malformations, notably affecting the heart. There is also an increased risk of cancers such as leukaemia, and numerous metabolic problems including diabetes and thyroid disease. Some of these problems may be life-threatening, or lead to considerable ill health, while some individuals with Down's syndrome have only mild problems and can lead a relatively normal life.

There is no cure for Down's syndrome, and antenatal diagnosis allows for preparation for the birth and subsequent care of a baby with Down's syndrome, or for the offer of a termination of pregnancy. Having a baby with Down's syndrome is likely to have a significant impact on family and social life, relationships and parents' work. Special provisions may need to be made for education and care of the child, as well as accommodating the possibility of periods of hospitalisation.

Definitive invasive tests (amniocentesis and chorionic villus sampling (CVS)) exist that allow the diagnosis of Down's syndrome before birth, but carry a risk of miscarriage. No test can predict the severity of problems a person with Down's syndrome will have. Noninvasive screening tests based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allow an estimate of the risk of a pregnancy being affected and provide parents with information to enable them to make choices about definitive testing. Such screening tests are used during the first and second trimester of pregnancy.

### *Screening tests for Down's syndrome*

Initially, screening was determined solely by using maternal age to classify a pregnancy as high or low risk for trisomy 21, as it was known that older women had a higher chance of carrying a baby with Down's syndrome (Penrose 1933).

Further advances in screening were made in the early 1980s, when Merkatz et al. investigated the possibility that low maternal serum alpha-fetoprotein (AFP), obtained from maternal blood in the second trimester of pregnancy could be associated with chromosomal abnormalities in the fetus. Their retrospective case-control study showed a statistically significant relationship between fetal trisomy, such as Down's syndrome, and lowered maternal serum AFP (Merkatz 1984). This was further explored by Cuckle et al in a larger retrospective trial using data collected as part of a neural tube defect (NTD) screening project (Cuckle 1984). This work was followed by calculation of risk estimates using maternal serum AFP values and maternal age, which ultimately led to the introduction of the two screening parameters in combination (Alfirevic 2004).

In 1987, in a small case-control study of women carrying fetuses with known chromosomal abnormalities, Bogart and colleagues investigated maternal serum levels of human chorionic gonadotrophin (hCG) as a possible screening tool for chromosomal abnormalities in the second trimester (Bogart 1987). This followed the observations that low hCG levels were associated with miscarriages, which are commonly associated with fetal chromosomal abnormalities. They concluded that high hCG levels were associated with Down's syndrome and because hCG levels plateau at 18 to 24 weeks, that this would be the most appropriate time for screening. Later work suggested that the  $\beta$  subunit of hCG was a more effective marker than total hCG (Macri 1990; Macri 1993). Second trimester unconjugated oestriol (uE3), produced by the fetal adrenals and the placenta, was also evaluated as a potential screening marker. In another retrospective case-control study, uE3 was shown to be lower in Down's syndrome pregnancies compared with unaffected pregnancies. When used in combination with AFP and maternal age, it appeared to identify more pregnancies affected by Down's syndrome than AFP and age alone (Canick 1988). Further work suggested that all three serum markers (AFP, hCG and uE3) showed even higher detection rates when combined with

maternal age (Wald 1988a; Wald 1988b) and appeared to be a cost-effective screening strategy (Wald 1992a).

Two other serum markers, produced by the placenta, have been linked with Down's syndrome, namely pregnancy associated plasma protein A or PAPP-A, and first trimester Inhibin A. PAPP-A has been shown to be reduced in the first trimester of Down's syndrome pregnancies, with its most marked reduction in the early first trimester (Bersinger 1995). Inhibin A is high in the second trimester in pregnancies affected by Down's syndrome (Cuckle 1995; Wallace 1995). There are some issues concerning the biological stability and hence reliability of this marker, and the effect this will have on individual risk.

### *Screening and parental choice*

Antenatal screening is used for several reasons (Alfirevic 2004), but the most important is to enable parental choice regarding pregnancy management and outcome. Before a woman and her partner opt to have a screening test, they need to be fully informed about the risks, benefits and possible consequences of such a test. This includes the choices they may have to face should the result show that the woman has a high risk of carrying a baby with Down's syndrome and implications of both false positive and false negative screening tests. They need to be informed of the risk of a miscarriage due to invasive diagnostic testing, and the possibility that a miscarried fetus may be chromosomally normal. If, following invasive diagnostic testing, the fetus is shown to have Down's syndrome, further decisions need to be made about continuation or termination of the pregnancy, the possibility of adoption and finally, preparation for parenthood. Equally, if a woman has a test that shows she is at a low risk of carrying a fetus with Down's syndrome, it does not necessarily mean that the baby will be born with a normal chromosomal make up. This possibility can only be excluded by an invasive diagnostic test (Alfirevic 2003). The decisions that may be faced by expectant parents inevitably engender a high level of anxiety at all stages of the screening process, and the outcomes of screening can be associated with considerable physical and psychological morbidity. No screening test can predict the severity of problems a person with Down's syndrome will have.

### **Index test(s)**

This review examined serum screening tests used in the first trimester of pregnancy (up to 14 weeks' gestation) comprised of the following 18 individual markers; a disintegrin and metalloprotease 12 (ADAM12), AFP, inhibin, PAPP-A, invasive trophoblast antigen (ITA), free  $\beta$ hCG, placental growth factor (PIGF), Schwangerschafts protein 1 (SP1), total hCG, progesterone, uE3, growth hormone binding protein (GHBP), placental growth hormone (PGH), hyperglycosylated hCG, proform of eosinophil major basic protein (ProMBP), human placental lacto-



gen (hPL), free alpha human chorionic gonadotrophin ( $\alpha$ hCG), and free  $\beta$ hCG to AFP ratio. These markers can be used individually, in combination with age, and can also be used in combination with each other. The risks are calculated by comparing a woman's test result for each marker with values for an unaffected population, and multiplying this with her age-related risk. Where several markers are combined, risks are computed using risk equations (often implemented in commercial software) that take into account the correlational relationships between the different markers and marker distributions in affected and unaffected populations.

### Alternative test(s)

Down's syndrome can be detected during pregnancy with invasive diagnostic tests such as amniocentesis or CVS, with or without prior screening. The ability to determine fetal chromosomal make up (also known as a karyotype) from amniotic fluid samples was demonstrated in 1966 by Steele and Breg (Steele 1966), and the first antenatal diagnosis of Down's syndrome was made in 1968 (Vaklenti 1968). Amniocentesis is an invasive procedure that involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks' gestation. CVS involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks' gestation. Amniocentesis and CVS are both methods of obtaining fetal chromosomal material, which are then used to diagnose Down's syndrome. Both tests use ultrasound scans to guide placement of the needle. Amniocentesis carries a risk of miscarriage in the order of 1%; transabdominal CVS may carry a similar risk (Alfirevic 2003).

### Rationale

This is one of a suite of Cochrane reviews, the aim of which is to identify all screening tests for Down's syndrome used in clinical practice, or evaluated in the research setting, in order to try to identify the most accurate test(s) available, and to provide clinicians, policy-makers and women with robust and balanced evidence on which to base decisions about interpreting test results and implementing screening policies to triage the use of invasive diagnostic testing.

There are many different screening tests which are available and offered which will be the subject of additional Cochrane reviews (currently in preparation or published (Alldred 2012)), and there are other reviews looking at this area. Tests to be assessed in Cochrane reviews include second trimester serum tests; urine tests; first trimester ultrasound markers; tests that combine serum and ultrasound markers; and tests that combine markers from the first trimester with markers from the second trimester. Second trimester

ultrasound markers have been assessed in a previous systematic review (Smith-Bindman 2001).

The topic has been split into several different reviews to allow for greater ease of reading and greater accessibility of data, and also to allow the reader to focus on separate groups of tests, for example, first trimester serum tests alone, first trimester serum and ultrasound, second trimester serum alone, first and second trimester serum combinations, with or without ultrasound markers; and urine markers alone. An overview review will compare the best tests, focusing on commonly used strategies and the best tests from each of these categories. This review is written with the global perspective in mind, rather than to conform with any specific local or national policy, as not all tests will be available in all areas where screening for Down's syndrome is carried out.

A systematic review of second trimester ultrasound markers in the detection of Down's syndrome fetuses was published in 2001 that concluded that nuchal fold thickening may be useful in detecting Down's syndrome, but that it was not sensitive enough to use as a screening test. The review concluded that the other second trimester ultrasound markers did not usefully distinguish between Down's syndrome and pregnancies without Down's syndrome (Smith-Bindman 2001). There has yet to be a systematic review and meta-analysis of the observed data on serum, urine and first trimester ultrasound markers, in order to draw rigorous and robust conclusions about the diagnostic accuracy of available Down's syndrome screening tests.

## OBJECTIVES

The aim of this review was to estimate and compare the accuracy of first trimester serum markers for the detection of Down's syndrome in the antenatal period, both as individual markers and as combinations of markers. Accuracy is described by the proportion of fetuses with Down's syndrome detected by screening before birth (sensitivity or detection rate), and the proportion of women with a low risk (normal) screening test result who subsequently had a baby unaffected by Down's syndrome (specificity).

### Investigation of sources of heterogeneity

We planned to investigate whether a uniform screening test is suitable for all women, or whether different screening methods are more applicable to different groups, defined by advanced maternal age, ethnic groups and aspects of the pregnancy and medical history such as multiple pregnancy, diabetes and family history of Down's syndrome. We also considered whether there existed evidence of overestimation of test accuracy in studies evaluating risk equations in the derivation sample rather than in a separate validation sample.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

We included studies in which all women from a given population had one or more index test(s) compared to a reference standard. Both consecutive series and diagnostic case-control study designs were included. Randomised trials where individuals were randomised to different screening strategies and all verified using a reference standard were also eligible for inclusion. Studies in which test strategies were compared head-to-head, either in the same women, or between randomised groups were identified for inclusion in separate comparisons of test strategies. Studies were excluded if they included less than five Down's syndrome cases, or more than 20% of participants were not followed up.

#### Participants

Pregnant women at less than 14 weeks' gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome in their pregnancy were eligible. Studies were included if the pregnant women were unselected, or if they represented groups with increased risk of Down's syndrome, or difficulty with conventional screening tests including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

#### Index tests

The following 18 index tests were examined; ADAM12, AFP, inhibin, PAPP-A, ITA, free  $\beta$ hCG, PIGF, SP1, total hCG, progesterone, uE3, GHBP, PGH, hyperglycosylated hCG, ProMBP, hPL, free  $\alpha$ hCG, and free  $\beta$ hCG to AFP ratio, and combinations of these markers combined with maternal age.

We looked at comparisons of tests used in isolation and in 78 various combinations. These included single (one marker), double (two markers), triple (three markers), quadruple (four markers) and quintuple (five markers) tests, some of which were adjusted for maternal age.

Where tests were used in combination, we looked at the performance of test combinations according to predicted probabilities computed using risk equations and dichotomised into high risk and low risk.

#### Target conditions

Down's syndrome in the fetus due to trisomy, translocation or mosaicism.

#### Reference standards

We considered several reference standards, involving chromosomal verification and postnatal macroscopic inspection. Chromosomal verification is considered preferential but because of the risks involved, often not feasible. Where macroscopic inspection or examination raises a question about the possibility of an individual being affected by Down's syndrome, in clinical practice this is usually confirmed or refuted by formal karyotyping.

Amniocentesis and CVS are invasive chromosomal verification tests undertaken during pregnancy. They are highly accurate but the process carries a 1% miscarriage rate, and therefore they are only used in pregnancies considered to be high risk of Down's, or on the mother's request. All other types of testing (postnatal examination, postnatal karyotyping, birth registers and Down's syndrome registers) are based on information available at the end of pregnancy. For the purposes of meta-analysis they are considered equivalent. The greatest concern is not their accuracy, but the loss of the pregnancy to miscarriage between the timing of serum testing and the reference standard. Miscarriage with cytogenetic testing of the fetus is included in the reference standard where available.

We anticipated that older studies, and studies undertaken in older women were more likely to have used invasive chromosomal verification tests in all women. Studies undertaken in younger women and more recent studies were likely to use differential verification as they often only used prenatal karyotypic testing on fetuses considered screen positive or high risk according to the screening test; the reference standard for most unaffected infants is likely to be observation of a phenotypically normal baby. Although the accuracy of this combined reference standard is considered high, it is methodologically a weaker approach because pregnancies that miscarry between the index test and birth are likely to be lost from the analysis, and miscarriage is more likely to occur in Down's than normal pregnancies.

#### Search methods for identification of studies

We used one generic search strategy to identify studies for all reviews in this series

#### Electronic searches

We applied a sensitive search strategy to search the following databases using the text words and MeSH terms detailed in [Appendix 1](#), adapting the search strategy for each different database.

Databases searched included:

- MEDLINE via OVID (1980 to 25 August 2011)
- Embase via Dialog Datastar (1980 to 25 August 2011)
- BIOSIS via EDINA (1985 to 25 August 2011)
- CINAHL via OVID (1982 to 25 August 2011)

- The Database of Abstracts of Reviews of Effectiveness (25 August 2011)
  - MEDION (25 August 2011)
- The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine ([www.ifcc.org/](http://www.ifcc.org/)) (25 August 2011)
  - [The National Research Register](#) (Archived 2007)
  - Health Services Research Projects in Progress database ([HSRPROJ](#)) (25 August 2011)

The search strategy combined three sets of search terms (see [Appendix 1](#)). The first set was made up of named tests, general terms used for screening/diagnostic tests and statistical terms. Note that the statistical terms were used to increase sensitivity and were not used as a methodological filter to increase specificity. The second set was made up of terms that encompass Down's syndrome and the third set made up of terms to limit the testing to pregnant women. All terms within each set were combined with the Boolean operator OR and then the three sets were combined using AND. The terms used were a combination of subject headings and free text terms. The search strategy was adapted to suit each database searched.

We attempted to identify cumulative papers which reported data from the same data set, and contacted authors to obtain clarification of the overlap between data presented in these papers, in order to prevent data from the same women being analysed more than once.

### Searching other resources

In addition, we examined references cited in studies identified as being potentially relevant, and those cited by previous reviews. We contacted authors of studies where further information was required.

We carried out forward citation searching of relevant items, using the search strategy in ISI citation indices, Google scholar and Pubmed 'related articles'.

We did not apply language restrictions to the search.

## Data collection and analysis

### Selection of studies

Two review authors screened the titles and abstracts (where available) of all studies identified by the search strategy. We obtained full-text versions of studies identified as being potentially relevant and two review authors independently assessed these for inclusion, using a study eligibility screening pro forma according to the pre-specified inclusion criteria. Any disagreement between the two review authors was settled by consensus, or where necessary, by a third party.

### Data extraction and management

A data extraction form was developed and piloted using a subset of 20 identified studies. Two review authors independently extracted data, and where disagreement or uncertainty existed, a third review author validated the information extracted.

Data on each marker were extracted as binary test positive/test negative results for Down's and non-Down's pregnancies, with a high-risk result-as defined by each individual study-being regarded as test positive (suggestive or diagnostic of Down's syndrome), and a low-risk result being regarded as test negative (suggestive of absence of Down's syndrome). Where results were reported at several thresholds, we extracted data at each threshold.

We made a note of those in special groups that posed either increased risk of Down's syndrome or difficulty with conventional screening tests, including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

### Assessment of methodological quality

We used a modified version of the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool ([Whiting 2003](#)), a quality assessment tool for use in systematic reviews of diagnostic accuracy studies, to assess the methodological quality of included studies. We anticipated that a key methodological issue would be the potential for bias arising from the differential use of invasive testing and follow-up for the reference standard according to index test results, bias arising due to higher loss to miscarriage if false negatives than true negatives. We chose to code this issue as originating from differential verification in the QUADAS tool: we are aware that it could also be coded under delay in obtaining the reference standard, and reporting of withdrawals. We omitted the QUADAS item assessing quality according to length of time between index and reference tests, as Down's syndrome is either present or absent rather than a condition that evolves and resolves, and disregarding the differential reference standard issue, thus any length of delay is acceptable. Two review authors assessed each included study separately. Any disagreement between the two authors was settled by consensus, or where necessary, by a third party. Each item in the QUADAS tool was marked as 'yes', 'no' or 'unclear', and scores are presented graphically and in tables. We did not use a summary quality score. See [Appendix 3](#) for QUADAS questionnaire.

### Statistical analysis and data synthesis

We initially examined each test or test strategy at each of the common risk thresholds used to define test positivity by plotting estimates of sensitivity and specificity from each study on forest plots and in receiver operating characteristic (ROC) space. Test strategies were selected for further investigation if they were evaluated in four or more studies or, if there were two or three studies, but the individual study results indicated performance likely to be superior to a sensitivity of 70% and specificity of 90%.

### Estimation of average sensitivity and specificity

The analysis for each test strategy was undertaken first restricting to studies that reported a common threshold to estimate average sensitivity and specificity for each test at each threshold. Although data on all thresholds were extracted, we present only key common thresholds from the literature, close to risks of 1:384, 1:250 and the 5% false positive rate (FPR), unless other thresholds were more commonly reported. Where combinations of tests were used in a risk score we extracted the result for the test combination using the risk score and not the individual components that made up the test.

We undertook meta-analyses using hierarchical summary ROC (HSROC) models, which included estimation of random-effects in accuracy and threshold parameters when there were four or more studies. Where there were fewer than four studies and the studies reported test performance at a common threshold, we computed average sensitivity and specificity values by using univariate fixed-effect or random-effects logistic regression models to average logit sensitivity and logit specificity separately because of insufficient number of studies to reliably estimate all the parameters in the HSROC model. It is common in this field for studies to report sensitivity for a fixed specificity (usually a 5% FPR). This removes the requirement to account for the correlation between sensitivity and specificity across studies by using a bivariate meta-analytical method since all specificities are the same value. Thus, at a fixed specificity value, logit sensitivities were pooled using a univariate random-effects logistic regression model. This model was further simplified to a fixed-effect model when there were only two or three studies and heterogeneity was not observed on the SROC plot. All analyses were undertaken using the NLMIXED procedure in SAS (version 9.2; SAS Institute, Cary, NC) and the `xtmelogit` command in Stata version 11.2 (Stata-Corp, College Station, TX, USA).

### Comparisons between tests

We made comparisons between tests, first by utilising all available studies, selecting one threshold from each study to estimate a summary ROC curve without restricting to a common threshold, and second, by making pair-wise comparisons using studies that compared tests in the same mothers (direct head-to-head comparison). The threshold was chosen for each study according to the following order of preference a) the risk threshold closest to 1 in 250; b) a multiples of the median (MoM) or presence/absence threshold; c) the performance closest to a 5% FPR or 95th percentile. The 5% FPR was chosen as a cut-off point as this is the cut-off most commonly reported in the literature.

For the analysis that included data from all studies, we compared test strategies in a single HSROC model, including two indicator terms for each test to allow for differences in accuracy and threshold. There was no indication of differing SROC curve shape between tests and so a single SROC shape parameter was included

in the model, such that the fitted SROC curves did not cross. The initial meta-analyses of individual test strategies indicated there were differences in the variability of the accuracy parameter such that the assumption of equal variances may not be justifiable. We attempted to fit a model with separate variance terms for each test strategy for the accuracy parameter but the model did not converge. We therefore restricted the meta-analysis that compared the accuracy of the different test strategies to only studies that used a 5% FPR threshold so that we could fit a univariate random effects logistic regression model that allowed for a separate variance term for the random-effects of logit sensitivity for each test. Using non-linear combinations of the parameter estimates from this model, we derived ratios of sensitivities for each pair of tests included in the model and obtained their corresponding 95% confidence interval (CI) by using the delta method. We used likelihood ratio tests to assess the statistical significance of differences in sensitivity between tests.

For direct comparisons between each pair of tests at the 5% FPR threshold, we used a separate model for each pair-wise comparison and pooled logit sensitivities using a univariate random-effects model. As studies rarely reported data cross-classified by both tests for Down's and normal pregnancies, the analytical method did not take full account of the pairing of test results, but the restriction to direct head-to-head comparisons should have removed the potential confounding of test comparisons with other features of the studies. The strength of evidence for differences in performance of test strategies relied on evidence from both the direct and indirect comparisons.

### Investigations of heterogeneity

We planned to undertake investigations of heterogeneity if there were 10 or more studies available for a test. We planned to investigate the effect of a covariate by adding covariate terms to the HSROC model to assess differences in accuracy and threshold.

### Sensitivity analyses

Mothers with pregnancies identified as high risk for Down's syndrome by serum testing are often offered immediate definitive testing by amniocentesis, whereas those considered low risk are assessed for Down's syndrome by inspection at birth. Such delayed and differential verification will introduce bias most likely through there being greater loss to miscarriage in the Down's syndrome pregnancies that were not detected by the serum testing (the false negative diagnoses). Testing and detection of miscarriages is impractical in many situations, and no clear data are available on the magnitude of these miscarriage rates.

To account for the possible bias introduced by such a mechanism, we planned to perform sensitivity analyses by increasing the percentage of false negatives in studies where delayed verification in test negatives occurred (Mol 1999). We planned to incrementally

increase the percentage from 10% to 50%, the final value representing a scenario where a third or more Down's pregnancies than normal pregnancies were likely to miscarry, thought to be higher than the likely value. We intended to conduct the sensitivity analyses on the analysis investigating the effect of maternal age on test sensitivity.

## RESULTS

### Results of the search

The search for the whole suite of reviews identified a total of 15,394 papers, once the results from each bibliographic database were combined and duplicates were removed. After screening out obviously inappropriate papers based on their title and abstract, 1145 papers remained and we obtained full-text copies for formal assessment of eligibility. From these, a total of 269 papers were deemed eligible and were included in the suite of reviews. We included a total of 56 studies (reported in 68 publications) in this review of first trimester serum screening, involving 204,759 pregnancies, of which 2113 were Down's syndrome pregnancies. A total of 78 different test strategies or combinations, at one or more thresholds, were evaluated in the 56 studies. These tests were produced from combinations of 18 different serum tests with and without maternal age; ADAM12, AFP, inhibin, PAPP-A, ITA, free  $\beta$ hCG, PIGF, SP1, total hCG, progesterone, uE3, GHBP, PGH, hyperglycosylated hCG, ProMBP, hPL, free  $\alpha$ hCG, and free  $\beta$ hCG to AFP ratio. Strategies evaluated included three quintuple tests, three quadruple tests, 12 triple tests, 27 double tests and 15 single tests in combination with maternal age, and three triple tests, five double tests and 10 single tests without maternal age. The following combinations evaluated included four or more studies.

### Double tests with maternal age

1. Free  $\beta$ hCG, AFP and maternal age (five studies; 5160 women including 174 Down's syndrome pregnancies)
2. Free  $\beta$ hCG, PAPP-A and maternal age (31 studies; 158,878 women including 1430 Down's syndrome pregnancies)

### Single tests with maternal age

1. Free  $\beta$ hCG and maternal age (nine studies; 16,656 women including 549 Down's syndrome pregnancies)
2. PAPP-A and maternal age (six studies; 13,742 women including 409 Down's syndrome pregnancies)

### Single tests without maternal age

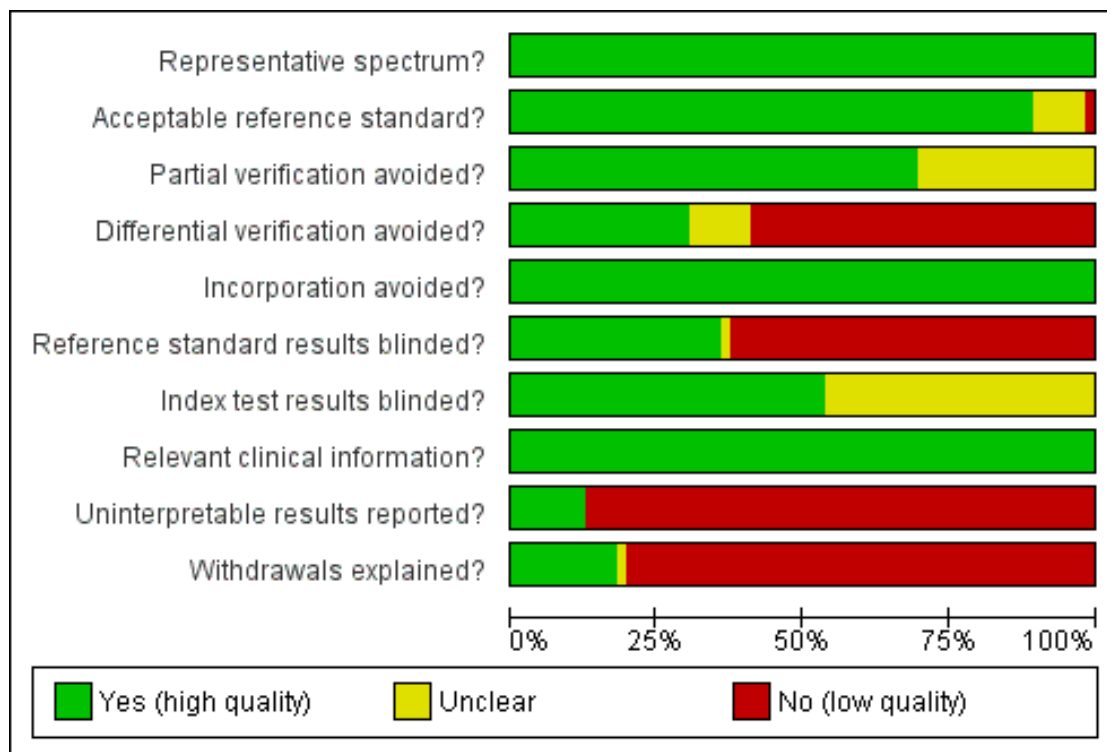
1. Free  $\beta$ hCG (four studies; 4280 women including 390 Down's syndrome pregnancies)
2. PAPP-A (six studies; 25,510 women including 430 Down's syndrome pregnancies)

Of the remaining test combinations, seven were evaluated in three studies, 17 were evaluated in two studies and the remainder were evaluated in single studies only.

### Methodological quality of included studies

We judged the methodological quality of the studies to be high in most categories (Figure 1). Due to the nature of testing for Down's syndrome screening and the potential side effects of invasive testing, differential verification is almost universal in the general screening population, as most women whose screening test result is defined as low risk will have their screening test verified at birth, rather than by invasive diagnosis in the antenatal period. Additionally, it was not always possible to ascertain from the included studies whether or not the results of index tests and reference standards were blinded. It would be difficult to blind clinicians performing invasive diagnostic tests (reference standards) to the index test result, unless all women received the same reference standard, which would not be appropriate in most scenarios. Any biases secondary to a lack of clinician blinding are likely to be minimal.

**Figure 1. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.**



Where details of completeness of follow-up were poorly reported, most studies seemed to assume 100% follow-up. However, there will inevitably be losses to follow-up due to women moving out of area, for example. Studies sometimes accounted for these and it is unlikely that there were enough losses to follow-up to have introduced significant bias. There was likely under-ascertainment of miscarriage, and very few papers accounted for miscarriage, or performed tissue karyotyping in pregnancies resulting in miscarriage. Some studies attempted to adjust for predicted miscarriage rate and the incidence of Down's syndrome in this specific population, but most did not. We have not attempted to adjust for expected miscarriage rate in this review. There is a higher natural miscarriage rate in the first trimester, however this will be uniform across studies and therefore unlikely to introduce significant bias. Some studies that provided estimates of risk using multivariable equations used the same data set to evaluate performance of the risk equation as was used to derive the equation. This is often thought to lead to over-estimation of test performance.

### Findings

The findings of the 21 most common and/or best performing test strategies are given in [Summary of findings 1](#). The remaining 57

strategies are briefly summarised in [Summary of findings 2](#). The test strategies evaluated by four or more studies are detailed below.

#### 1) Free $\beta$ HCG, PAPP-A and maternal age (double test)

Results for this double test were derived from 31 studies (Biagiotti 1998; Brambati 1994; Christiansen 2005; Christiansen 2007a; Christiansen 2009; Christiansen 2010; Cowans 2010; Crossley 2002a; De Graaf 1999a; Forest 1997; Gyselaers 2005; Haddow 1998; Kagan 2009; Kozlowski 2007 GC; Kozlowski 2007 PC; Krantz 2000; Muller 2003a; Niemimaa 2001a; O'Leary 2006; Orlandi 1997; Sahota 2010; Schaelike 2009; Scott 2004; Spencer 1999a; Topping 2010; Tsukerman 1999; Valinen 2007; Wald 2003a; Wapner 2003; Wojdemann 2005; Zaragoza 2009), and included 158,878 women in whom 1430 pregnancies were known to be affected by Down's syndrome. Seven studies contributed over 10,000 pregnancies each to the data (Crossley 2002a; Gyselaers 2005; Kagan 2009; Krantz 2000; O'Leary 2006; Sahota 2010; Schaelike 2009). Studies presented data for cut-points of 5% FPR (Biagiotti 1998; Brambati 1994; Cowans 2010; De Graaf 1999a; Forest 1997; Haddow 1998; Kagan 2009; Sahota 2010; Spencer 1999a; Sahota 2010; Topping 2010; Tsukerman 1999; Wald

2003a; Wapner 2003; Zaragoza 2009), 1:250 risk (Christiansen 2005; Christiansen 2007a; Christiansen 2009; Christiansen 2010; Crossley 2002a; Kagan 2009; Muller 2003a; Niemimaa 2001a; Torring 2010; Valinen 2007; Wojdemann 2005), and 1:300 risk (Kozłowski 2007 GC; Kozłowski 2007 PC; Schaelike 2009). At a cut-point of 5% FPR (17 studies), the sensitivity was estimated as 68% (95% confidence interval (CI) 65 to 71) and the specificity at 95% (95% CI 95 to 95). At a cut-point of 1:250 FPR (11 studies), the sensitivity was estimated as 73% (95% CI 67 to 79) and the specificity as 93% (95% CI 91 to 94).

## 2) Free $\beta$ hCG, AFP and maternal age (double test)

Results for this double test were derived from five studies (Benattar 1999; Biagiotti 1995; Forest 1995; Tsukerman 1999; Wald 2003a), and included 5160 women in whom 174 pregnancies were known to be affected by Down's syndrome. Two contributed over 1000 pregnancies each to the data (Benattar 1999; Tsukerman 1999). Studies presented data for cut-points of 5% FPR (Biagiotti 1995; Tsukerman 1999; Wald 2003a), 1:250 risk (Benattar 1999) and 1:384 risk (Forest 1995). At a cut-point of 5% FPR (three studies), the sensitivity was estimated as 49% (95% CI 39 to 60) and the specificity as 95% (95% CI 94 to 96).

## 3) PAPP-A and maternal age (single test)

Results for this single test were derived from six studies (Biagiotti 1998; Brambati 1993; Forest 1997; Krantz 2000; Spencer 1999a; Wald 2003a), and included 13,742 women in whom 409 pregnancies were known to be affected by Down's syndrome. Krantz 2000 was the largest study, contributing over 10,000 pregnancies to the data. Studies presented data for cut-points of 5% FPR (Biagiotti 1998; Brambati 1993; Forest 1997; Spencer 1999a; Wald 2003a) and 1:105 risk (Krantz 2000). At a cut-point of 5% FPR (five studies), the sensitivity was estimated as 55% (95% CI 46 to 63) and the specificity as 95% (95% CI 94 to 96).

## 4) Free $\beta$ hCG and maternal age (single test)

Results for this single test were derived from nine studies (Biagiotti 1995; Biagiotti 1998; Brambati 1994; Forest 1995; Forest 1997; Krantz 2000; Noble 1995; Spencer 1999a; Wald 2003a), and included 16,656 women in whom 549 pregnancies were known to be affected by Down's syndrome. Krantz 2000 contributed over 10,000 pregnancies to the data. Studies presented data for cut-points of 5% FPR (Biagiotti 1995; Biagiotti 1998; Brambati 1994; Forest 1997; Noble 1995; Spencer 1999a; Wald 2003a), 1:384 risk (Forest 1995) and 1:105 risk (Krantz 2000). At a cut-point of 5% FPR (seven studies), the sensitivity was estimated as 42% (95% CI 36 to 48) and the specificity as 95% (95% CI 94 to 96).

## 5) PAPP-A alone (single test without maternal age)

Results for this single test were derived from six studies (Brambati 1993; Brameld 2008; Brizot 1994; Casals 1996; Spencer 1999a; Wald 2003a), and included 25,510 women in whom 430 pregnancies were known to be affected by Down's syndrome. Brameld 2008 was the largest study contributing over 20,000 pregnancies to the data. Studies presented data for cut-points of 5% FPR (Brambati 1993; Brizot 1994; Casals 1996; Spencer 1999a; Wald 2003a) and  $\leq$  5th percentile (Brameld 2008). At a cut-point of 5% FPR (four studies), the sensitivity was estimated as 52% (95% CI 39 to 65) and the specificity as 95% (95% CI 94 to 96).

## 6) Free $\beta$ hCG alone (single test without maternal age)

Results for this single test were derived from four studies (Casals 1996; Noble 1997; Spencer 1999a; Wald 2003a), and included 4280 women in whom 390 pregnancies were known to be affected by Down's syndrome. Studies were all of a similar size. Studies presented data at a 5% FPR. At this cut-point, the sensitivity was estimated as 25% (95% CI 18 to 34) and the specificity as 95% (95% CI 94 to 96).

## 7) Other test combinations

Of the 73 test combinations evaluated in three or fewer studies, several test combinations demonstrated estimated sensitivities of more than 70% and estimated specificities of more than 90%. Twelve of these were evaluated in single studies (Summary of findings 2), however, three test combinations were evaluated in two or more studies.

1. A triple test of **PAPP-A, free  $\beta$ hCG, AFP and maternal age** was evaluated in three studies (Muller 2003a; Tsukerman 1999; Wald 2003a), had an estimated sensitivity of 74% (95% CI 65 to 81) at a cut-point of 5% FPR.
2. A triple test of **ADAM 12, PAPP-A, free  $\beta$ hCG and maternal age** was evaluated in three studies (Christiansen 2010; Torring 2010; Valinen 2009), had an estimated sensitivity of 74% (95% CI 63 to 83) at a cut-point of 5% FPR.
3. A triple test of **PIGE, PAPP-A, free  $\beta$ hCG and maternal age** was evaluated in two studies (Cowans 2010; Zaragoza 2009), had an estimated sensitivity of 76% (95% CI 69 to 82) at a cut-point of 5% FPR.

## Comparative analysis of the nine selected test strategies

We chose to estimate detection rates at a 5% FPR, in common with much of the literature. Figure 2 shows point estimates of detection rates for a 5% FPR based on all available data for all nine test combinations described above, and the confidence intervals at a fixed 5% FPR. For example, the plot shows that for the double test with a marker combination of free  $\beta$ hCG, AFP and maternal age, the

estimated detection rate at a 5% FPR was 49% (95% CI 39 to 60) based on data from three studies with 157 affected cases and 2992 total participants. The test combinations in Figure 2 are ordered according to decreasing detection rates. The single test strategies with and without maternal age (PAPP-A alone; free  $\beta$ hCG alone, PAPP-A and maternal age, and free  $\beta$ hCG and maternal age) have the worst performance, whereas, the triple test strategies (ADAM 12, PAPP-A, free  $\beta$ hCG and maternal age; PAPP-A, free  $\beta$ hCG, AFP and maternal age) have the highest performance. In between lie the double tests (free  $\beta$ hCG, PAPP-A and maternal age; free  $\beta$ hCG, AFP and maternal age). However, it should be noted that the confidence intervals on these estimates are wide and overlap for the lower performing five strategies, suggesting that any of the differences observed may be explicable by chance.

**Figure 2. Detection rates (sensitivity) at a 5% false positive rate for the nine selected test strategies. Each circle represents the summary sensitivity for a test strategy and the size of each circle is proportional to the number of Down's cases. The estimates are shown with 95% confidence intervals. The test strategies are ordered on the plot according to decreasing detection rate. The number of studies, cases and women included for each test strategy are shown on the horizontal axis. A = Age, PIGF, PAPP-A and free  $\beta$ hCG; B = Age, PAPP-A, free  $\beta$ hCG and AFP; C = Age, ADAM 12, PAPP-A and free  $\beta$ hCG; D = Age, PAPP-A and free  $\beta$ hCG; E = Age, PAPP-A; F = PAPP-A; G = Age, free  $\beta$ hCG and AFP; H = Age, free  $\beta$ hCG; I = Free  $\beta$ hCG**

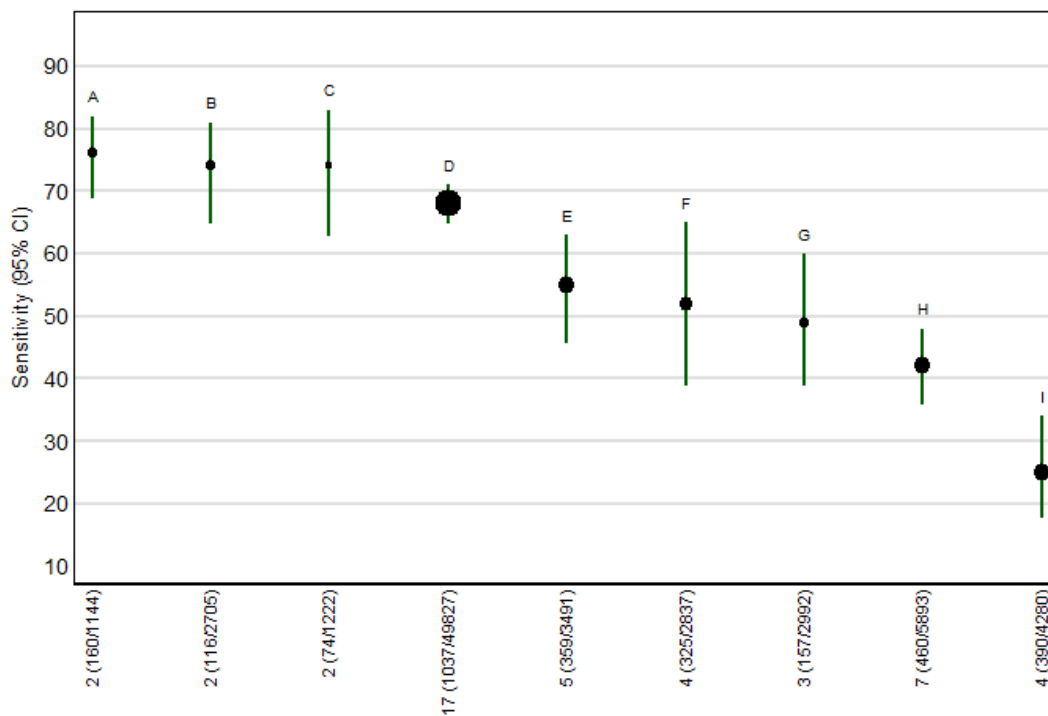




Table 1 shows pair-wise direct comparisons (head-to-head) where studies were available. Such comparisons are regarded as providing the strongest evidence as they compare tests within pregnancies and are thus unconfounded. The table shows the ratios of sensitivities with 95% CIs and P values ( $P < 0.05$  being considered a statistically significant difference) for each test comparison, the number of studies ( $K$ ) for which data were available. The table shows that the sensitivity of the single test combinations (PAPP-A alone, free  $\beta$ hCG alone, PAPP-A and maternal age, and free  $\beta$ hCG and maternal age) tended to be significantly worse ( $P < 0.05$ ) than the double and triple tests where data are available. The double test comprised of PAPP-A, free  $\beta$ hCG and maternal age appears to have significantly better ( $P = 0.004$ ) test accuracy than the double test comprised of free  $\beta$ hCG, AFP and maternal age. Otherwise, there was no strong evidence of significant improvements in sensitivity with the addition of a third marker. However, most comparisons in this table are based on only single studies and are unlikely to be powered to detect differences in detection rates.

Table 2 shows the same comparisons made using all available data (as used to create Figure 2). Results are in agreement with the direct comparisons, and in addition, showed that the triple test comprised of PIGE, PAPP-A, free  $\beta$ hCG and maternal age is significantly better ( $P = 0.024$ ) than the double test comprised of PAPP-A, free  $\beta$ hCG and maternal age. However, these comparisons are potentially confounded by differences between the studies, and are based on small numbers of studies.

#### Investigation of heterogeneity and sensitivity analyses

The key characteristics of the 56 included studies is summarised in Table 3 with further details available in the Characteristics of included studies table. Only one test combination- PAPP-A, free  $\beta$ hCG and maternal age (17 studies) was evaluated by 10 or more studies but there were no data for investigation of the effect of maternal age or any other potential source of heterogeneity. The planned sensitivity analyses were also not possible.

## Summary of findings

<b>Review Question</b>	What is the accuracy of serum-based markers for Down's syndrome screening in the first trimester?				
<b>Population</b>	Pregnant women at less than 14 weeks' gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome. Most studies were undertaken in women identified to be high risk based on maternal age				
<b>Settings</b>	All settings				
<b>Numbers of studies, pregnancies and Down's syndrome cases</b>	56 studies (68 publications) involving 204,759 pregnancies of which 2113 were Down's syndrome pregnancies				
<b>Index tests</b>	18 serum markers (ADAM12, AFP, inhibin, PAPP-A, ITA, free $\beta$ hCG, PIGF, SP1, total hCG, progesterone, uE3, GHBP, PGH, hyperglycosylated hCG, ProMBP, hPL, free $\alpha$ hCG, and free $\beta$ hCG to AFP ratio) singly or in combination with or without maternal age				
<b>Reference standards</b>	Chromosomal verification (amniocentesis and CVS undertaken during pregnancy, and postnatal karyotyping) and postnatal macroscopic inspection				
<b>Study limitations</b>	35 studies used selective chromosomal verification during pregnancy, and were at risk of under-ascertainment of Down's syndrome cases due loss of the pregnancy to miscarriage between the serum test and the reference standard				
<b>Tests with at least 70% sensitivity and at least 95% specificity</b>					
<b>Test strategy</b>	<b>Studies</b>	<b>Women (cases)</b>	<b>Sensitivity (95% CI)</b>	<b>Specificity (95% CI)</b>	<b>Test*</b>
<b>Test strategies (with or without maternal age) evaluated by a single study</b>					
<b>Without maternal age</b>					
<b>Double tests</b>					
PAPP-A and AFP	1	96 (16)	81 (54 to 96)	95 (88 to 99)	
PAPP-A and ITA	1	344 (24)	71 (49 to 87)	95 (92 to 97)	
<b>Triple tests</b>					

PAPP-A, free $\beta$ hCG and ITA	1	344 (24)	75 (53 to 90)	95 (92 to 97)
PIGF, PAPP-A and free $\beta$ hCG	1	699 (90)	72 (62 to 81)	95 (93 to 97)
<b>With maternal age</b>				
<b>Double tests</b>				
Free $\beta$ hCG and SP1	1	60 (14)	71 (42 to 92)	96 (85 to 99)
PAPP-A and Hyperglycosylated hCG	1	10775 (23)	74 (52 to 90)	95 (95 to 95)
<b>Triple tests</b>				
PAPP-A, free $\beta$ hCG and Inhibin	1	1110 (85)	74 (63 to 83)	95 (94 to 96)
PAPP-A, SP1 and ProMBP	1	192 (15)	73 (45 to 92)	95 (91 to 98)
hPL, PAPP-A and free $\beta$ hCG (1:250 risk)	1	183 (47)	77 (62 to 88)	95 (90 to 98)
<b>Quadruple tests</b>				
GHBP, PGH, PAPP-A and free $\beta$ hCG (1:250 risk)	1	335 (74)	76 (64 to 85)	95 (91 to 97)
<b>Quintuple tests</b>				
PAPP-A, free $\beta$ hCG, AFP, uE3 and Inhibin	1	1110 (85)	78 (67 to 86)	95 (94 to 96)
PAPP-A, total hCG, AFP, uE3 and Inhibin	1	1110 (85)	73 (62 to 82)	95 (94 to 96)
<b>Test strategies (with or without maternal age) evaluated by at least two studies</b>				

Free $\beta$ hCG	4	4280 (390)	25 (18 to 34)	95 (94 to 96)	P <0.001
PAPP-A	4	2837 (325)	52 (39 to 65)	95 (94 to 96)	
Age, free $\beta$ hCG	7	5893 (460)	42 (36 to 48)	95 (94 to 96)	
Age, PAPP-A	5	3491 (359)	55 (46 to 63)	95 (94 to 96)	
Age, free $\beta$ hCG and AFP	3	2992 (157)	49 (39 to 60)	95 (94 to 96)	
Age, PAPP-A and free $\beta$ hCG	17	49827 (1037)	68 (65 to 71)	95 (95 to 95)	
Age, PAPP-A, free $\beta$ hCG and AFP	2	2705 (116)	74 (65 to 81)	95 (94 to 96)	
Age, ADAM 12, PAPP-A and free $\beta$ hCG	2	1222 (74)	74 (63 to 83)	95 (94 to 96)	
Age, PIGF, PAPP-A and free $\beta$ hCG	2	1144 (160)	76 (69 to 82)	95 (93 to 96)	

\*Likelihood ratio test for the difference in sensitivity between the nine test strategies that were formally compared in a single meta-analytic model.

**ADAM12:** a disintegrin and metalloprotease; **AFP:** alpha-fetoprotein;  $\alpha$ hCG: alpha human chorionic gonadotrophin;  **$\beta$ hCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **CVS:** chorionic villus sampling; **GHBP:** growth hormone binding protein; **hCG:** human chorionic gonadotrophin; **hPL:** human placental lactogen; **ITA:** invasive trophoblast antigen; **PAPP-A:** pregnancy-associated plasma protein A; **PGH:** placental growth hormone; **PIGF:** placental growth factor; **PROMBP:** proform of eosinophil major basic protein; **SPI:** Schwangerschafts protein 1; **uE3:** unconjugated oestriol

Test strategy	Studies	Women (cases)	Sensitivity (95% CI)	Specificity (95% CI)	Threshold
<b>Without maternal age</b>					
<b>Single tests</b>					
AFP	2	2248 (104)	10 (4 to 21)	95	5% FPR
ADAM 12	1	579 (17)	41 (18 to 67)	95 (93 to 97)	5% FPR
Free $\beta$ hCG to AFP ratio	1	476 (9)	11 (0 to 48)	98 (96 to 99)	0.25 MoM
Inhibin	3	2098 (184)	19 (4 to 58)	95	5% FPR
PIGF	1	699 (90)	28 (19 to 38)	95 (93 to 97)	5% FPR
Total hCG	3	2098 (184)	19 (4 to 58)	95	5% FPR
SP1	3	1080 (53)	32 (1 to 96)	95	5% FPR
uE3	1	1110 (85)	13 (7 to 22)	95 (94 to 96)	5% FPR
<b>Double tests</b>					
Free $\beta$ hCG and AFP	1	1138 (19)	16 (3 to 40)	95 (94 to 96)	5% FPR
Free $\beta$ hCG and Inhibin	1	876 (76)	30 (20 to 42)	95 (93 to 96)	5% FPR
PAPP-A and free $\beta$ hCG	2	795 (106)	64 (50 to 76)	95	5% FPR
<b>Triple tests</b>					
Total hCG, free $\alpha$ hCG and progesterone	1	129 (17)	53 (28 to 77)	96 (90 to 99)	0.34 MoM
<b>With maternal age</b>					

<b>Single tests</b>					
ADAM 12	2	703 (46)	67 (46 to 83)	91 (87 to 94)	1:400 risk
AFP	2	1397 (126)	33 (23 to 46)	95	5% FPR
Free $\alpha$ hCG	1	512 (12)	25 (5 to 57)	89 (86 to 91)	1:384 risk
GHBP	1	335 (74)	27 (17 to 39)	95 (91 to 97)	1:250 risk
hPL	1	183 (47)	45 (30 to 60)	93 (88 to 97)	1:250 risk
Inhibin	1	1110 (85)	32 (22 to 43)	95 (94 to 96)	5% FPR
ITA	1	278 (54)	48 (34 to 62)	95 (91 to 98)	5% FPR
PGH	1	335 (74)	41 (29 to 53)	94 (91 to 97)	1:250 risk
PIGF	1	699 (90)	43 (33 to 54)	95 (93 to 97)	5% FPR
ProMBP	1	181 (25)	36 (18 to 57)	94 (89 to 97)	1:250 risk
SP1	2	804 (29)	38 (22 to 56)	95	5% FPR
Total hCG	1	512 (12)	33 (10 to 65)	94 (92 to 96)	1:384 risk
uE3	1	512 (12)	33 (10 to 65)	86 (83 to 89)	1:384 risk
<b>Double tests</b>					
ADAM 12 and PAPP-A	1	691 (46)	61 (45 to 75)	95 (93 to 97)	5% FPR
AFP and free $\alpha$ hCG	1	512 (12)	33 (10 to 65)	87 (83 to 89)	1:384 risk
AFP and total hCG	1	512 (12)	33 (10 to 65)	93 (90 to 95)	1:384 risk

AFP and uE3	1	512 (12)	42 (15 to 72)	87 (84 to 90)	1:384 risk
Free $\beta$ hCG and free $\alpha$ hCG	1	512 (12)	42 (15 to 72)	94 (91 to 96)	1:384 risk
Free $\beta$ hCG and Inhibin	1	1110 (85)	44 (33 to 55)	95 (94 to 96)	5% FPR
Free $\beta$ hCG and total hCG	1	512 (12)	25 (5 to 57)	93 (90 to 95)	1:384 risk
Free $\beta$ hCG and uE3	1	287 (41)	61 (45 to 76)	95 (92 to 97)	5% FPR
GHBP and free $\beta$ hCG	1	335 (74)	61 (49 to 72)	92 (88 to 95)	1:250 risk
GHBP and PAPP-A	1	335 (74)	66 (54 to 77)	93 (89 to 96)	1:250 risk
GHBP and PGH	1	335 (74)	47 (36 to 59)	93 (90 to 96)	1:250 risk
hPL and free $\beta$ hCG	1	183 (47)	68 (53 to 81)	94 (89 to 97)	1:250 risk
hPL and PAPP-A	1	183 (47)	55 (40 to 70)	94 (89 to 97)	1:250 risk
PAPP-A and AFP	2	2705 (116)	63 (50 to 74)	95	5% FPR
PAPP-A and Inhibin	1	1110 (85)	68 (57 to 78)	95 (94 to 96)	5% FPR
PAPP-A and ITA	2	622 (78)	62 (46 to 75)	95	5% FPR
PGH and free $\beta$ hCG	1	335 (74)	64 (52 to 74)	93 (89 to 96)	1:250 risk
PGH and PAPP-A	1	335 (74)	65 (53 to 76)	93 (89 to 96)	1:250 risk
Total hCG and free $\alpha$ hCG	1	512 (12)	42 (15 to 72)	92 (89 to 94)	1:384 risk
Total hCG and Inhibin	1	1110 (85)	34 (24 to 45)	95 (94 to 96)	5% FPR
Total hCG and PAPP-A	2	4327 (133)	66 (54 to 76)	95	5% FPR

Total hCG and uE3	1	512 (12)	42 (15 to 72)	92 (89 to 94)	1:384 risk
uE3 and free $\alpha$ hCG	1	512 (12)	33 (10 to 65)	89 (86 to 91)	1:384 risk
<b>Triple tests</b>					
AFP, free $\alpha$ hCG and uE3	1	512 (12)	58 (28 to 85)	82 (79 to 85)	1:384 risk
Free $\beta$ hCG, AFP and uE3	1	287 (41)	66 (49 to 80)	95 (92 to 97)	5% FPR
GHBP, PAPP-A and free $\beta$ hCG	1	335 (74)	76 (64 to 85)	94 (91 to 97)	1:250 risk
PAPP-A, total hCG and Inhibin	1	1110 (85)	69 (58 to 79)	95 (94 to 96)	5% FPR
PGH, PAPP-A and free $\beta$ hCG	1	335 (74)	76 (64 to 85)	94 (91 to 97)	1:250 risk
Total hCG, AFP and uE3	1	512 (12)	42 (15 to 72)	91 (88 to 94)	1:384 risk
<b>Quadruple tests</b>					
Free $\beta$ hCG, total hCG, AFP and uE3	1	512 (12)	50 (21 to 79)	92 (89 to 94)	1:384 risk
Total hCG, AFP, uE3 and free $\alpha$ hCG	1	512 (12)	50 (21 to 79)	90 (87 to 92)	1:384 risk
<b>Quintuple tests</b>					
Free $\beta$ hCG, total hCG, AFP, uE3 and free $\alpha$ hCG	1	512 (12)	33 (10 to 65)	90 (87 to 92)	1:384 risk

**AFP:** alpha-fetoprotein;  **$\alpha$ hCG:** alpha human chorionic gonadotrophin;  **$\beta$  hCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **FPR:** false positive rate; **GHBP:** growth hormone binding protein; **hCG:** human chorionic gonadotrophin; **hPL:** human placental lactogen; **ITA:** invasive trophoblast antigen; **PAPP-A:** pregnancy-associated plasma protein A; **PGH:** placental growth hormone; **PIGF:** placental growth factor; **PROMBP:** proform of eosinophil major basic protein; **SPI:** Schwangerschafts protein 1; **uE3:** unconjugated oestriol



## DISCUSSION

### Summary of main results

The systematic review found a large number of studies evaluating first trimester Down's syndrome serum screening tests, including studies evaluating the commonly used double test. Few studies were available to evaluate the performance of test strategies involving newer markers, such as ADAM 12, and few studies provided unconfounded comparisons of test strategies by applying and comparing several strategies using the same serum sample, the majority of studies only evaluating a single test combination. A summary of results for the nine most common and best performing strategies is given in [Summary of findings 1](#), briefer details for the remaining strategies are given in [Summary of findings 2](#). Three key findings were noted.

1. The double test comprised of PAPP-A, free  $\beta$ hCG and maternal age appears to have significantly better ( $P < 0.05$ ) test accuracy than the double test comprised of free  $\beta$ hCG, AFP and maternal age, and the single tests (both the markers alone and in combination with maternal age). This test detects around seven out of every 10 Down's affected pregnancies for a fixed 5% FPR. By comparison, the double test comprised of free  $\beta$ hCG, AFP and maternal age, and single tests alone and in combination with maternal age detects between two and five out of every 10 Down's affected pregnancies for a fixed 5% FPR.

2. Whilst the triple test combinations show the highest detection rates, they were not shown to be statistically superior to the double test comprised of PAPP-A, free  $\beta$ hCG and maternal age. Whilst some significant differences between these categories of tests were noted in the indirect comparisons, the potential for confounding is of concern. Estimates suggest that triple test combinations may detect between seven and eight out of every 10 Down's syndrome pregnancies at a 5% FPR, however these estimates are based on data from two or three studies evaluating small numbers of women. It is difficult to make strong recommendations on the use of triple tests, as we cannot rule out possible differences due to the limited power there is to detect a difference.

3. The evidence for higher numbers of markers shows similar detection rates to double and triple markers, but are based on data from one study only, therefore further evaluation of these tests is required. Furthermore, there are other combinations of double markers that show similar detection rates to standard double markers commonly used in clinical practice, which may warrant further study.

### Strengths and weaknesses of the review

This review is the first comprehensive review of first trimester serum screening. We examined papers from around the world,

covering a wide cross-section of women in varying populations. We contacted authors to verify data where necessary to give as complete a picture as possible, while trying to avoid replication of data.

There were a number of factors that made meta-analysis of the data difficult, which we tried to adapt for, in order to allow for comparability of data presented in different studies.

1. There were many different cut-points used to define pregnancies as high or low risk for Down's syndrome. This means that direct comparison is more difficult than if all studies used the same cut-point to dichotomise their populations. This is less of an issue for first trimester serum screening, compared to second trimester serum screening, as the majority of authors chose a cut-point of 5% FPR.

2. There were many different risk equations and software applications in use for combination of multiple markers, which were often not described in the papers. This means that risks may be calculated by different formulae and they may not be directly comparable for this reason. It is possible that this is responsible for unexplained heterogeneity in results.

3. Different laboratories and clinics run different assays and use different machines and methods. This may influence raw results and subsequent risk calculations. Many laboratories have a quality assessment or audit trail, however, this may not necessarily be standard across the board. For example, how many assays are run, how often medians are calculated and adjusted for a given population and how quickly samples are tested from initially being taken.

4. Few papers made direct comparisons between tests, making it difficult to detect if a real difference exists between tests (i.e. how different tests perform in the same population). There were differences in populations, with assay medians being affected, for example, by race. It is not certain whether it is appropriate to make comparisons between populations which are inherently different.

5. We were unable to perform the investigations of heterogeneity that we had originally intended to because the data simply were not available. The vast majority of papers looking at pregnancies conceived by IVF, affected by diabetes, multiple gestation or a family history of Down's syndrome involved unaffected pregnancies only.

In addition, the search for this review was last updated in August 2011, and it is possible that new studies may have been published which have not been included. Since the search was completed we have kept a watching brief on outputs and are not aware of any studies with substantial sample sizes which could substantially affect the findings.

### Applicability of findings to the review question

Potentially, when planning screening policy or a clinical screening programme, clinicians and policy makers need to make decisions

about a finite number of tests or type of tests that can be offered. These policies are often driven by both the needs of a specific population and by financial resources. Economic analysis was considered to be outside of the scope of this review. Many of the tests examined as part of this review are already commercially available and in use in the clinical setting. The studies were carried out on populations of typical pregnant women and therefore, the results should be considered comparable with most pregnant populations encountered in every day clinical practice.

We were unable to extract information about harms of testing, information about miscarriage rates and uptake of definitive testing as the data were not available the majority of the time. Whilst it is unlikely that major differences between the tests evaluated here exist in terms of direct harms of testing, as they are all based on a single blood sample, differences in accuracy may lead to differences in the use of definitive testing and its consequent adverse outcomes.

In some countries with a defined screening policy (i.e. the UK), first trimester screening plays a major role, although usually in combination with first trimester ultrasound scanning. In others however, there may only be a limited range of tests or markers available, often second trimester markers, rather than first trimester markers. The results of this review should be interpreted and applied in the context of test availability and local restrictions, populations or policies.

## AUTHORS' CONCLUSIONS

### Implications for practice

The evidence supports the use of the first trimester double test comprised of PAPP-A, free  $\beta$ hCG and maternal age, there is little evidence to recommend the use of first trimester serum tests with three or more markers, however the data available on these tests are limited, and based on generally small populations of women. We would not recommend that these tests be introduced into wider clinical practice without careful consideration of cost.

The review has shown that tests involving two or three markers in combination with maternal age are significantly better than those involving one marker. We would therefore recommend that one marker tests are not used for Down's syndrome screening. The choice of multiple markers will depend on the availability of certain assays in local laboratories. On the basis of this review we would recommend the combination of PAPP-A, free  $\beta$ hCG and maternal age, as it significantly outperforms free  $\beta$ hCG, AFP and maternal age, and is widely available. The data for other test combinations limits our ability to make any other recommendations about specific test combinations. Alternative screening methods

should also be considered (i.e. use of ultrasound markers in the first trimester) when making policy decisions, and are the subject of other reviews in this suite.

The screening blood tests themselves have no adverse effects for the woman, over and above the risks of a routine blood test. However some women who have a 'high risk' screening test result, and are given amniocentesis or CVS have a risk of miscarrying a baby unaffected by Down's. Parents will need to weigh up this risk when deciding whether or not to have an amniocentesis or CVS following a 'high risk' screening test result.

### Implications for research

Further evaluations of test combinations involving three or more markers are required to determine whether their apparent advantages are not chance findings.

Future studies should ensure that adequate sample sizes are recruited, and take opportunities to make comparisons of test performance testing several alternative test combinations on the same serum samples. Such direct comparison removes issues of confounding when making test comparisons, and allows a clear focus on testing the incremental benefit of increasingly complex and expensive testing strategies. The reporting of studies of test accuracy can be improved and more closely adhere to the standards for the reporting of diagnostic accuracy studies (STARD) guideline. Three key aspects of this are 1) formally testing the statistical significance of differences in test performance in direct comparisons and estimating incremental changes in detection rates (together with confidence intervals), 2) clearly reporting the number of mothers studied and their results, and 3) reporting the numbers of women who are lost to follow-up. Many authors reported results of extrapolating findings to age-standardised national cohorts to demonstrate the performance of the test, and failed to report the actual numbers studied and evaluated.

For the purposes of meta-analysis and to allow for comparisons to be made between different tests and combinations, we would recommend the publication of consensus standard algorithms for estimating risk, and reporting of test performance at a standard set of thresholds. This would be difficult to achieve and implement, but an attempt at consensus should be made.

## ACKNOWLEDGEMENTS

We acknowledge the assistance of the Pregnancy and Childbirth Cochrane Review Group Editorial base with writing the searches (detailed in the generic protocol [Alldred 2010](#)) and other aspects of this review.

## REFERENCES

### References to studies included in this review

#### Baviera 2010 *{published data only}*

Baviera G, Chemicata S, De Domenico R, Granese R, Carbone C, Dugo N, et al. First- and second-trimester ADAM12s in Down syndrome screening. *Clinical Chemistry* 2010;**56**(8):1355–7.

#### Benattar 1999 *{published data only}*

Benattar C, Audibert F, Taieb J, Ville Y, Roberto A, Lindenbaum A, et al. Efficiency of ultrasound and biochemical markers for Down's syndrome risk screening. A prospective study. *Fetal Diagnosis and Therapy* 1999;**14**(2): 112–7.

#### Biagiotti 1995 *{published data only}*

Biagiotti R, Cariati E, Brizzi L, d'Agata A. Maternal serum screening for Down's syndrome in the first trimester of pregnancy. *British Journal of Obstetrics and Gynaecology* 1995;**102**(8):660–2.

#### Biagiotti 1998 *{published data only}*

Biagiotti R, Brizzi L, Periti E, d'Agata A, Vanzi E, Cariati E. First trimester screening for Down's syndrome using maternal serum PAPP-A and free beta-hCG in combination with fetal nuchal translucency thickness. *British Journal of Obstetrics and Gynaecology* 1998;**105**(8):917–20.

#### Brambati 1993 *{published data only}*

Brambati B, Macintosh MC, Teisner B, Maguiness S, Shrimanker K, Lanzani A, et al. Low maternal serum levels of pregnancy associated plasma protein A (PAPP-A) in the first trimester in association with abnormal fetal karyotype. *British Journal of Obstetrics and Gynaecology* 1993;**100**(4): 324–6.

#### Brambati 1994 *{published data only}*

Brambati B, Tului L, Bonacchi I, Shrimanker K, Suzuki Y, Grudzinskas JG. Serum PAPP-A and free beta-hCG are first-trimester screening markers for Down syndrome. *Prenatal Diagnosis* 1994;**14**(11):1043–7.

#### Brameld 2008 *{published data only}*

Brameld KJ, Dickinson JE, O'Leary P, Bower C, Goldblatt J, Hewitt B, et al. First trimester predictors of adverse pregnancy outcomes. *Australian & New Zealand Journal of Obstetrics & Gynaecology* 2008;**48**(6):529–35.

#### Brizot 1994 *{published data only}*

Brizot ML, Snijders RJ, Bersinger NA, Kuhn P, Nicolaides KH. Maternal serum pregnancy-associated plasma protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. *Obstetrics and Gynecology* 1994;**84**(6):918–22.

#### Casals 1996 *{published data only}*

Casals E, Fortuny A, Grudzinskas JG, Suzuki Y, Teisner B, Comas C, et al. First-trimester biochemical screening for Down syndrome with the use of PAPP-A, AFP, and beta-hCG. *Prenatal Diagnosis* 1996;**16**(5):405–10.

#### Christiansen 1999 *{published data only}*

Christiansen M, Oxvig C, Wagner JM, Qin QP, Nguyen TH, Overgaard MT, et al. The proform of eosinophil major basic protein: a new maternal serum marker for Down syndrome. *Prenatal Diagnosis* 1999;**19**(10):905–10.

#### Christiansen 2004 *{published data only}*

Christiansen M, Larsen SO, Oxvig C, Qin QP, Wagner JM, Overgaard MT, et al. Screening for Down's syndrome in early and late first and second trimester using six maternal serum markers. *Clinical Genetics* 2004;**65**(1):11–6.

#### Christiansen 2005 *{published data only}*

Christiansen M, Norgaard-Pedersen B. Inhibin A is a maternal serum marker for Down's syndrome early in the first trimester. *Clinical Genetics* 2005;**68**(1):35–9.

#### Christiansen 2007a *{published data only}*

Christiansen M, Sorensen TL, Norgaard-Pedersen B. Human placental lactogen is a first-trimester maternal serum marker of Down syndrome. *Prenatal Diagnosis* 2007;**27**(1):1–5.

#### Christiansen 2009 *{published data only}*

Christiansen M. Placental growth hormone and growth hormone binding protein are first trimester maternal serum markers of Down syndrome. *Prenatal Diagnosis* 2009;**29**(13):1249–55.

#### Christiansen 2010 *{published data only}*

Christiansen M, Pihl K, Hedley PL, Gjerris AC, Lind PO, Larsen SO, et al. ADAM 12 may be used to reduce the false positive rate of first trimester combined screening for Down syndrome. *Prenatal Diagnosis* 2010;**30**(2):110–4.

#### Cowans 2010 *{published data only}*

Cowans NJ, Stamatopoulou A, Spencer K. First trimester maternal serum placental growth factor in trisomy 21 pregnancies. *Prenatal Diagnosis* 2010;**30**(5):449–53.

#### Crandall 1993 *{published data only}*

Crandall BF, Hanson FW, Keener S, Matsumoto M, Miller W. Maternal serum screening for alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin between 11 and 15 weeks of pregnancy to detect fetal chromosome abnormalities.[see comment]. *American Journal of Obstetrics and Gynecology* 1993;**168**(6 Pt 1):1864–7; discussion 1867–9.

#### Crossley 2002a *{published data only}*

Crossley JA, Aitken DA, Cameron AD, McBride E, Connor JM. Combined ultrasound and biochemical screening for Down's syndrome in the first trimester: a Scottish multicentre study. *BJOG: an international journal of obstetrics and gynaecology* 2002;**109**(6):667–76.

#### De Graaf 1999a *{published data only}*

De Graaf I, Pajkrt E, Bilardo CM, Leschot NJ, Cuckle HS, Van Lith JM. Early pregnancy screening for fetal aneuploidy with serum markers and nuchal translucency. *Prenatal Diagnosis* 1999;**19**(5):458–62.

**Forest 1995 {published data only}**

Forest JC, Masse J, Rousseau F, Moutquin JM, Brideau NA, Belanger M. Screening for Down syndrome during the first and second trimesters: impact of risk estimation parameters. *Clinical Biochemistry* 1995;**28**(4):443–9.

**Forest 1997 {published data only}**

Forest JC, Masse J, Moutquin JM. Screening for Down syndrome during first trimester: a prospective study using free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Clinical Biochemistry* 1997;**30**(4):333–8.

**Gyselaers 2005 {published data only}**

Gyselaers WJ, Vereecken AJ, Van Herck EJ, Straetmans DP, de Jonge ET, Ombelet WU, et al. Population screening for fetal trisomy 21: easy access to screening should be balanced against a uniform ultrasound protocol. *Prenatal Diagnosis* 2005;**25**(11):984–90.

**Haddow 1998 {published data only}**

Haddow JE, Palomaki GE, Knight GJ, Williams J, Miller WA, Johnson A. Screening of maternal serum for fetal Down's syndrome in the first trimester. *New England Journal of Medicine* 1998;**338**(14):955–61.

**Kagan 2009 {published data only}**

Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. *Ultrasound in Obstetrics & Gynecology* 2009;**33**(3):259–64.

\* Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. Prospective validation of first-trimester combined screening for trisomy 21. *Ultrasound in Obstetrics & Gynecology* 2009;**34**(1):14–8.

Kagan KO, Staboulidou I, Cruz J, Wright D, Nicolaides KH. Two-stage first-trimester screening for trisomy 21 by ultrasound assessment and biochemical testing. *Ultrasound in Obstetrics & Gynecology* 2010;**36**(5):542–7.

Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6 weeks of gestation. *Ultrasound in Obstetrics & Gynecology* 2009;**33**(1):18–22.

Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound in Obstetrics & Gynecology* 2008;**31**(6):618–24.

Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound in Obstetrics & Gynecology* 2008;**31**(5):493–502.

Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. *Human Reproduction* 2008;**23**(9):1968–75.

**Kornman 1998 {published data only}**

Kornman LH, Morssink LP, Ten Hoor KA, de Wolf BT, Kloosterman MD, Beekhuis JR, et al. Schwangerschaftsprotein 1 (SP1) adds little to the Age-related detection of fetal Down syndrome in the first trimester of pregnancy. *Prenatal Diagnosis* 1998;**18**(10):1086–90.

**Kozlowski 2007 GC {published data only}**

Kozlowski P, Knippel AJ, Stressig R. Comparing first trimester screening performance: routine care gynaecologists' practices vs. prenatal centre. *Ultraschall in der Medizin* 2007;**28**(3):291–5.

**Kozlowski 2007 PC {published data only}**

Kozlowski P, Knippel AJ, Stressig R. Comparing first trimester screening performance: routine care gynaecologists' practices vs. prenatal centre. *Ultraschall in der Medizin* 2007;**28**(3):291–5.

**Krantz 2000 {published data only}**

Krantz DA, Hallahan TW, Orlandi F, Buchanan P, Larsen JW Jr, Macri JN. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstetrics and Gynecology* 2000;**96**(2):207–13.

**Kratzer 1991 {published data only}**

Kratzer PG, Golbus MS, Monroe SE, Finkelstein DE, Taylor RN. First-trimester un euploidy screening using serum human chorionic gonadotropin (hCG), free alphaCG, and progesterone. *Prenatal Diagnosis* 1991;**11**(10):751–63.

**Laigaard 2003 {published data only}**

Laigaard J, Sorensen T, Frohlich C, Pedersen BN, Christiansen M, Schiott K, et al. ADAM12: a novel first-trimester maternal serum marker for Down syndrome. *Prenatal Diagnosis* 2003;**23**(13):1086–91.

**Macintosh 1993 {published data only}**

Macintosh MC, Brambati B, Chard T, Grudzinskas JG. First-trimester maternal serum Schwangerschaftsprotein 1 (SP1) in pregnancies associated with chromosomal anomalies. *Prenatal Diagnosis* 1993;**13**(7):563–8.

**Muller 2003a {published data only}**

Muller F, Benattar C, Audibert F, Roussel N, Dreux S, Cuckle H. First-trimester screening for Down syndrome in France combining fetal nuchal translucency measurement and biochemical markers. *Prenatal Diagnosis* 2003;**23**(10):833–6.

**Nebiolo 1990 {published data only}**

Milunsky A, Wands J, Brambati B, Bonacchi I, Currie K. First-trimester maternal serum alpha-fetoprotein screening for chromosome defects. *American Journal of Obstetrics and Gynecology* 1988;**159**(5):1209–13.

\* Nebiolo L, Ozturk M, Brambati B, Miller S, Wands J, Milunsky A. First-trimester maternal serum alpha-fetoprotein and human chorionic gonadotropin screening for chromosome defects. *Prenatal Diagnosis* 1990;**10**(9):575–81.

**Niemimaa 2001a {published data only}**

Niemimaa M, Suonpaa M, Perheentupa A, Seppala M, Heinonen S, Laitinen P, et al. Evaluation of first trimester

- maternal serum and ultrasound screening for Down's syndrome in Eastern and Northern Finland. *European Journal of Human Genetics* 2001;**9**(6):404–8.
- Noble 1995** *{published data only}*  
Brizot ML, Snijders RJM, Butler J, Bersinger NA, Nicolaides KH. Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. *British Journal of Obstetrics and Gynaecology* 1995;**102**:127–32.  
\* Noble PL, Abraha HD, Snijders RJ, Sherwood R, Nicolaides KH. Screening for fetal trisomy 21 in the first trimester of pregnancy: maternal serum free beta-hCG and fetal nuchal translucency thickness. *Ultrasound in Obstetrics & Gynecology* 1995;**6**(6):390–5.
- Noble 1997** *{published data only}*  
Noble PL, Wallace EM, Snijders RJ, Groome NP, Nicolaides KH. Maternal serum 1T Inhibin-A and free beta-hCG concentrations in trisomy 21 pregnancies at 10 to 14 weeks of gestation. *British Journal of Obstetrics and Gynaecology* 1997;**104**(3):367–71.
- O'Leary 2006** *{published data only}*  
O'Leary P, Breheny N, Dickinson JE, Bower C, Goldblatt J, Hewitt B, et al. First-trimester combined screening for Down syndrome and other fetal anomalies. *Obstetrics and Gynecology* 2006;**107**(4):869–76.
- Orlandi 1997** *{published data only}*  
Orlandi F, Damiani G, Hallahan TW, Krantz DA, Macri JN. First-trimester screening for fetal aneuploidy: biochemistry and nuchal translucency. *Ultrasound in Obstetrics & Gynecology* 1997;**10**(6):381–6.
- Palomaki 2007** *{published data only}*  
Palomaki GE, Neveux LM, Haddow JE, Wyatt P. Hyperglycosylated-hCG (h-hCG) and Down syndrome screening in the first and second trimesters of pregnancy. *Prenatal Diagnosis* 2007;**27**(9):808–13.
- Qin 1997** *{published data only}*  
Qin QP, Christiansen M, Nguyen TH, Sorensen S, Larsen SO, Norgaard-Pedersen B. Schwangerschaftsprotein 1 (SP1) as a maternal serum marker for Down syndrome in the first and second trimesters. *Prenatal Diagnosis* 1997;**17**(2): 101–8.
- Sahota 2010** *{published data only}*  
\* Sahota DS, Leung TY, Chan LW, Law LW, Fung TY, Chen M, et al. Comparison of first-trimester contingent screening strategies for Down syndrome. *Ultrasound in Obstetrics & Gynecology* 2010;**35**(3):286–91.  
Sahota DS, Leung TY, Chen M, Chan LW, Fung TY, Lau TK. Comparison of likelihood ratios of first-trimester nuchal translucency measurements: multiples of median, delta or mixture. *Ultrasound in Obstetrics & Gynecology* 2010;**36**(1):15–9.
- Schaelike 2009** *{published data only}*  
Schaelike M, Kossakiewicz M, Kossakiewicz A, Schild RL. Examination of a first-trimester Down syndrome screening concept on a mix of 11,107 high- and low-risk patients at a private center for prenatal medicine in Germany. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 2009;**144**(2):140–5.
- Scott 2004** *{published data only}*  
Scott F, Peters H, Bonifacio M, McLennan A, Boogert A, Kesby G, et al. Prospective evaluation of a first trimester screening program for Down syndrome and other chromosomal abnormalities using maternal age, nuchal translucency and biochemistry in an Australian population. *Australian & New Zealand Journal of Obstetrics & Gynaecology* 2004;**44**(3):205–9.
- Spencer 1999a** *{published data only}*  
Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A.[see comment]. *Ultrasound in Obstetrics & Gynecology* 1999;**13**(4):231–7.
- Spencer 2002a** *{published data only}*  
Spencer K, Talbot JA, Abushoufa RA. Maternal serum hyperglycosylated human chorionic gonadotropin (HhCG) in the first trimester of pregnancies affected by Down syndrome, using a sialic acid-specific lectin immunoassay.[see comment]. *Prenatal Diagnosis* 2002;**22** (8):656–62.
- Torring 2010** *{published data only}*  
Torring N, Ball S, Wright D, Sarkissian G, Guitton M, Darbouret B. First trimester screening for trisomy 21 in gestational week 8–10 by ADAM12-S as a maternal serum marker. *Reproductive Biology & Endocrinology* 2010;**8**:129.
- Tsukerman 1999** *{published data only}*  
Tsukerman GL, Gusina NB, Cuckle HS. Maternal serum screening for Down syndrome in the first trimester: experience from Belarus. *Prenatal Diagnosis* 1999;**19**(6): 499–504.
- Valinen 2007** *{published data only}*  
Valinen Y, Rapakko K, Kokkonen H, Laitinen P, Tekay A, Ahola T, et al. Clinical first-trimester routine screening for Down syndrome in singleton pregnancies in northern Finland. *American Journal of Obstetrics and Gynecology* 2007;**196**(3):278–5.
- Valinen 2009** *{published data only}*  
Valinen Y, Laitinen P, Ranta J, Ignatius J, Jarvela I, Ryyanen M. Effect of a new marker, ADAM12, on Down risk figures in first trimester screening. *Journal of Maternal-fetal & Neonatal Medicine* 2009;**22**(7):602–7.
- Van Lith 1992** *{published data only}*  
Van Lith JM, Mantingh A, Kloosterman MD, Kanhai HHH, Wolf H, Everhardt E, et al. First-trimester maternal serum human chorionic gonadotropin as a marker for fetal chromosomal disorders. *Prenatal Diagnosis* 1992;**12**(6): 495–504.

**Wald 2003a {published data only}**

Wald NJ, Rodeck C, Hackshaw AK, Rudnicka A. SURUSS in perspective. *Seminars in Perinatology* 2005;**29**(4):225–35.

Wald NJ, Rodeck C, Hackshaw AK, Rudnicka A. SURUSS in perspective.[see comment]. *BJOG: an international journal of obstetrics and gynaecology* 2004;**111**(6):521–31.

Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Journal of Medical Screening* 2003;**10**(2):56–104.

\* Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM, et al. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Health Technology Assessment (Winchester, England)* 2003;**7**(11):1–77.

**Wallace 1995 {published data only}**

Wallace EM, Grant VE, Swanston IA, Groome NP. Evaluation of maternal serum dimeric IT Inhibin A as a first-trimester marker of Down's syndrome. *Prenatal Diagnosis* 1995;**15**(4):359–62.

**Wapner 2003 {published data only}**

\* Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, et al. First-trimester screening for trisomies 21 and 18.[see comment]. *New England Journal of Medicine* 2003;**349**(15):1405–13.

Wapner RJ. First trimester screening: the BUN study. *Seminars in Perinatology* 2005;**29**(4):236–9.

**Weinans 2005 {published data only}**

Weinans MJ, Sancken U, Pandian R, van de Ouweland JM, de Buijn HW, Holm JB, et al. Invasive trophoblast antigen (hyperglycosylated human chorionic gonadotropin) as a first-trimester serum marker for Down syndrome. *Clinical Chemistry* 2005;**51**(7):1276–9.

**Wojdemann 2005 {published data only}**

Wojdemann KR, Shalmi AC, Christiansen M, Larsen SO, Sundberg K, Brocks V, et al. Improved first-trimester Down syndrome screening performance by lowering the false-positive rate: a prospective study of 9941 low-risk women. *Ultrasound in Obstetrics & Gynecology* 2005;**25**(3):227–33.

**Zaragoza 2009 {published data only}**

Zaragoza E, Akolekar R, Poon LC, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11-13 weeks in chromosomally abnormal pregnancies. *Ultrasound in Obstetrics & Gynecology* 2009;**33**(4):382–6.

**References to studies excluded from this review****Abbas 1995 {published data only}**

Abbas A, Chard T, Nicolaides K. Fetal and maternal hCG concentration in aneuploid pregnancies. *British Journal of Obstetrics and Gynaecology* 1995;**102**(7):561–3.

**Abdul-Hamid 2004 {published data only}**

Abdul-Hamid S, Fox R, Martin I. Maternal serum screening for trisomy 21 in women with a false positive result in last

pregnancy. *Journal of Obstetrics and Gynaecology* 2004;**24**(4):374–6.

**Abraha 1999 {published data only}**

Abraha HD, Noble PL, Nicolaides KH, Sherwood RA. Maternal serum S100 protein in normal and Down syndrome pregnancies. *Prenatal Diagnosis* 1999;**19**(4):334–6.

**Abu-Rustum 2010 {published data only}**

Abu-Rustum RS, Daou L, Abu-Rustum SE. Role of first-trimester sonography in the diagnosis of aneuploidy and structural fetal anomalies. *Journal of Ultrasound in Medicine* 2010;**29**(10):1445–52.

**Achiron 2010 {published data only}**

Achiron R, Gindes L, Gilboa Y, Weissmann-Brenner A, Berkenstadt M. Umbilical vein anomaly in fetuses with Down syndrome. *Ultrasound in Obstetrics & Gynecology* 2010;**35**(3):297–301.

**Adekunle 1999 {published data only}**

Adekunle O, Gopee A, el-Sayed M, Thilaganathan B. Increased first trimester nuchal translucency: pregnancy and infant outcomes after routine screening for Down's syndrome in an unselected antenatal population. *British Journal of Radiology* 1999;**72**(857):457–60.

**Agaard-Tillery 2010 {published data only}**

Agaard-Tillery KM, Flint Porter T, Malone FD, Nyberg DA, Collins J, Comstock CH, et al. Influence of maternal BMI on genetic sonography in the FaSTER trial. *Prenatal Diagnosis* 2010;**30**(1):14–22.

**Aitken 1993 {published data only}**

Aitken DA, McCaw G, Crossley JA, Berry E, Connor JM, Spencer K, et al. First-trimester biochemical screening for fetal chromosome abnormalities and neural tube defects. *Prenatal Diagnosis* 1993;**13**(8):681–9.

**Aitken 1996a {published data only}**

Aitken DA, Syvertsen BS, Crossley JA, Berry E, Connor JM. Heat-stable and immunoreactive placental alkaline phosphatase in maternal serum from Down's syndrome and trisomy 18 pregnancies.[see comment]. *Prenatal Diagnosis* 1996;**16**(11):1051–4.

**Aitken 1996b {published data only}**

Aitken DA, Wallace EM, Crossley JA, Swanston IA, Van Pareren Y, Van Maarle M, et al. Dimeric Inhibin A as a marker for Down's syndrome in early pregnancy. *New England Journal of Medicine* 1996;**334**(19):1231–6.

**Ajayi 2011 {published data only}**

Ajayi GO. Is there any effect of fetal gender on the markers of first trimester Down's syndrome screening?. *Clinical and Experimental Obstetrics & Gynecology* 2011;**38**(2):162–4.

**Akbas 2001 {published data only}**

Akbas SH, Ozben T, Alper O, Ugur A, Yucel G, Luleci G. Maternal serum screening for Down's syndrome, open neural tube defects and trisomy 18. *Clinical Chemistry and Laboratory Medicine* 2001;**39**(6):487–90.

- Alexioly 2009** *{published data only}*  
Alexioly E, Alexioly E, Trakakis E, Kassanos D, Farmakidis G, Kondyliou A, et al. Predictive value of increased nuchal translucency as a screening test for the detection of fetal chromosomal abnormalities. *Journal of Maternal-fetal & Neonatal Medicine* 2009;**22**(10):857–62.
- Allingham-Hawkins 2011** *{published data only}*  
Allingham-Hawkins DJ, Chitayat D, Cirigliano V, Summers A, Tokunaga J, Winsor E, et al. Prospective validation of quantitative fluorescent polymerase chain reaction for rapid detection of common aneuploidies. *Genetics in Medicine* 2011;**13**(2):140–7.
- American College 2009** *{published data only}*  
American CofN-M. [Share with women. Prenatal tests for Down syndrome]. [Spanish]. *Journal of Midwifery & Women's Health* 2009;**54**(6):527–8.
- Antona 1998** *{published data only}*  
Antona D, Wallace EM, Shearing C, Ashby JP, Groome NP. Inhibin A and pro-alphaC Inhibin A in Down syndrome and normal pregnancies. *Prenatal Diagnosis* 1998;**18**(11):1122–6.
- Antsaklis 1999** *{published data only}*  
Antsaklis A, Papantoniou N, Mesogitis S, Michalas S, Aravantinos D. Pregnant women of 35 years of age or more: Maternal serum markers or amniocentesis?. *Journal of Obstetrics and Gynaecology* 1999;**19**(3):253–6.
- Anuwutnavin 2009** *{published data only}*  
Anuwutnavin S, Wanitpongpan P, Chanprapaph P. Specificity of fetal tricuspid regurgitation in prediction of Down syndrome in Thai fetuses at 17-23 weeks of gestation. *Journal of the Medical Association of Thailand* 2009;**92**(9):1123–30.
- Ashwood 1987** *{published data only}*  
Ashwood ER, Cheng E, Luthy DA. Maternal serum alpha-fetoprotein and fetal trisomy-21 in women 35 years and older: implications for alpha-fetoprotein screening programs. *American Journal of Medical Genetics* 1987;**26**(3):531–9.
- Asrani 2005** *{published data only}*  
Asrani CH. Triple marker. *National Journal of Homoeopathy* 2005;**7**(3):174.
- Audibert 2001** *{published data only}*  
Audibert F, Dommergues M, Benattar C, Taieb J, Thalabard JC, Frydman R. Screening for Down syndrome using first-trimester ultrasound and second-trimester maternal serum markers in a low-risk population: a prospective longitudinal study. *Ultrasound in Obstetrics & Gynecology* 2001;**18**(1):26–31.
- Axt-Fleidner 2006** *{published data only}*  
Axt Fleidner, Schwarze A, Kreislermaier P, Krapp M, Smrcek J, Diedrich K. Umbilical cord diameter at 11-14 weeks of gestation: Relationship to nuchal translucency, ductus venous blood flow and chromosomal defects. *Fetal Diagnosis and Therapy* 2006;**21**(4):390–5.
- Azuma 2002** *{published data only}*  
Azuma M, Yamamoto R, Wakui Y, Minobe S, Satomura S, Fujimoto S. A novel method for the detection of Down syndrome with the use of four serum markers. *American Journal of Obstetrics and Gynecology* 2002;**187**(1):197–201.
- Baghagho 2004** *{published data only}*  
Baghagho EE, Kharboush IF, El-Kaffash DM, KarKour TA, Ismail SR, Mortada MM. Maternal serum alpha fetoprotein among pregnant females in Alexandria. *Journal of the Egyptian Public Health Association* 2004;**79**(1-2):59–81.
- Bahado-Singh 1995** *{published data only}*  
Bahado-Singh RO, Goldstein I, Uerpaiojkit B, Copel JA, Mahoney MJ, Baumgarten A. Normal nuchal thickness in the midtrimester indicates reduced risk of Down syndrome in pregnancies with abnormal triple-screen results. *American Journal of Obstetrics and Gynecology* 1995;**173**(4):1106–10.
- Bahado-Singh 1996** *{published data only}*  
Bahado-Singh RO, Tan A, Deren O, Hunter D, Copel J, Mahoney MJ. Risk of Down syndrome and any clinically significant chromosome defect in pregnancies with abnormal triple-screen and normal targeted ultrasonographic results. *American Journal of Obstetrics and Gynecology* 1996;**175**(4 I):824–9.
- Bahado-Singh 1999** *{published data only}*  
Bahado-Singh RO, Oz AU, Flores D, Cermik D, Acuna E, Mahoney MJ, et al. Nuchal thickness, urine  $\beta$ -core fragment level, and maternal age for down syndrome screening. *American Journal of Obstetrics and Gynecology* 1999;**180**(2 I):491–5.
- Bahado-Singh 2002** *{published data only}*  
Bahado-Singh R, Shahabi S, Karaca M, Mahoney MJ, Cole L, Oz UA. The comprehensive midtrimester test: high-sensitivity Down syndrome test. *American Journal of Obstetrics and Gynecology* 2002;**186**(4):803–8.
- Bahado-Singh 2003** *{published data only}*  
Bahado-Singh R, Cheng CC, Matta P, Small M, Mahoney MJ. Combined serum and ultrasound screening for detection of fetal aneuploidy. *Seminars in Perinatology* 2003;**27**(2):145–51.
- Ball 2007** *{published data only}*  
Ball RH, Caughey AB, Malone FD, Nyberg DA, Comstock CH, Saade GR, et al. First- and second-trimester evaluation of risk for Down syndrome. *Obstetrics and Gynecology* 2007;**110**(1):10–7.
- Bar-Hava 2001** *{published data only}*  
Bar-Hava I, Yitzhak M, Krissi H, Shohat M, Shalev J, Czitron B, et al. Triple-test screening in in vitro fertilization pregnancies. *Journal of Assisted Reproduction and Genetics* 2001;**18**(4):226–9.
- Barkai 1996** *{published data only}*  
Barkai G, Goldman B, Ries L, Chaki R, Dor J, Cuckle H. Down's syndrome screening marker levels following assisted reproduction. *Prenatal Diagnosis* 1996;**16**(12):1111–4.
- Barnabei 1995** *{published data only}*  
Barnabei VM, Krantz DA, Macri JN, Larsen JW Jr. Enhanced twin pregnancy detection within an open neural

- tube defect and Down syndrome screening protocol using free- $\beta$  hCG and AFP. *Prenatal Diagnosis* 1995;**15**(12): 1131–4.
- Bartels 1988** *{published data only}*  
Bartels I, Lindemann A. Maternal levels of pregnancy-specific  $\beta$  1-glycoprotein (SP-1) are elevated in pregnancies affected by Down's syndrome. *Human Genetics* 1988;**80**(1): 46–8.
- Bartels 1993** *{published data only}*  
Bartels I, Hoppe-Sievert B, Bockel B, Herold S, Caesar J. Adjustment formulae for maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated oestriol to maternal weight and smoking. *Prenatal Diagnosis* 1993; **13**(2):123–30.
- Barth 1991** *{published data only}*  
Barth WH Jr, Frigoletto FD Jr, Krauss CM, MacMillin MD, Stryker JM, Benacerraf BR. Ultrasound detection of fetal aneuploidy in women with elevated maternal serum alpha-fetoprotein. *Obstetrics and Gynecology* 1991;**77**(6): 897–900.
- Bas-Budecka 2007** *{published data only}*  
Bas-Budecka E, Perenc M, Sieroszewski P. [Abnormal second trimester screening for fetal chromosomal abnormalities as a predictor of adverse pregnancy outcome]. [Polish]. *Ginekologia Polska* 2007;**78**(11):877–80.
- Baviera 2004** *{published data only}*  
Baviera G, Carbone C, Corrado F, Mastrantonio P. Placental growth hormone in Down's syndrome screening. *Journal of Maternal-fetal & Neonatal Medicine* 2004;**16**(4):241–3.
- Bazzett 1998** *{published data only}*  
Bazzett LB, Yaron Y, O'Brien JE, Critchfield G, Kramer RL, Ayoub M, et al. Fetal gender impact on multiple-marker screening results. *American Journal of Medical Genetics* 1998;**76**(5):369–71.
- Beke 2008** *{published data only}*  
Beke A, Barakonyi E, Belics Z, Joo JG, Csaba A, Papp C, et al. Risk of chromosome abnormalities in the presence of bilateral or unilateral choroid plexus cysts. *Fetal Diagnosis and Therapy* 2008;**23**(3):185–91.
- Bellver 2005** *{published data only}*  
Bellver J, Lara C, Soares SR, Ramirez A, Pellicer A, Remohi J, et al. First trimester biochemical screening for Down's syndrome in singleton pregnancies conceived by assisted reproduction. *Human Reproduction* 2005;**20**(9):2623–7.
- Benn 1995** *{published data only}*  
Benn PA, Horne D, Briganti S, Greenstein RM. Prenatal diagnosis of diverse chromosome abnormalities in a population of women identified by triple-marker testing as screen positive for Down syndrome. *American Journal of Obstetrics and Gynecology* 1995;**173**(2):496–501.
- Benn 1996** *{published data only}*  
Benn PA, Horne D, Craffey A, Collins R, Ramsdell L, Greenstein R. Maternal serum screening for birth defects: results of a Connecticut regional program. *Connecticut Medicine* 1996;**60**(6):323–7.
- Benn 1997** *{published data only}*  
Benn PA, Clive JM, Collins R. Medians for second-trimester maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol; differences between races or ethnic groups. *Clinical Chemistry* 1997;**43**(2):333–7.
- Benn 1998** *{published data only}*  
Benn PA. Preliminary evidence for associations between second-trimester human chorionic gonadotropin and unconjugated oestriol levels with pregnancy outcome in Down syndrome pregnancies. *Prenatal Diagnosis* 1998;**18**(4):319–24.
- Benn 2001** *{published data only}*  
Benn PA, Ying J, Beazoglou T, Egan JF. Estimates for the sensitivity and false-positive rates for second trimester serum screening for Down syndrome and trisomy 18 with adjustment for cross-identification and double-positive results. *Prenatal Diagnosis* 2001;**21**(1):46–51.
- Benn 2002** *{published data only}*  
Benn PA, Kaminsky LM, Ying J, Borgida AF, Egan JF. Combined second-trimester biochemical and ultrasound screening for Down syndrome. *Obstetrics and Gynecology* 2002;**100**(6):1168–76.
- Benn 2003a** *{published data only}*  
Benn PA, Fang M, Egan JFX, Horne D, Collins R. Incorporation of inhibin-A in second-trimester screening for Down syndrome. *Obstetrics and Gynecology* 2003;**101**(3):451–4.
- Benn 2003b** *{published data only}*  
Benn P. Improved antenatal screening for Down's syndrome. *Lancet* 2003;**361**(9360):794–5.
- Benn 2005a** *{published data only}*  
Benn P, Wright D, Cuckle H. Practical strategies in contingent sequential screening for Down syndrome. *Prenatal Diagnosis* 2005;**25**(8):645–52.
- Benn 2005b** *{published data only}*  
Benn P, Donnenfeld AE. Sequential Down syndrome screening: the importance of first and second trimester test correlations when calculating risk. *Journal of Genetic Counseling* 2005;**14**(6):409–13.
- Benn 2007** *{published data only}*  
Benn PA, Campbell WA, Zelop CM, Ingardia C, Egan JF. Stepwise sequential screening for fetal aneuploidy. *American Journal of Obstetrics and Gynecology* 2007;**197**(3):312–5.
- Berry 1995** *{published data only}*  
Berry E, Aitken DA, Crossley JA, Macri JN, Connor JM. Analysis of maternal serum alpha-fetoprotein and free  $\beta$  human chorionic gonadotrophin in the first trimester: implications for Down's syndrome screening. *Prenatal Diagnosis* 1995;**15**(6):555–65.
- Berry 1997** *{published data only}*  
Berry E, Aitken DA, Crossley JA, Macri JN, Connor JM. Screening for Down's syndrome: changes in marker levels and detection rates between first and second trimesters.



- British Journal of Obstetrics and Gynaecology* 1997;**104**(7): 811–7.
- Bersinger 1994** *{published data only}*  
Bersinger NA, Brizot ML, Johnson A, Snijders RJ, Abbott J, Schneider H, et al. First trimester maternal serum pregnancy-associated plasma protein A and pregnancy-specific  $\beta$  1-glycoprotein in fetal trisomies. *British Journal of Obstetrics and Gynaecology* 1994;**101**(11):970–4.
- Bersinger 2000** *{published data only}*  
Bersinger NA, Xin WZ. Glycosylation of pregnancy-associated plasma protein a (PAPP-A) and pregnancy-specific ( $\beta$ )(1)-glycoprotein (SP1): Relevance for fetal down syndrome screening and for placental function studies. *Immuno-Analyse et Biologie Specialisee* 2000;**15**(6):402–8.
- Bersinger 2001** *{published data only}*  
Bersinger NA, Chanson A, Crazzolara S, Hänggi W, Pescia G, Scheier M, et al. Serum levels of placenta protein markers: The relevance of differences between spontaneous and after in vitro fertilization pregnancies for fetal trisomy screening. *Journal für Fertilität und Reproduktion* 2001;**11**(3):7–13.
- Bersinger 2003** *{published data only}*  
Bersinger NA, Noble P, Nicolaides KH. First-trimester maternal serum PAPP-A, SP1 and M-CSF levels in normal and trisomic twin pregnancies. *Prenatal Diagnosis* 2003;**23**(2):157–62.
- Bersinger 2004** *{published data only}*  
Bersinger NA, Wunder D, Vanderlick F, Chanson A, Pescia G, Janecek P, et al. Maternal serum levels of placental proteins after in vitro fertilisation and their implications for prenatal screening. *Prenatal Diagnosis* 2004;**24**(6):471–7.
- Bersinger 2005** *{published data only}*  
Bersinger NA, Vanderlick F, Birkhäuser MH, Janecek P, Wunder D. First trimester serum concentrations of placental proteins in singleton and multiple IVF pregnancies: Implications for Down syndrome screening. *Immuno-Analyse et Biologie Specialisee* 2005;**20**(1):21–7.
- Bestwick 2008** *{published data only}*  
Bestwick JP, Huttly WJ, Wald NJ. First trimester Down's syndrome screening marker values and cigarette smoking: new data and a meta-analysis on free beta human chorionic gonadotrophin, pregnancy-associated plasma protein-A and nuchal translucency. *Journal of Medical Screening* 2008;**15**(4):204–6.
- Biggio 2004** *{published data only}*  
Biggio Jr, Morris TC, Owen J, Stringer JSA. An outcomes analysis of five prenatal screening strategies for trisomy 21 in women younger than 35 years. *American Journal of Obstetrics and Gynecology* 2004;**190**(3):721–9.
- Bilardo 2011** *{published data only}*  
Bilardo CM, Timmerman E, De Medina PG, Clur SA. Low-resistance hepatic artery flow in first-trimester fetuses: an ominous sign. *Ultrasound in Obstetrics & Gynecology* 2011;**37**(4):438–43.
- Bindra 2002** *{published data only}*  
Bindra R, Heath V, Nicolaides KH. Screening for chromosomal defects by fetal nuchal translucency at 11 to 14 weeks. *Clinical Obstetrics and Gynecology* 2002;**45**(3): 661–70.
- Blundell 1999** *{published data only}*  
Blundell G, Ashby JP, Martin C, Shearing CH, Langdale-Brown B, Keeling J, et al. Clinical follow-up of high mid-trimester maternal serum intact human chorionic gonadotrophin concentrations in singleton pregnancies. *Prenatal Diagnosis* 1999;**19**(3):219–23.
- Boormans 2010** *{published data only}*  
Boormans EM, Birnie E, Oepkes D, Galjaard RJ, Schuring-Blom GH, van Lith JM, et al. Comparison of multiplex ligation-dependent probe amplification and karyotyping in prenatal diagnosis. *Obstetrics and Gynecology* 2010;**115**(2 Pt 1):297–303.
- Boots 1989** *{published data only}*  
Boots LR, Davis RO, Foster JM, Goldenberg RL. Maternal serum alpha-fetoprotein prenatal screening for Down syndrome. *Alabama Medicine* 1989;**59**(1):25–7.
- Bornstein 2009a** *{published data only}*  
Bornstein E, Lenchner E, Donnenfeld A, Barnhard Y, Seubert D, Divon MY. Advanced maternal age as a sole indication for genetic amniocentesis; risk-benefit analysis based on a large database reflecting the current common practice. *Journal of Perinatal Medicine* 2009;**37**(2):99–102.
- Bornstein 2009b** *{published data only}*  
Bornstein E, Lenchner E, Donnenfeld A, Kapp S, Keeler SM, Divon MY. Comparison of modes of ascertainment for mosaic vs complete trisomy 21. *American Journal of Obstetrics and Gynecology* 2009;**200**(4):440–5.
- Bornstein 2010** *{published data only}*  
Bornstein E, Lenchner E, Donnenfeld A, Jodicic C, Keeler SM, Kapp S, et al. Complete trisomy 21 vs translocation Down syndrome: a comparison of modes of ascertainment. *American Journal of Obstetrics and Gynecology* 2010;**203**(4): 391–5.
- Borowski 2007** *{published data only}*  
Borowski D, Czuba B, Cnota W, Hincz P, Czekerowski A, Gajewska J, et al. [Evaluation of pregnancy-associated plasma protein A (PAPP-A) and free beta subunit of human chorionic gonadotrophin (beta hCG) levels and sonographic assessment of fetal nuchal translucency (NT) in singleton pregnancies between 11 and 14 weeks of gestation--Polish multi-centre research]. [Polish]. *Ginekologia Polska* 2007; **78**(5):384–7.
- Borrell 2007** *{published data only}*  
Borrell A, Mercade I, Casals E, Borobio V, Seres A, Soler A, et al. Combining fetal nuchal fold thickness with second-trimester biochemistry to screen for trisomy 21. *Ultrasound in Obstetrics & Gynecology* 2007;**30**(7):941–5.
- Borruto 2002** *{published data only}*  
Borruto F, Comparetto C, Acanfora L, Bertini G, Rubaltelli FF. Role of ultrasound evaluation of nuchal translucency in

- prenatal diagnosis. *Clinical and Experimental Obstetrics & Gynecology* 2002;**29**(4):235–41.
- Bottalico 2009** *{published data only}*  
Bottalico JN, Chen X, Tartaglia M, Rosario B, Yarabothu D, Nelson L. Second-trimester genetic sonogram for detection of fetal chromosomal abnormalities in a community-based antenatal testing unit. *Ultrasound in Obstetrics & Gynecology* 2009;**33**(2):161–8.
- Boue 1990** *{published data only}*  
Boue A, Muller F. Screening for Down's syndrome with maternal serum human chorionic gonadotropin at midtrimester. *Current Opinion in Pediatrics* 1990;**2**(6):1157–60.
- Bradley 1994** *{published data only}*  
Bradley LA, Horwitz JA, Dowman AC, Ponting NR, Peterson LM. Triple marker screening for fetal Down syndrome. *International Pediatrics* 1994;**9**(3):168–74.
- Braithwaite 1996** *{published data only}*  
Braithwaite JM, Economides DL. Nuchal translucency and screening for Down's syndrome. *Contemporary Reviews in Obstetrics and Gynaecology* 1996;**8**(2):75–81.
- Brambati 1995** *{published data only}*  
Brambati B, Cislighi C, Tului L, Alberti E, Amidani M, Colombo U, et al. First-trimester Down's syndrome screening using nuchal translucency: a prospective study in women undergoing chorionic villus sampling. *Ultrasound in Obstetrics & Gynecology* 1995;**5**(1):9–14.
- Brambati 1996** *{published data only}*  
Brambati B, Tului L, Alberti E. Sonography in the first trimester screening of trisomy 21 and other fetal aneuploidies. *Early Pregnancy* 1996;**2**(3):155–67.
- Brizot 1995a** *{published data only}*  
Brizot ML, Bersinger NA, Xydias G, Snijders RJ, Nicolaides KH. Maternal serum Schwangerschafts protein-1 (SP1) and fetal chromosomal abnormalities at 10-13 weeks' gestation. *Early Human Development*. 1995;**43**(1):31–6.
- Brizot 1995b** *{published data only}*  
Brizot ML, Kuhn P, Bersinger NA, Snijders RJ, Nicolaides KH. First trimester maternal serum alpha-fetoprotein in fetal trisomies. *British Journal of Obstetrics and Gynaecology* 1995;**102**(1):31–4.
- Brizzi 1989** *{published data only}*  
Brizzi L, Cariati E, Periti E, Nannini R, Torricelli F, Cappelli G, et al. Evaluation of maternal serum alpha-fetoprotein and ultrasound examination to screen fetal chromosomal abnormalities. *Journal of Nuclear Medicine and Allied Sciences* 1989;**33**(3 Suppl):85–8.
- Brock 1990** *{published data only}*  
Brock DJ, Barron L, Holloway S, Liston WA, Hillier SG, Seppala M. First-trimester maternal serum biochemical indicators in Down syndrome. *Prenatal Diagnosis* 1990;**10**(4):245–51.
- Calda 2010** *{published data only}*  
Calda P, Sipek A, Gregor V. Gradual implementation of first trimester screening in a population with a prior screening strategy: population based cohort study. *Acta Obstetrica et Gynecologica Scandinavica* 2010;**89**(8):1029–33.
- Campogrande 2001** *{published data only}*  
Campogrande M, Viora E, Errante G, Bastonero S, Sciarrone A, Grassi Pirrone P, et al. Correlations between first and second trimester markers for Down's syndrome screening. *Journal of Medical Screening* 2001;**8**(3):163–4.
- Canick 1988** *{published data only}*  
Canick JA, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Wald NJ. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. *British Journal of Obstetrics and Gynaecology* 1988;**95**(4):330–3.
- Canick 1995** *{published data only}*  
Canick JA, Kellner LH, Saller DN Jr, Palomaki GE, Walker RP, Osathanondh R. Second-trimester levels of maternal urinary gonadotropin peptide in down syndrome pregnancy. *Prenatal Diagnosis* 1995;**15**(8):739–44.
- Canini 2002** *{published data only}*  
Canini S, Prefumo F, Famularo L, Venturini PL, Palazzese V, De Biasio P. Comparison of first trimester, second trimester and integrated Down's syndrome screening results in unaffected pregnancies. *Clinical Chemistry and Laboratory Medicine* 2002;**40**(6):600–3.
- Cans 1998** *{published data only}*  
Cans C, Amblard F, Devillard F, Pison H, Jalbert P, Jouk PS. Population screening for aneuploidy using maternal age and ultrasound. *Prenatal Diagnosis* 1998;**18**(7):683–92.
- Carreras 1991** *{published data only}*  
Carreras de Paz JJ, Silva Mendoza JM, Violante Diaz M, Cerrillo Hinojosa M, Ahued Ahued JR. [Proposed normal values for alpha fetoprotein in maternal serum for the detection of neural tube closure defects and Down syndrome. Preliminary study]. [Spanish]. *Ginecologia y Obstetricia de Mexico* 1991;**59**:261–4.
- Caughy 2007** *{published data only}*  
Caughy AB, Musci TJ, Belluomini J, Main D, Otto C, Goldberg J. Nuchal translucency screening: how do women actually utilize the results?. *Prenatal Diagnosis* 2007;**27**(2):119–23.
- Cebesoy 2008** *{published data only}*  
Cebesoy FB. Combining 'nasal bone length assessment as MoM' with other markers for trisomy 21 screening: could it be more effective?. *American Journal of Obstetrics and Gynecology* 2008;**198**(6):726–7.
- Chelli 2008** *{published data only}*  
Chelli D, Dimassi K, Chaabouni M, Ben Saad M, Mssaed H, Bchir F, et al. [Prenatal diagnosis of trisomy 21: the Tunisian experience]. [French]. *Sante* 2008;**18**(4):199–203.
- Chen 1999** *{published data only}*  
Chen FM. Integrated screening for Down's syndrome. *Journal of Family Practice* 1999;**48**(11):846–7.

- Chen 2002** *{published data only}*  
Chen M, Lam YH, Tang MH, Lee CP, Sin SY, Tang R, et al. The effect of ethnic origin on nuchal translucency at 10-14 weeks of gestation. *Prenatal Diagnosis* 2002;**22**(7):576-8.
- Chen 2004** *{published data only}*  
Chen M, Lam YH, Lee CP, Tang MHY. Ultrasound screening of fetal structural abnormalities at 12 to 14 weeks in Hong Kong. *Prenatal Diagnosis* 2004;**24**(2):92-7.
- Chen 2005** *{published data only}*  
Chen CP, Lin CJ, Wang W. Impact of second-trimester maternal serum screening on prenatal diagnosis of Down syndrome and the use of amniocentesis in the Taiwanese population. *Taiwanese Journal of Obstetrics and Gynecology* 2005;**44**(1):31-5.
- Chen 2008** *{published data only}*  
Chen M, Lee CP, Lam YH, Tang RY, Chan BC, Wong SF, et al. Comparison of nuchal and detailed morphology ultrasound examinations in early pregnancy for fetal structural abnormality screening: a randomized controlled trial. *Ultrasound in Obstetrics & Gynecology* 2008;**31**(2):136-46.
- Cheng 1993** *{published data only}*  
Cheng EY, Luthy DA, Zebelman AM, Williams MA, Lieppman RE, Hickok DE. A prospective evaluation of a second-trimester screening test for fetal Down syndrome using maternal serum alpha-fetoprotein, hCG, and unconjugated estriol. *Obstetrics and Gynecology* 1993;**81**(1):72-7.
- Cheng 1999** *{published data only}*  
Cheng PJ, Liu CM, Chang SD, Lin YT, Soong YK. Elevated second-trimester maternal serum hCG in women undergoing haemodialysis. *Prenatal Diagnosis* 1999;**19**(10):955-8.
- Cheng 2004a** *{published data only}*  
Cheng CC, Bahado-Singh RO, Chen SC, Tsai MS. Pregnancy outcomes with increased nuchal translucency after routine Down syndrome screening. *International Journal of Gynaecology and Obstetrics* 2004;**84**(1):5-9.
- Cheng 2004b** *{published data only}*  
Cheng PJ, Chu DC, Chueh HY, See LC, Chang HC, Weng DR. Elevated maternal midtrimester serum free  $\beta$ -human chorionic gonadotropin levels in vegetarian pregnancies that cause increased false-positive Down syndrome screening results. *American Journal of Obstetrics and Gynecology* 2004;**190**(2):442-7.
- Chitayat 2002** *{published data only}*  
Chitayat D, Farrell SA, Huang T, Meier C, Wyatt PR, Summers AM. Double-positive maternal serum screening results for down syndrome and open neural tube defects: An indicator for fetal structural or chromosomal abnormalities and adverse obstetric outcomes. *American Journal of Obstetrics and Gynecology* 2002;**187**(3):758-63.
- Chiu 2011** *{published data only}*  
Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ* 2011;**342**:c7401.
- Cho 2009** *{published data only}*  
Cho EH, Park BY, Kang YS, Lee EH. Validation of QF-PCR in a Korean population. *Prenatal Diagnosis* 2009;**29**(3):213-6.
- Chou 2009** *{published data only}*  
Chou CY, Hsieh FJ, Cheong ML, Lee FK, She BQ, Tsai MS. First-trimester Down syndrome screening in women younger than 35 years old and cost-effectiveness analysis in Taiwan population. *Journal of Evaluation in Clinical Practice* 2009;**15**(5):789-96.
- Christiansen 2002** *{published data only}*  
Christiansen M, Hogdall EV, Larsen SO, Hogdall C. The variation of risk estimates through pregnancy in second trimester maternal serum screening for Down syndrome. *Prenatal Diagnosis* 2002;**22**(5):385-7.
- Christiansen 2007b** *{published data only}*  
Christiansen M, Spencer K, Laigaard J, Cowans NJ, Larsen SO, Wewer UM. ADAM 12 as a second-trimester maternal serum marker in screening for Down syndrome. *Prenatal Diagnosis* 2007;**27**(7):611-5.
- Christiansen 2008** *{published data only}*  
Christiansen M, Sorensen TL, Larsen SO, Norgaard-Pedersen B. First-trimester maternal serum progesterone in aneuploid pregnancies. *Prenatal Diagnosis* 2008;**28**(4):319-22.
- Chung 2000** *{published data only}*  
Chung BL, Kim YP, Nam MH. The application of three-dimensional ultrasound to nuchal translucency thickness measurement at 10-14 weeks of gestation. *Prenatal and Neonatal Medicine* 2000;**5**(1):17-21.
- CNGOF 1996** *{published data only}*  
Anon. Blood screening of Down's syndrome (Trisomy 21) and reimbursement of karyotype for women under 38. *Revue Francaise de Gynecologie et d'Obstetrique* 1996;**91**(11):575-7.
- Cole 1996** *{published data only}*  
Cole L, Isozaki T, Palomaki G, Canick J, Iles R, Kellner L, et al. Detection of  $\beta$ -core fragment in second trimester Down's syndrome pregnancies. *Early Human Development* 1996;**47** Suppl:S47-S8.
- Comas 2001** *{published data only}*  
Comas C, Antolin E, Torrents M, Muñoz A, Figueras F, Echevarría M, et al. Early screening for chromosomal abnormalities: New strategies combining biochemical, sonographic and doppler parameters. *Prenatal and Neonatal Medicine* 2001;**6**(2):95-102.
- Comas 2002a** *{published data only}*  
Comas C, Torrents M, Munoz A, Antolin E, Figueras F, Echevarria M. Measurement of nuchal translucency as a single strategy in trisomy 21 screening: should we use any other marker?. *Obstetrics and Gynecology* 2002;**100**(4):648-54.

- Comas 2002b** *{published data only}*  
Comas C, Carrera JM. Early sonographic screening for chromosomal abnormalities. *Ultrasound Review of Obstetrics and Gynecology* 2002;**2**(2):88–91.
- Comstock 2006** *{published data only}*  
Comstock CH, Malone FD, Ball RH, Nyberg DA, Saade GR, Berkowitz RL, et al. Is there a nuchal translucency millimeter measurement above which there is no added benefit from first trimester serum screening?. *American Journal of Obstetrics and Gynecology* 2006;**195**(3):843–7.
- Conde 1998** *{published data only}*  
Conde Agudelo A, Kafury-Goeta AC. Triple-marker test as screening for down syndrome: a meta-analysis. *Obstetrical and Gynecological Survey* 1998;**53**(6):369–76.
- Cowans 2011** *{published data only}*  
Cowans NJ, Stamatopoulou A, Topping N, Spencer K. Early first-trimester maternal serum placental growth factor in trisomy 21 pregnancies. *Ultrasound in Obstetrics & Gynecology* 2011;**37**(5):515–9.
- Crossley 1991** *{published data only}*  
Crossley JA, Aitken DA, Connor JM. Prenatal screening for chromosome abnormalities using maternal serum chorionic gonadotrophin, alpha-fetoprotein, and age. *Prenatal Diagnosis* 1991;**11**(2):83–101.
- Crossley 1993** *{published data only}*  
Crossley JA, Aitken DA, Connor JM. Second-trimester unconjugated oestriol levels in maternal serum from chromosomally abnormal pregnancies using an optimized assay.[see comment]. *Prenatal Diagnosis* 1993;**13**(4):271–80.
- Crossley 1996** *{published data only}*  
Crossley JA, Berry E, Aitken DA, Connor JM. Insulin-dependent diabetes mellitus and prenatal screening results: current experience from a regional screening programme. *Prenatal Diagnosis* 1996;**16**(11):1039–42.
- Crossley 2002b** *{published data only}*  
Crossley JA, Aitken DA, Waugh SM, Kelly T, Connor JM. Maternal smoking: age distribution, levels of alpha-fetoprotein and human chorionic gonadotrophin, and effect on detection of Down syndrome pregnancies in second-trimester screening. *Prenatal Diagnosis* 2002;**22**(3):247–55.
- Cuckle 1984** *{published data only}*  
Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. *Lancet* 1984;**i**(8383):926–9.
- Cuckle 1987a** *{published data only}*  
Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *British Journal of Obstetrics and Gynaecology* 1987;**94**(5):387–402.
- Cuckle 1987b** *{published data only}*  
Cuckle HS, Nanchahal K, Wald NJ. Maternal serum alpha-fetoprotein and ethnic origin. *British Journal of Obstetrics and Gynaecology* 1987;**94**(11):1111–2.
- Cuckle 1990** *{published data only}*  
Cuckle HS, Wald NJ, Densen JW, Royston P, Knight GJ, Haddow JE, et al. The effect of smoking in pregnancy on maternal serum alpha-fetoprotein, unconjugated oestriol, human chorionic gonadotrophin, progesterone and dehydroepiandrosterone sulphate levels. *British Journal of Obstetrics and Gynaecology* 1990;**97**(3):272–4.
- Cuckle 1996** *{published data only}*  
Cuckle HS, Holding S, Jones R, Groome NP, Wallace EM. Combining Inhibin A with existing second-trimester markers in maternal serum screening for Down's syndrome. *Prenatal Diagnosis* 1996;**16**(12):1095–100.
- Cuckle 1999a** *{published data only}*  
Cuckle HS, Sehmi I, Jones R, Evans LW. Maternal serum activin A and follistatin levels in pregnancies with Down syndrome. *Prenatal Diagnosis* 1999;**19**(6):513–6.
- Cuckle 1999b** *{published data only}*  
Cuckle HS, Van Lith JM. Appropriate biochemical parameters in first-trimester screening for Down syndrome.[see comment]. *Prenatal Diagnosis* 1999;**19**(6):505–12.
- Cullen 1990** *{published data only}*  
Cullen MT, Gabrielli S, Green JJ, Rizzo N, Mahoney MJ, Salafia C, et al. Diagnosis and significance of cystic hygroma in the first trimester. *Prenatal Diagnosis* 1990;**10**(10):643–51.
- Cusick 2004** *{published data only}*  
Cusick W, Provenzano J, Sullivan CA, Gallousis FM, Rodis JF. Fetal nasal bone length in euploid and aneuploid fetuses between 11 and 20 weeks' gestation: a prospective study. *Journal of Ultrasound in Medicine* 2004;**23**(10):1327–33.
- Cusick 2007** *{published data only}*  
Cusick W, Shevell T, Duchan LS, Lupinacci CA, Terranova J, Crombleholme WR. Likelihood ratios for fetal trisomy 21 based on nasal bone length in the second trimester: how best to define hypoplasia?. *Ultrasound in Obstetrics & Gynecology* 2007;**30**(3):271–4.
- D'Ottavio 1997** *{published data only}*  
D'Ottavio G, Meir YJ, Rustico MA, Pecile V, Fischer-Tamaro L, Conoscenti G, et al. Screening for fetal anomalies by ultrasound at 14 and 21 weeks. *Ultrasound in Obstetrics & Gynecology* 1997;**10**(6):375–80.
- Dancoine 2001** *{published data only}*  
Dancoine F, Couplet G, Mainardi A, Sukno F, Jaumain P, Nowak E, et al. Antenatal screening for Dawn's syndrome with serum markers: Influence of maternal weight, smoking habits and diabetes. *Immuno-Analyse et Biologie Specialisee* 2001;**16**(6):381–9.
- Dane 2008** *{published data only}*  
Dane B, Dane C, Cetin A, Kiray M, Sivri D, Yayla M. Pregnancy outcome in fetuses with increased nuchal translucency. *Journal of Perinatology* 2008;**28**(6):400–4.
- De Biasio, 1999** *{published data only}*  
De Biasio, Siccardi M, Volpe G, Famularo L, Santi F, Canini S. First-trimester screening for down syndrome using nuchal

- translucency measurement with free  $\beta$ -hCG and PAPP-A between 10 and 13 weeks of pregnancy - The combined test. *Prenatal Diagnosis* 1999;**19**(4):360–3.
- De Biasio, 2001** *{published data only}*  
De Biasio, Ferrero S, Prefumo F, Canini S, Marchini P, Bruzzone I, et al. Down's syndrome: First trimester approach. *Italian Journal of Gynaecology and Obstetrics* 2001;**13**(1):22–6.
- De Biasio 2000** *{published data only}*  
De Biasio P, Canini S, Prefumo F, Famularo L, Venturini PL. Extent of correlation between first and second trimester markers for Down's syndrome screening. *Journal of Medical Screening* 2000;**7**(3):163.
- De Graaf 1991** *{published data only}*  
De Graaf I, Cuckle HS, Pajkrt E, Leschot NJ, Bleker OP, Van Lith JM. Co-variables in first trimester maternal serum screening. *Prenatal Diagnosis* 1991;**20**(3):186–9.
- De Graaf 1999b** *{published data only}*  
De Graaf I, Pajkrt E, Bilardo CM, Leschot NJ, Cuckle HS, Van Lith JM. Early pregnancy screening for fetal aneuploidy with serum markers and nuchal translucency. *Prenatal Diagnosis*. 1999;**19**(5):458–62.
- Del Carmen Saucedo 2009** *{published data only}*  
Del Carmen Saucedo M, DeVigan C, Vodovar V, Lelong N, Goffinet F, Khoshnood B. Measurement of nuchal translucency and the prenatal diagnosis of Down syndrome. *Obstetrics and Gynecology* 2009;**114**(4):829–38.
- DeVore 2001** *{published data only}*  
DeVore GR, Romero R. Combined use of genetic sonography and maternal serum triple-marker screening: an effective method for increasing the detection of trisomy 21 in women younger than 35 years.[see comment]. *Journal of Ultrasound in Medicine* 2001;**20**(6):645–54.
- Dhaifalah 2007a** *{published data only}*  
Dhaifalah I, Mickova I, Vrbicka D, Santavy J, Curtisova V. [Advanced maternal age as an indication for invasive prenatal diagnostics?]. [Czech]. *Ceska Gynecologie* 2007;**72**(3):181–4.
- Dhaifalah 2007b** *{published data only}*  
Dhaifalah I, Mickova I, Santavy J, Vrbicka D, Zapletalova D, Curtisova V. [Efficiency of measuring nasal bone as an ultrasound marker of Down syndrome in 11th to 13th+6 week of pregnancy]. [Czech]. *Ceska Gynecologie* 2007;**72**(1):19–23.
- Dhallan 2007** *{published data only}*  
Dhallan R, Guo X, Emche S, Damewood M, Bayliss P, Cronin M, et al. A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. *Lancet* 2007;**369**(9560):474–81.
- Dickerson 1994** *{published data only}*  
Dickerson VM. Multiple marker screening. *Western Journal of Medicine* 1994;**161**(2):161.
- Dimaio 1987** *{published data only}*  
Dimaio MS, Baumgarten A, Greenstein RM, Saal HM, Mahoney MJ. Screening for fetal Down's syndrome in pregnancy by measuring maternal serum alpha-fetoprotein levels. *New England Journal of Medicine* 1987;**317**(6):342–6.
- Doran 1986** *{published data only}*  
Doran TA, Cadesky K, Wong PY, Mastrogiamoco C, Capello T. Maternal serum alpha-fetoprotein and fetal autosomal trisomies. *American Journal of Obstetrics and Gynecology* 1986;**154**(2):277–81.
- Dreux 2008** *{published data only}*  
Dreux S, Olivier C, Dupont JM, Leporrier N, Study G, Oury JF, et al. Maternal serum screening in cases of mosaic and translocation Down syndrome. *Prenatal Diagnosis* 2008;**28**(8):699–703.
- Drugan 1996a** *{published data only}*  
Drugan A, Reichler A, Bronstein M, Johnson MP, Sokol RJ, Evans MI. Abnormal biochemical serum screening versus 2nd-trimester ultrasound-detected minor anomalies as predictors of aneuploidy in low-risk women. *Fetal Diagnosis and Therapy* 1996;**11**(5):301–5.
- Drugan 1996b** *{published data only}*  
Drugan A, O'Brien JE, Dvorin E, Krivchenia EL, Johnson MP, Sokol RJ, et al. Multiple marker screening in multifetal gestations: failure to predict adverse pregnancy outcomes. *Fetal Diagnosis and Therapy* 1996;**11**(1):16–9.
- Drysdale 2002** *{published data only}*  
Drysdale K, Ridley D, Walker K, Higgins B, Dean T. First-trimester pregnancy scanning as a screening tool for high-risk and abnormal pregnancies in a district general hospital setting. *Journal of Obstetrics and Gynaecology* 2002;**22**(2):159–65.
- Dugoff 2008** *{published data only}*  
Dugoff L, Cuckle HS, Hobbins JC, Malone FD, Belfort MA, Nyberg DA, et al. Prediction of patient-specific risk for fetal loss using maternal characteristics and first- and second-trimester maternal serum Down syndrome markers. *American Journal of Obstetrics and Gynecology* 2008;**199**(3):290–6.
- Ebell 1999** *{published data only}*  
Ebell M. Is the integrated test better for screening for Down's syndrome than the traditional triple test?. *Evidence-Based Practice* 1999;**2**(11):4–5.
- Economides 1998** *{published data only}*  
Economides DL, Whitlow BJ, Kadir R, Lazanakis M, Verdin SM. First trimester sonographic detection of chromosomal abnormalities in an unselected population. *British Journal of Obstetrics and Gynaecology* 1998;**105**(1):58–62.
- Erickson 2004** *{published data only}*  
Erickson JA, Ashwood ER, Gin CA. Evaluation of a dimeric inhibin-A assay for assessing fetal Down syndrome: establishment, comparison, and monitoring of median concentrations for normal pregnancies. *Archives of Pathology & Laboratory Medicine* 2004;**128**(4):415–20.
- Evans 1996** *{published data only}*  
Evans MI, O'Brien JE, Dvorin E, Krivchenia EL, Drugan A, Hume RF Jr, et al. Similarity of insulin-dependent

- diabetics' and non-insulin-dependent diabetics' levels of  $\beta$ -hCG and unconjugated estriol with controls: no need to adjust as with alpha-fetoprotein. *Journal of the Society for Gynecologic Investigation* 1996;**3**(1):20–2.
- Evans 2007** *{published data only}*  
Evans MI, Galen RS. Comparison of serum markers in first-trimester down syndrome screening. *Obstetrics and Gynecology* 2007;**109**(3):782.
- Falcon 2005** *{published data only}*  
Falcon O, Cavoretto P, Peralta CF, Csapo B, Nicolaides KH. Fetal head-to-trunk volume ratio in chromosomally abnormal fetuses at 11 + 0 to 13 + 6 weeks of gestation. *Ultrasound in Obstetrics & Gynecology* 2005;**26**(7):755–60.
- Falcon 2006** *{published data only}*  
Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. Fetal tricuspid regurgitation at the 11 + 0 to 13 + 6-week scan: association with chromosomal defects and reproducibility of the method. *Ultrasound in Obstetrics & Gynecology* 2006;**27**(6):609–12.
- Ford 1998** *{published data only}*  
Ford C, Moore AJ, Jordan PA, Bartlett WA, Wyldes MP, Jones AF, et al. The value of screening for Down's syndrome in a socioeconomically deprived area with a high ethnic population.[see comment]. *British Journal of Obstetrics and Gynaecology* 1998;**105**(8):855–9.
- Frishman 1997** *{published data only}*  
Frishman GN, Canick JA, Hogan JW, Hackett RJ, Kellner LH, Saller DN Jr. Serum triple-marker screening in in vitro fertilization and naturally conceived pregnancies. *Obstetrics and Gynecology* 1997;**90**(1):98–101.
- Fukada 2000** *{published data only}*  
Fukada Y, Takizawa M, Amemiya A, Yoda H, Kohno K, Hoshi K. Detection of aneuploidy with fetal nuchal translucency and maternal serum markers in Japanese women. *Acta Obstetrica et Gynecologica Scandinavica* 2000;**79**(12):1124–5.
- Gaudry 2009** *{published data only}*  
Gaudry P, Lebbar A, Choiset A, Girard S, Lewin F, Tsatsaris V, et al. Is rapid aneuploidy screening used alone acceptable in prenatal diagnosis? An evaluation of the possible role of ultrasound examination. *Fetal Diagnosis and Therapy* 2009;**25**(2):285–90.
- Gebb 2009** *{published data only}*  
Gebb J, Dar P. Should the first-trimester aneuploidy screen be maternal age adjusted? Screening by absolute risk versus risk adjusted to maternal age. *Prenatal Diagnosis* 2009;**29**(3):245–7.
- Geerts 2008** *{published data only}*  
Geerts L. Prenatal diagnosis of chromosomal abnormalities in a resource-poor setting. *International Journal of Gynaecology and Obstetrics* 2008;**103**(1):16–21.
- Geipel 2010** *{published data only}*  
Geipel A, Willruth A, Vieten J, Gembruch U, Berg C. Nuchal fold thickness, nasal bone absence or hypoplasia, ductus venosus reversed flow and tricuspid valve regurgitation in screening for trisomies 21, 18 and 13 in the early second trimester. *Ultrasound in Obstetrics & Gynecology* 2010;**35**(5):535–9.
- Gekas 2009** *{published data only}*  
Gekas J, Gagne G, Bujold E, Douillard D, Forest JC, Reinharz D, et al. Comparison of different strategies in prenatal screening for Down's syndrome: cost effectiveness analysis of computer simulation. *BMJ* 2009;**338**:b138.
- Gekas 2011a** *{published data only}*  
Gekas J, van den Berg DG, Durand A, Vallee M, Wildschut HI, Bujold E, et al. Rapid testing versus karyotyping in Down's syndrome screening: cost-effectiveness and detection of clinically significant chromosome abnormalities. *European Journal of Human Genetics* 2011;**19**(1):3–9.
- Gekas 2011b** *{published data only}*  
Gekas J, Durand A, Bujold E, Vallee M, Forest JC, Rousseau F, et al. Cost-effectiveness and accuracy of prenatal Down syndrome screening strategies: should the combined test continue to be widely used?. *American Journal of Obstetrics and Gynecology* 2011;**204**(2):175–8.
- Gerovassili 2007** *{published data only}*  
Gerovassili A, Garner C, Nicolaides KH, Thein SL, Rees DC. Free fetal DNA in maternal circulation: a potential prognostic marker for chromosomal abnormalities?. *Prenatal Diagnosis* 2007;**27**(2):104–10.
- Ghidini 1998** *{published data only}*  
Ghidini A, Spong CY, Grier RE, Walker CN, Pezzullo JC. Is maternal serum triple screening a better predictor of Down syndrome in female than in male fetuses?. *Prenatal Diagnosis* 1998;**18**(2):123–6.
- Goetzinger 2010** *{published data only}*  
Goetzinger KR, Dicke JM, Gray DL, Stamilio DM, Macones GA, Odibo AO. The effect of fetal gender in predicting Down syndrome using long bone ultrasonographic measurements. *Prenatal Diagnosis* 2010;**30**(10):950–5.
- Goldie 1995** *{published data only}*  
Goldie DJ, Astley JP, Beaman JM, Bickley DA, Gunneberg A, Jones SR. Screening for Down's syndrome: the first two years experience in Bristol. *Journal of Medical Screening* 1995;**2**(4):207–10.
- Gollo 2008** *{published data only}*  
Gollo CA, Murta CG, Bussamra LC, Santana RM, Moron AF. [Predictive value for fetal outcome of Doppler velocimetry of the ductus venosus between the 11th and the 14th gestation week]. [Portuguese]. *Revista Brasileira de Ginecologia e Obstetricia* 2008;**30**(1):5–11.
- Gonçalves 2004** *{published data only}*  
Gonçalves LF, Espinoza J, Lee W, Schoen ML, Devers P, Mazor M, et al. Phenotypic characteristics of absent and hypoplastic nasal bones in fetuses with down syndrome: Description by 3-dimensional ultrasonography and clinical significance. *Journal of Ultrasound in Medicine* 2004;**23**(12):1619–27.

- Goodburn 1994** {published data only}  
 Goodburn SF, Yates JR, Raggatt PR, Carr C, Ferguson-Smith ME, Kershaw AJ, et al. Second-trimester maternal serum screening using alpha-fetoprotein, human chorionic gonadotrophin, and unconjugated oestriol: experience of a regional programme. *Prenatal Diagnosis* 1994;**14**(5): 391–402.
- Gorduza 2007** {published data only}  
 Gorduza EV, Onofriescu M, Martiniuc V, Grigore M, Mihalceanu E, Iliev G. [FISH technique in aneuploidies prenatal diagnosis]. [Romanian]. *Revista Medico-Chirurgicala a Societatii de Medici Si Naturalisti Din Iasi* 2007;**111**(4):990–5.
- Grace 2010** {published data only}  
 Grace D, Eggers P, Glantz JC, Ozcan T. Mitral valve-tricuspid valve distance as a sonographic marker of trisomy 21. *Ultrasound in Obstetrics & Gynecology* 2010;**35**(2): 172–7.
- Grati 2010** {published data only}  
 Grati FR, Barlocco A, Grimi B, Milani S, Frascoli G, Di Meco AM, et al. Chromosome abnormalities investigated by non-invasive prenatal testing account for approximately 50% of fetal unbalances associated with relevant clinical phenotypes. *American Journal of Medical Genetics* 2010;**Part A. 152A**(6):1434–42.
- Gray 2009** {published data only}  
 Gray DL, Dicke JM, Dickerson R, McCourt C, Odibo AO. Reevaluating humeral length for the detection of fetal trisomy 21. *Journal of Ultrasound in Medicine* 2009;**28**(10): 1325–30.
- Gregor 2007** {published data only}  
 Gregor V, Sipek A, Horacek J. [Birth defects in the Czech Republic--the prenatal diagnostic]. [Czech]. *Ceska Gynekologie* 2007;**72**(4):262–8.
- Gregor 2009** {published data only}  
 Gregor V, Sipek A, Sipek AJ, Horacek J, Langhammer P, Petrilkova L, et al. [Prenatal diagnostics of chromosomal aberrations Czech Republic: 1994-2007]. [Czech]. *Ceska Gynekologie* 2009;**74**(1):44–54.
- Grether 2009** {published data only}  
 Grether González P, Aguinaga Ríos M, Colegio Mexicano de Especialistas en Ginecología y Obstetricia. [Prenatal genetic screening: biochemical markers of the first and second quarter]. [Spanish]. *Ginecología y Obstetricia de Mexico* 2009;**77**(2):S27–46.
- Grozdea 2002** {published data only}  
 Grozdea J, De La Farge F, Bourrouillou G, Calot M, Cambus JP, Valdiguie P. Maternal serum urea resistant alkaline phosphatase in Down syndrome pregnancy. *Early Human Development* 2002;**67**(1-2):55–9.
- Guo 2010** {published data only}  
 Guo Q, Zhou Y, Wang X, Li Q. Simultaneous detection of trisomies 13, 18, and 21 with multiplex ligation-dependent probe amplification-based real-time PCR. *Clinical Chemistry* 2010;**56**(9):1451–9.
- Gyselaers 2004a** {published data only}  
 Gyselaers WJ, Vereecken AJ, Van Herck EJ, Straetmans DP, Martens GE, De Jonge ET, et al. Screening for trisomy 21 in Flanders: a 10 years review of 40.490 pregnancies screened by maternal serum. *European Journal of Obstetrics, Gynecology, & Reproductive Biology* 2004;**115**(2):185–9.
- Gyselaers 2004b** {published data only}  
 Gyselaers WJA, Vereecken AJ, Van Herck, Straetmans DPL, De Jonge, Ombelet WUA, et al. Single-step maternal serum screening for trisomy 21 in the era of combined or integrated screening. *Gynecologic and Obstetric Investigation* 2004;**58**(4):221–4.
- Gyselaers 2006a** {published data only}  
 Gyselaers WJ, Vereecken AJ, Van Herck EJ, Straetmans DP, Ombelet WU, Nijhuis JG. Nuchal translucency thickness measurements for fetal aneuploidy screening: Log NT-MoM or Delta-NT, performer-specific medians and ultrasound training. *Journal of Medical Screening* 2006;**13**(1):4–7.
- Gyselaers 2006b** {published data only}  
 Gyselaers WJ, Roets ER, Van Holsbeke CD, Vereecken AJ, Van Herck EJ, Straetmans DP, et al. Sequential triage in the first trimester may enhance advanced ultrasound scanning in population screening for trisomy 21. *Ultrasound in Obstetrics & Gynecology* 2006;**27**(6):622–7.
- Hackshaw 1995** {published data only}  
 Hackshaw AK, Densem J, Wald NJ. Repeat maternal serum testing for Down's syndrome screening using multiple markers with special reference to free alpha and free  $\beta$ -hCG. *Prenatal Diagnosis* 1995;**15**(12):1125–30.
- Hackshaw 2001** {published data only}  
 Hackshaw AK, Wald NJ. Repeat testing in antenatal screening for Down syndrome using dimeric inhibin-A in combination with other maternal serum markers. *Prenatal Diagnosis* 2001;**21**(1):58–61.
- Haddow 1992** {published data only}  
 Haddow JE, Palomaki GE, Knight GJ, Williams J, Pulkkinen A, Canick J, et al. Prenatal screening for Down's syndrome with use of maternal serum markers. *New England Journal of Medicine* 1992;**327**(9):588–93.
- Hadzsiev 2007** {published data only}  
 Hadzsiev K, Czako M, Veszpremi B, Kosztolanyi G. [Rapid diagnosis of fetal chromosomal abnormalities by fluorescence in situ hybridization]. [Hungarian]. *Orvosi Hetilap* 2007;**148**(30):1401–4.
- Hafner 1995** {published data only}  
 Hafner E, Schuchter K, Philipp K. Screening for chromosomal abnormalities in an unselected population by fetal nuchal translucency. *Ultrasound in Obstetrics & Gynecology* 1995;**6**(5):330–3.
- Hallahan 1998** {published data only}  
 Hallahan TW, Krantz DA, Tului L, Alberti E, Buchanan PD, Orlandi F, et al. Comparison of urinary free  $\beta$  (hCG) and  $\beta$ -core (hCG) in prenatal screening for chromosomal abnormalities. *Prenatal Diagnosis* 1998;**18**(9):893–900.

- Han 2008** *{published data only}*  
Han SH, An JW, Jeong GY, Yoon HR, Lee A, Yang YH, et al. Clinical and cytogenetic findings on 31,615 mid-trimester amniocenteses. *Korean Journal of Laboratory Medicine* 2008;**28**(5):378–85.
- Harper 2010** *{published data only}*  
Harper LM, Gray D, Dicke J, Stamilio DM, Macones GA, Odibo AO. Do race-specific definitions of short long bones improve the detection of down syndrome on second-trimester genetic sonograms?. *Journal of Ultrasound in Medicine* 2010;**29**(2):231–5.
- Harrison 2006** *{published data only}*  
Harrison G, Goldie D. Second-trimester Down's syndrome serum screening: double, triple or quadruple marker testing?. *Annals of Clinical Biochemistry* 2006;**43**(1):67–72.
- Harry 2006** *{published data only}*  
Harry WG, Reed KL. Nuchal translucency and first-trimester screening. *Journal of the Society for Gynecologic Investigation* 2006;**13**(3):153–4.
- Hayashi 1995** *{published data only}*  
Hayashi M, Kozu H. Maternal urinary  $\beta$ -core fragment of hCG/creatinine ratios and fetal chromosomal abnormalities in the second trimester of pregnancy. *Prenatal Diagnosis* 1995;**15**(1):11–6.
- Hayashi 1996** *{published data only}*  
Hayashi M, Kozu H, Takei H. Maternal urinary free  $\beta$ -subunit of human chorionic gonadotrophin: Creatinine ratios and fetal chromosomal abnormalities in the second trimester of pregnancy. *British Journal of Obstetrics and Gynaecology* 1996;**103**(6):577–80.
- Heikkila 1997** *{published data only}*  
Heikkila A, Ryyananen M, Kirkinen P, Saarikoski S. Results and views of women in population-wide pregnancy screening for trisomy 21 in east Finland. *Fetal Diagnosis and Therapy* 1997;**12**(2):93–6.
- Heinig 2007** *{published data only}*  
Heinig J, Steinhard J, Schmitz R, Nofer JR, Kiesel L, Klockenbusch W. Maternal serum free beta-hCG and PAPP-A in patients with habitual abortion-influence on first-trimester screening for chromosomal abnormalities. *Prenatal Diagnosis* 2007;**27**(9):814–6.
- Heinonen 1996** *{published data only}*  
Heinonen S, Ryyananen M, Kirkinen P, Hippelainen M, Saarikoski S. Effect of in vitro fertilization on human chorionic gonadotropin serum concentrations and Down's syndrome screening. *Fertility and Sterility* 1996;**66**(3):398–403.
- Herman 2000** *{published data only}*  
Herman A, Weinraub Z, Dreazen E, Arieli S, Rozansky S, Bukovsky I, et al. Combined first trimester nuchal translucency and second trimester biochemical screening tests among normal pregnancies. *Prenatal Diagnosis* 2000;**20**(10):781–4.
- Herman 2003** *{published data only}*  
Herman A, Dreazen E, Tovbin Y, Reish O, Bukovsky I, Maymon R. Correlation and overlapping between nuchal translucency and triple test among Down syndrome-affected pregnancies. *Fetal Diagnosis and Therapy* 2003;**18**(3):196–200.
- Herrou 1992** *{published data only}*  
Herrou M, Leporrier N, Leymarie P. Screening for fetal Down syndrome with maternal serum hCG and oestriol: a prospective study. *Prenatal Diagnosis* 1992;**12**(11):887–92.
- Hershey 1985** *{published data only}*  
Hershey DW, Crandall BF, Schroth PS. Maternal serum alpha-fetoprotein screening of fetal trisomies. *American Journal of Obstetrics and Gynecology* 1985;**153**(2):224–5.
- Hershey 1986** *{published data only}*  
Hershey DW, Crandall BF, Perdue S. Combining maternal age and serum alpha-fetoprotein to predict the risk of Down syndrome. *Obstetrics and Gynecology* 1986;**68**(2):177–80.
- Hewitt 1993** *{published data only}*  
Hewitt B. Nuchal translucency in the first trimester. *Australian & New Zealand Journal of Obstetrics & Gynaecology* 1993;**33**(4):389–91.
- Hills 2010** *{published data only}*  
Hills A, Donaghue C, Waters J, Waters K, Sullivan C, Kulkarni A, et al. QF-PCR as a stand-alone test for prenatal samples: the first 2 years' experience in the London region. *Prenatal Diagnosis* 2010;**30**(6):509–17.
- Ho 2010** *{published data only}*  
Ho SS, Choolani MA. FlashFISH: "same day" prenatal diagnosis of common chromosomal aneuploidies. *Methods in Molecular Biology* 2010;**659**:261–8.
- Hogdall 1992** *{published data only}*  
Hogdall CK, Hogdall EV, Arends J, Norgaard-Pedersen B, Smidt-Jensen S, Larsen SO. CA-125 as a maternal serum marker for Down's syndrome in the first and second trimesters. *Prenatal Diagnosis* 1992;**12**(3):223–7.
- Hong Kong Practitioner** *{published data only}*  
Anon. Screening tests in pregnancy. *Hong Kong Practitioner* 2001;**23**(10):461–5.
- Hoogendoorn 2008** *{published data only}*  
Hoogendoorn M, Evers SM, Schielen PC, van Genugten ML, de Wit GA, Ament AJ. Costs and effects of prenatal screening methods for Down syndrome and neural tube defects. *Community Genetics* 2008;**11**(6):359–67.
- Howe 2000** *{published data only}*  
Howe DT, Gornall R, Wellesley D, Boyle T, Barber J. Six year survey of screening for Down's syndrome by maternal age and mid-trimester ultrasound scans. *BMJ* 2000;**320**(7235):606–10.
- Hsiao 1991** *{published data only}*  
Hsiao KJ, Lee SY, Chuang HC. [Antenatal screening of maternal alpha-fetoprotein with dried-blood spot samples on filter paper]. [Chinese]. *Journal of the Formosan Medical Association* 1991;**90**(6):598–604.
- Hsieh 1999** *{published data only}*  
Hsieh TT, Hsu JJ, Lo LM, Liou JD, Soong YK. Maternal urine alpha-fetoprotein concentrations between 14 and 21



- weeks of gestation. *Changgeng Yi Xue Za Zhi* 1999;**22**(2):234–9.
- Hsu 1997** *{published data only}*  
Hsu JJ, Hsieh TT, Soong YK. Influence of maternal age and weight on second-trimester serum alpha-fetoprotein, total and free  $\beta$  human chorionic gonadotropin levels. *Changgeng Yi Xue Za Zhi* 1997;**20**(3):181–6.
- Hsu 1998** *{published data only}*  
Hsu JJ, Hsieh TT, Hung TH, Chiang CH. Midtrimester maternal serum free  $\beta$ -human chorionic gonadotropin levels: normal reference values for Taiwanese women. *Changgeng Yi Xue Za Zhi* 1998;**21**(3):277–82.
- Hsu 1999** *{published data only}*  
Hsu JJ, Hsieh TT, Hung TH, Chen KC, Soong YK. Urine free  $\beta$ -human chorionic gonadotropin levels between 14 and 21 weeks of gestation in Taiwanese pregnancies. *Changgeng Yi Xue Za Zhi* 1999;**22**(1):11–6.
- Hu 2007** *{published data only}*  
Hu YL, Birth Defect Intervention Group of Jiangsu Province. [Serum screening of fetal chromosome abnormality during second pregnancy trimester: results of 26,803 pregnant women in Jiangsu Province]. [Chinese]. *Chung-Hua i Hsueh Tsa Chih [Chinese Medical Journal]* 2007;**87**(35):2476–80.
- Huang 2003** *{published data only}*  
Huang T, Summers AM, Wyatt PR, Meier C, Cote GB. Maternal serum marker medians in Aboriginal Canadian women. *Prenatal Diagnosis* 2003;**23**(2):98–100.
- Huang 2007a** *{published data only}*  
Huang T, Boucher K, Summers AM. Second trimester prenatal screening for Down syndrome: the associations between the levels of serum markers in successive pregnancies. *Prenatal Diagnosis* 2007;**27**(12):1138–42.
- Huang 2007b** *{published data only}*  
Huang T, Wang FL, Boucher K, O'Donnell A, Rashid S, Summers AM. Racial differences in first trimester nuchal translucency. *Prenatal Diagnosis* 2007;**27**(12):1174–6.
- Huggon 2004** *{published data only}*  
Huggon IC, Turan O, Allan LD. Doppler assessment of cardiac function at 11–14 weeks' gestation in fetuses with normal and increased nuchal translucency. *Ultrasound in Obstetrics & Gynecology* 2004;**24**(4):390–8.
- Hui 2003** *{published data only}*  
Hui PW, Tang MH, Lam YH, Ng EH, Yeung WS, Ho PC. Maternal serum hCG and alpha-fetoprotein levels in pregnancies conceived after IVF or ICSI with fresh and frozen-thawed embryos. *Human Reproduction* 2003;**18**(3):572–5.
- Hui 2005** *{published data only}*  
Hui PW, Tang MH, Lam YH, Yeung WS, Ng EH, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. *Ultrasound in Obstetrics & Gynecology* 2005;**25**(3):234–8.
- Hultén 2004** *{published data only}*  
Hultén M. Combined serum and nuchal translucency screening in the first trimester achieves 85% to 90% detection rate for Down and Edward syndromes. *Evidence-Based Healthcare* 2004;**8**(2):82–4.
- Hung 2003** *{published data only}*  
Hung JH, Fu CY, Yuan CC, Chen CL, Yang ML, Shu LP, et al. Nuchal translucency incorporated into a one-stage multifactorial screening model for Down syndrome prediction at second-trimester pregnancy. *Ultrasound in Medicine & Biology* 2003;**29**(12):1667–74.
- Hung 2008** *{published data only}*  
Hung JH, Fu CY, Chen CY, Chao KC, Hung J. Fetal nasal bone length and Down syndrome during the second trimester in a Chinese population. *Journal of Obstetrics and Gynaecology Research* 2008;**34**(4):518–23.
- Hurley 1993** *{published data only}*  
Hurley PA, Ward RH, Teisner B, Iles RK, Lucas M, Grudzinskas JG. Serum PAPP-A measurements in first-trimester screening for Down syndrome. *Prenatal Diagnosis* 1993;**13**(10):903–8.
- Huttly 2004** *{published data only}*  
Huttly W, Rudnicka A, Wald NJ. Second-trimester prenatal screening markers for Down syndrome in women with insulin-dependent diabetes mellitus. *Prenatal Diagnosis* 2004;**24**(10):804–7.
- Hwa 2004** *{published data only}*  
Hwa HL, Yen MF, Hsieh FJ, Ko TM, Chen TH. Evaluation of second trimester maternal serum screening for Down's Syndrome using the Spiegelhalter-Knill-Jones (S-KJ) approach. *Journal of Perinatal Medicine* 2004;**32**(5):407–12.
- Iles 1996** *{published data only}*  
Iles RK. Urinary analysis for Down's syndrome: Is the measurement of urinary  $\beta$ -core the future of biochemical screening for Down's syndrome. *Early Human Development* 1996;**47**(Suppl):S41–S45.
- Ind 1994** *{published data only}*  
Ind TEJ, Iles RK, Cuckle HS, Chard T. Second trimester maternal serum placental alkaline phosphatase concentrations in Down's syndrome. *Journal of Obstetrics and Gynaecology* 1994;**14**(5):305–8.
- Ivorra-Deleuze 2010** *{published data only}*  
Ivorra-Deleuze D, Bretelle F, Heinemann M, Levy A, Toga C, Philip N, et al. [Combined screening for Down syndrome in Marseille multidisciplinary prenatal centers]. [French]. *Gynecologie, Obstetrique & Fertilité* 2010;**38**(12):786–8.
- Jakobsen 2011** *{published data only}*  
Jakobsen TR, Sogaard K, Tabor A. Implications of a first trimester Down syndrome screening program on timing of malformation detection. *Acta Obstetrica et Gynecologica Scandinavica* 2011;**90**(7):728–36.

- Jean-Pierre 2005** *{published data only}*  
Jean-Pierre C. Fetal nasal bone: review of first trimester findings. *Ultrasound Review of Obstetrics and Gynecology* 2005;**5**(2):102–4.
- Johnson 1991** *{published data only}*  
Johnson A, Cowchock FS, Darby M, Wapner R, Jackson LG. First-trimester maternal serum alpha-fetoprotein and chorionic gonadotropin in aneuploid pregnancies. *Prenatal Diagnosis* 1991;**11**(7):443–50.
- Johnson 1993** *{published data only}*  
Johnson MP, Johnson A, Holzgreve W, Isada NB, Wapner RJ, Treadwell MC, et al. First-trimester simple hygroma: cause and outcome. *American Journal of Obstetrics and Gynecology* 1993;**168**(1):156–61.
- Jorgensen 1999** *{published data only}*  
Jorgensen FS, Valentin L, Salvesen KA, Jorgensen C, Jensen FR, Bang J, et al. MULTISCAN—a Scandinavian multicenter second trimester obstetric ultrasound and serum screening study. *Acta Obstetrica et Gynecologica Scandinavica* 1999;**78**(6):501–10.
- Jorgez 2007** *{published data only}*  
Jorgez CJ, Dang DD, Wapner R, Farina A, Simpson JL, Bischoff FZ. Elevated levels of total (maternal and fetal) beta-globin DNA in maternal blood from first trimester pregnancies with trisomy 21. *Human Reproduction* 2007;**22**(8):2267–72.
- Josefsson 1998** *{published data only}*  
Josefsson A, Molander E, Selbing A. Nuchal translucency as a screening test for chromosomal abnormalities in a routine first trimester ultrasound examination. *Acta Obstetrica et Gynecologica Scandinavica* 1998;**77**(5):497–9.
- Jou 2001** *{published data only}*  
Jou HJ, Shih JC, Wu SC, Li TC, Tzeng CY, Hsieh FJ. First-trimester Down's syndrome screening by fetal nuchal translucency measurement in Taiwan. *Journal of the Formosan Medical Association* 2001;**100**(4):257–61.
- Jung 2007** *{published data only}*  
Jung E, Won HS, Lee PR, Kim A. Ultrasonographic measurement of fetal nasal bone length in the second trimester in Korean population. *Prenatal Diagnosis* 2007;**27**(2):154–7.
- Kagan 2006** *{published data only}*  
Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation between increased fetal nuchal translucency thickness and chromosomal defects.[see comment]. *Obstetrics and Gynecology* 2006;**107**(1):6–10.
- Kagan 2007** *{published data only}*  
Kagan KO, Frisova V, Nicolaides KH, Spencer K. Dose dependency between cigarette consumption and reduced maternal serum PAPP-A levels at 11-13+6 weeks of gestation. *Prenatal Diagnosis* 2007;**27**(9):849–53.
- Kagan 2008** *{published data only}*  
Kagan KO, Anderson JM, Anwandter G, Neksasova K, Nicolaides KH. Screening for triploidy by the risk algorithms for trisomies 21, 18 and 13 at 11 weeks to 13 weeks and 6 days of gestation. *Prenatal Diagnosis* 2008;**28**(13):1209–13.
- Kalelioglu 2007** *{published data only}*  
Kalelioglu IH. Humerus length measurement in Down syndrome screening. *Clinical and Experimental Obstetrics & Gynecology* 2007;**34**(2):93–5.
- Kautzmann 1995** *{published data only}*  
Kautzmann M, Solis RL, Luberta A, Fernandez JL, Navarro J, Rodriguez L, et al. Study of the efficiency of screening for trisomy 21 based on maternal serum levels of AFP and hCG combined with maternal age. *Journal of Clinical Ligand Assay* 1995;**18**(3):181–5.
- Kazerouni 2009** *{published data only}*  
Kazerouni NN, Currier B, Malm L, Riggle S, Hodgkinson C, Smith S, et al. Triple-marker prenatal screening program for chromosomal defects. *Obstetrics and Gynecology* 2009;**114**(1):50–8.
- Keith 1992** *{published data only}*  
Keith D. Maternal serum screening for neural tube defects and Down syndrome. *Clinical Laboratory Science* 1992;**5**(5):274–6.
- Kelekci 2004** *{published data only}*  
Kelekci S, Yazicioglu HF, Oguz S, Inan I, Yilmaz B, Sonmez S. Nasal bone measurement during the 1st trimester: is it useful?. *Gynecologic and Obstetric Investigation* 2004;**58**(2):91–5.
- Kellner 1995a** *{published data only}*  
Kellner LH, Weiner Z, Weiss RR, Neuer M, Martin GM, Mueenuddin M, et al. Triple marker (alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin) versus alpha-fetoprotein plus free-β subunit in second-trimester maternal serum screening for fetal Down syndrome: a prospective comparison study.[see comment]. *American Journal of Obstetrics and Gynecology* 1995;**173**(4):1306–9.
- Kellner 1995b** *{published data only}*  
Kellner LH, Weiss RR, Weiner Z, Neuer M, Martin GM, Schulman H, et al. The advantages of using triple-marker screening for chromosomal abnormalities. *American Journal of Obstetrics and Gynecology* 1995;**172**(3):831–6.
- Kellner 1997** *{published data only}*  
Kellner LH, Canick JA, Palomaki GE, Neveux LM, Saller DN Jr, Walker RP, et al. Levels of urinary β-core fragment, total oestriol, and the ratio of the two in second-trimester screening for Down syndrome. *Prenatal Diagnosis* 1997;**17**(12):1135–41.
- Kirkegaard 2008** *{published data only}*  
Kirkegaard I, Petersen OB, Uldbjerg N, Topping N. Improved performance of first-trimester combined screening for trisomy 21 with the double test taken before a gestational age of 10 weeks. *Prenatal Diagnosis* 2008;**28**(9):839–44.
- Kjaergaard 2008** *{published data only}*  
Kjaergaard S, Hahnemann JM, Skibsted L, Jensen LN, Sperling L, Zingenberg H, et al. [Prenatal diagnosis of chromosome aberrations after implementation of screening

- for Down's syndrome]. [Danish]. *Ugeskrift for Laeger* 2008;**170**(14):1152–6.
- Knight 1990** *{published data only}*  
Knight GJ, Palomaki GE. Maternal serum alpha fetoprotein screening for fetal down syndrome. *Journal of Clinical Immunoassay* 1990;**13**(1):23–9.
- Knight 2001** *{published data only}*  
Knight GJ, Palomaki GE, Neveux LM, Haddow JE, Lambert-Messerlian GM. Clinical validation of a new dimeric inhibin-A assay suitable for second trimester Down's syndrome screening. *Journal of Medical Screening* 2001;**8**(1):2–7.
- Knight 2005** *{published data only}*  
Knight GJ, Palomaki GE, Neveux LM, Smith DE, Kloza EM, Pulkkinen A, et al. Integrated serum screening for Down syndrome in primary obstetric practice. *Prenatal Diagnosis* 2005;**25**(12):1162–7.
- Koos 2006** *{published data only}*  
Koos BJ. First-trimester screening: Lessons from clinical trials and implementation. *Current Opinion in Obstetrics and Gynecology* 2006;**18**(2):152–5.
- Kornman 1996** *{published data only}*  
Kornman LH, Morssink LP, Beekhuis JR, de Wolf BT, Heringa MP, Mantingh A. Nuchal translucency cannot be used as a screening test for chromosomal abnormalities in the first trimester of pregnancy in a routine ultrasound practice.[see comment]. *Prenatal Diagnosis* 1996;**16**(9):797–805.
- Kornman 1997** *{published data only}*  
Kornman LH, Morssink LP, Wortelboer MJ, Beekhuis JR, de Wolf BT, Pratt JJ, et al. Maternal urinary  $\beta$ -core hCG in chromosomally abnormal pregnancies in the first trimester. *Prenatal Diagnosis* 1997;**17**(2):135–9.
- Kotaska 2007** *{published data only}*  
Kotaska A. Prenatal screening for fetal aneuploidy. *Journal of Obstetrics & Gynaecology Canada: JOGC* 2007;**29**(6):499–500.
- Kramer 1998** *{published data only}*  
Kramer RL, Yaron Y, O'Brien JE, Critchfield G, Ayoub M, Johnson MP, et al. Effect of adjustment of maternal serum alpha-fetoprotein levels in insulin-dependent diabetes mellitus. *American Journal of Medical Genetics* 1998;**75**(2):176–8.
- Krantz 1996** *{published data only}*  
Krantz DA, Larsen JW, Buchanan PD, Macri JN. First-trimester Down syndrome screening: free  $\beta$ -human chorionic gonadotropin and pregnancy-associated plasma protein A. *American Journal of Obstetrics and Gynecology* 1996;**174**(2):612–6.
- Krantz 2005** *{published data only}*  
Krantz DA, Hallahan TW, Macri VJ, Macri JN. Maternal weight and ethnic adjustment within a first-trimester Down syndrome and trisomy 18 screening program. *Prenatal Diagnosis* 2005;**25**(8):635–40.
- Krantz 2007** *{published data only}*  
Krantz DA, Hallahan TW, Macri VJ, Macri JN. Genetic sonography after first-trimester Down syndrome screening. *Ultrasound in Obstetrics & Gynecology* 2007;**29**(6):666–70.
- Kulch 1993** *{published data only}*  
Kulch P, Keener S, Matsumoto M, Crandall BF. Racial differences in maternal serum human chorionic gonadotropin and unconjugated oestriol levels. *Prenatal Diagnosis* 1993;**13**(3):191–5.
- Lai 1998** *{published data only}*  
Lai FM, Yeo GS. Down syndrome screening in Singapore—the effectiveness of a second trimester serum screening policy modelled on 29,360 pregnancies in KK Women's and Children's Hospital. *Singapore Medical Journal* 1998;**39**(2):69–75.
- Lai 2003** *{published data only}*  
Lai TH, Chen SC, Tsai MS, Lee FK, Wei CF. First-trimester screening for Down syndrome in singleton pregnancies achieved by intrauterine insemination. *Journal of Assisted Reproduction and Genetics* 2003;**20**(8):327–31.
- Laigaard 2006a** *{published data only}*  
Laigaard J, Cuckle H, Wewer UM, Christiansen M. Maternal serum ADAM12 levels in Down and Edwards' syndrome pregnancies at 9–12 weeks' gestation. *Prenatal Diagnosis* 2006;**26**(8):689–91.
- Laigaard 2006b** *{published data only}*  
Laigaard J, Spencer K, Christiansen M, Cowans NJ, Larsen SO, Pedersen BN, et al. ADAM 12 as a first-trimester maternal serum marker in screening for Down syndrome. *Prenatal Diagnosis* 2006;**26**(10):973–9.
- Lam 1997** *{published data only}*  
Lam YH, Tang MH, Tang LC, Lee CP, Ho PK. Second-trimester maternal urinary gonadotrophin peptide screening for fetal Down syndrome in Asian women. *Prenatal Diagnosis* 1997;**17**(12):1101–6.
- Lam 1998** *{published data only}*  
Lam YH, Ghosh A, Tang MH, Tang LC, Lee CP, Sin SY, et al. Second-trimester maternal serum alpha-fetoprotein and human chorionic gonadotrophin screening for Down's syndrome in Hong Kong. *Prenatal Diagnosis* 1998;**18**(6):585–9.
- Lam 1999a** *{published data only}*  
Lam YH, Yeung WS, Tang MH, Ng EH, So WW, Ho PC. Maternal serum alpha-fetoprotein and human chorionic gonadotrophin in pregnancies conceived after intracytoplasmic sperm injection and conventional in-vitro fertilization. *Human Reproduction* 1999;**14**(8):2120–3.
- Lam 1999b** *{published data only}*  
Lam YH, Tang MH. Second-trimester maternal serum inhibin-A screening for fetal Down syndrome in Asian women. *Prenatal Diagnosis* 1999;**19**(5):463–7.
- Lam 2000** *{published data only}*  
Lam YH, Tang MH, Lee CP, Sin SY, Tang R, Wong HS, et al. Acceptability of serum screening as an alternative to cytogenetic diagnosis of down syndrome among women 35

- years or older in Hong Kong. *Prenatal Diagnosis* 2000;**20**(6):487–90.
- Lam 2001** *{published data only}*  
Lam YH, Tang MH. The effect of fetal gender on second-trimester maternal serum inhibin-A concentration. *Prenatal Diagnosis* 2001;**21**(8):662–4.
- Lambert-Messerlian 1996** *{published data only}*  
Lambert-Messerlian GM, Canick JA, Palomaki GE, Schneyer AL. Second trimester levels of maternal serum inhibin A, total inhibin, alpha inhibin precursor, and activin in Down's syndrome pregnancy. *Journal of Medical Screening* 1996;**3**(2):58–62.
- Lambert-Messerlian 1998** *{published data only}*  
Lambert-Messerlian GM, Luisi S, Florio P, Mazza V, Canick JA, Petraglia F. Second trimester levels of maternal serum total activin A and placental inhibin/activin alpha and  $\beta$ A subunit messenger ribonucleic acids in Down syndrome pregnancy. *European Journal of Endocrinology* 1998;**138**(4):425–9.
- Lauria 2007** *{published data only}*  
Lauria MR, Branch MD, LaCroix VH, Harris RD, Baker ER. Clinical impact of systematic genetic sonogram screening in a low-risk population. *Journal of Reproductive Medicine* 2007;**52**(5):359–64.
- Lehavi 2005** *{published data only}*  
Lehavi O, Aizenstein O, Evans MI, Yaron Y. 2nd-trimester maternal serum human chorionic gonadotropin and alpha-fetoprotein levels in male and female fetuses with Down syndrome. *Fetal Diagnosis and Therapy* 2005;**20**(3):235–8.
- Leung 2006** *{published data only}*  
Leung TY, Spencer K, Leung TN, Fung TY, Lau TK. Higher median levels of free  $\beta$ -hCG and PAPP-A in the first trimester of pregnancy in a Chinese ethnic group. Implication for first trimester combined screening for Down's syndrome in the Chinese population. *Fetal Diagnosis and Therapy* 2006;**21**(1):140–3.
- Leymarie 1993** *{published data only}*  
Leymarie P, Leporrier N. Maternal serum markers and prenatal screening for Down syndrome. *Archives Francaises de Pediatrie* 1993;**50**(5):455–7.
- Li 1998** *{published data only}*  
Li G, Huang X. [Clinical uses of maternal serum markers in the prenatal diagnosis] [Chinese]. *Chung-Hua Fu Chan Ko Tsa Chih* 1998;**33**(4):252–4.
- Li 1999** *{published data only}*  
Li W, Zhou Y. [Measurement of pregnancy-associated plasma protein A in maternal peripheral blood and Down syndrome] [Chinese]. *Chung-Hua Fu Chan Ko Tsa Chih* 1999;**34**(10):631–3.
- Li 2010** *{published data only}*  
Li HW, Hui PW, Tang MH, Lau ET, Yeung WS, Ho PC, et al. Maternal serum anti-Mullerian hormone level is not superior to chronological age in predicting Down syndrome pregnancies. *Prenatal Diagnosis* 2010;**30**(4):320–4.
- Liao 1997** *{published data only}*  
Liao S, Wang Y, Ye G. [AFP, uE3,  $\beta$ -hCG levels applied for prenatal diagnosis of Down's syndrome]. [Chinese]. *Chung-Hua Fu Chan Ko Tsa Chih* 1997;**32**(11):655–8.
- Liao 2001** *{published data only}*  
Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Human Reproduction* 2001;**16**(7):1501–4.
- Lim 2002** *{published data only}*  
Lim KI, Pugash D, Dansereau J, Wilson RD. Nuchal index: a gestational age independent ultrasound marker for the detection of Down syndrome. *Prenatal Diagnosis* 2002;**22**(13):1233–7.
- Lippman 1987** *{published data only}*  
Lippman A, Evans JA. Screening for maternal serum alpha-fetoprotein: what about the low side?. *Canadian Medical Association Journal* 1987;**136**(8):801–4.
- Liu 2003** *{published data only}*  
Liu JT, Hao N, Sun NH, Wang FY, Xu YH, Gai MY, et al. [Screening by maternal serum markers for Down's syndrome]. [Chinese]. *Chung-Kuo i Hsueh Ko Hsueh Yuan Hsueh Pao Acta Academiae Medicinae Sinicae* 2003;**25**(2):156–9.
- Liu 2010** *{published data only}*  
Liu YH, Li LF, Wu YM. [Analysis of Down syndrome screening by maternal serum detection in mid-pregnancy]. [Chinese]. *Nan Fang Yi Ke Da Xue Xue Bao = Journal of Southern Medical University* 2010;**30**(3):532–4.
- Lo 2010** *{published data only}*  
Lo TK, Lai FK, Leung WC, Lau WL, Tang LC, Chin RK. A new policy for prenatal screening and diagnosis of Down syndrome for pregnant women with advanced maternal age in a public hospital. *Journal of Maternal-fetal & Neonatal Medicine* 2010;**23**(8):914–9.
- Lustig 1988** *{published data only}*  
Lustig L, Clarke S, Cunningham G, Schonberg R, Tompkinson G. California's experience with low MS-AFP results. *American Journal of Medical Genetics* 1988;**31**(1):211–22.
- Luthgens 2008** *{published data only}*  
Luthgens K. Comparison of the new PRC software with the established algorithm of the FMF UK for the detection of trisomy 21 and 18/13. *Fetal Diagnosis and Therapy* 2008;**24**(4):376–84.
- MacDonald 1991** *{published data only}*  
MacDonald ML, Wagner RM, Slotnick RN. Sensitivity and specificity of screening for Down syndrome with alpha-fetoprotein, hCG, unconjugated estriol, and maternal age.[see comment]. *Obstetrics and Gynecology* 1991;**77**(1):63–8.
- Macintosh 1994** *{published data only}*  
Macintosh MCM, Iles R, Teisner B, Sharma K, Chard T, Grudzinskas J, et al. Maternal serum human chorionic gonadotrophin and pregnancy-associated plasma protein A,

- markers for fetal Down syndrome at 8-14 weeks. *Prenatal Diagnosis* 1994;**14**(3):203–8.
- Macintosh 1997** *{published data only}*  
Macintosh MCM, Nicolaides KH, Noble P, Chard T, Gunn L, Iles R. Urinary  $\beta$ -core hCG: Screening for aneuploidies in early pregnancy (11-14 weeks' gestation). *Prenatal Diagnosis* 1997;**17**(5):401–5.
- MacRae 2010** *{published data only}*  
MacRae AR, Chodirker BN, Davies GA, Palomaki GE, Knight GJ, Minett J, et al. Second and first trimester estimation of risk for Down syndrome: implementation and performance in the SAFER study. *Prenatal Diagnosis* 2010;**30**(5):459–66.
- Macri 1994** *{published data only}*  
Macri JN, Kasturi RV, Krantz DA, Cook EJ, Moore ND, Young JA, et al. Maternal serum Down syndrome screening: free  $\beta$ -protein is a more effective marker than human chorionic gonadotropin.[see comment]. *American Journal of Obstetrics and Gynecology* 1990;**163**(4):1248–53.  
\* Macri JN, Spencer K, Garver K, Buchanan PD, Say B, Carpenter NJ, et al. Maternal serum free  $\beta$  hCG screening: results of studies including 480 cases of Down syndrome.[see comment]. *Prenatal Diagnosis* 1994;**14**(2):97–103.  
Spencer K, Macri JN. Early detection of Down's syndrome using free  $\beta$  human choriogonadotropin. *Annals of Clinical Biochemistry* 1992;**19**(3):349–50.
- Macri 1996** *{published data only}*  
Macri JN, Anderson RW, Krantz DA, Larsen JW, Buchanan PD. Prenatal maternal dried blood screening with alpha-fetoprotein and free  $\beta$ -human chorionic gonadotropin for open neural tube defect and Down syndrome. *American Journal of Obstetrics and Gynecology* 1996;**174**(2):566–72.
- Malone 1998** *{published data only}*  
Malone FD, D'Alton ME. Ultrasound clinics. Fetal nuchal fold translucency screening. *Contemporary OB/GYN* 1998;**43**(3):117–8.
- Malone 2003** *{published data only}*  
Malone FD, D'Alton ME. First-trimester sonographic screening for Down syndrome. *Obstetrics and Gynecology* 2003;**102**(5):1066–79.
- Mandryka-Stankewycz 2009** *{published data only}*  
Mandryka-Stankewycz S, Perenc M, Dec G, Sieroszewski P. [Noninvasive prenatal test in the first trimester of pregnancy (NT and estimation of beta-hCG and PAPP-A) in the diagnosis of fetal abnormalities in Polish population--comparison of the biochemistry own normal ranges and literature reported data]. [Polish]. *Ginekologia Polska* 2009;**80**(11):851–5.
- Mangione 2001** *{published data only}*  
Mangione R, Guyon F, Taine L, Wen ZQ, Roux D, Vergnaud A, et al. Pregnancy outcome and prognosis in fetuses with increased first-trimester nuchal translucency. *Fetal Diagnosis and Therapy* 2001;**16**(6):360–3.
- Markov 2008** *{published data only}*  
Markov D, Dimitrova V. [Ultrasound screening for chromosomal anomalies by assessment of the fetal nasal bone during 11-14 weeks of gestation--a pilot study]. [Bulgarian]. *Akusberstvo i Ginekologija* 2008;**47**(1):3–9.
- Maymon 2001a** *{published data only}*  
Maymon R, Shulman A. Comparison of triple serum screening and pregnancy outcome in oocyte donation versus IVF pregnancies. *Human Reproduction* 2001;**16**(4):691–5.
- Maymon 2001b** *{published data only}*  
Maymon R, Dreazen E, Buckovsky I, Weinraub Z, Herman A. Does a 'notched' nuchal translucency indicate Down syndrome fetuses or other adverse pregnancy outcome?. *Prenatal Diagnosis* 2001;**21**(5):403–8.
- Maymon 2002** *{published data only}*  
Maymon R, Shulman A. Serial first- and second-trimester Down's syndrome screening tests among IVF-versus naturally-conceived singletons. *Human Reproduction* 2002;**17**(4):1081–5.
- Maymon 2004** *{published data only}*  
Maymon R, Shulman A. Integrated first- and second-trimester Down syndrome screening test among unaffected IVF pregnancies. *Prenatal Diagnosis* 2004;**24**(2):125–9.
- Maymon 2005** *{published data only}*  
Maymon R, Cuckle H, Jones R, Reish O, Sharony R, Herman A. Predicting the result of additional second-trimester markers from a woman's first-trimester marker profile: a new concept in Down syndrome screening. *Prenatal Diagnosis* 2005;**25**(12):1102–6.
- McDuffie 1996** *{published data only}*  
McDuffie RS Jr, Haverkamp AD, Stark CF, Haverkamp C, Barth CK. Prenatal screening using maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol: two-year experience in a health maintenance organization. *Journal of Maternal-fetal Medicine* 1996;**5**(2):70–3.
- Meier 2002** *{published data only}*  
Meier C, Huang T, Wyatt PR, Summers AM. Accuracy of expected risk of Down syndrome using the second-trimester triple test. *Clinical Chemistry* 2002;**48**(4):653–5.
- Merkatz 1984** *{published data only}*  
Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. *American Journal of Obstetrics and Gynecology* 1984;**148**(7):886–94.
- Merz 2005** *{published data only}*  
Merz E. The fetal nasal bone in the first trimester - Precise assessment using 3D sonography. *Ultraschall in der Medizin* 2005;**26**(5):365–6.
- Merz 2008** *{published data only}*  
Merz E, Thode C, Alkier A, Eiben B, Hackeloer BJ, Hansmann M, et al. A new approach to calculating the risk of chromosomal abnormalities with first-trimester screening data. *Ultraschall in der Medizin* 2008;**29**(6):639–45.

- Metzenbauer 2001** *{published data only}*  
Metzenbauer M, Hafner E, Hoefinger D, Schuchter K, Stangl G, Ogris E, et al. Three-dimensional ultrasound measurement of the placental volume in early pregnancy: method and correlation with biochemical placenta parameters. *Placenta* 2001;**22**(6):602–5.
- Metzenbauer 2002** *{published data only}*  
Metzenbauer M, Hafner E, Schuchter K, Philipp K. First-trimester placental volume as a marker for chromosomal anomalies: preliminary results from an unselected population. *Ultrasound in Obstetrics & Gynecology* 2002;**19**(3):240–2.
- Mikic 1999** *{published data only}*  
Mikic TS, Johnson P. Second trimester maternal serum  $\beta$  human chorionic gonadotrophin and pregnancy outcome. *British Journal of Obstetrics and Gynaecology* 1999;**106**(6): 598–600.
- Miller 1991** *{published data only}*  
Miller CH, O'Brien TJ, Chatelain S, Butler BB, Quirk JG. Alteration in age-specific risks for chromosomal trisomy by maternal serum alpha-fetoprotein and human chorionic gonadotropin screening. *Prenatal Diagnosis* 1991;**11**(3): 153–8.
- Milunsky 1989** *{published data only}*  
Milunsky A, Jick SS, Bruell CL, Maclaughlin DS, Tsung Y-K, Jick H, et al. Predictive values relative risks and overall benefits of high and low maternal serum alpha fetoprotein screening in singleton pregnancies - new epidemiological data. *American Journal of Obstetrics and Gynecology* 1989; **161**(2):291–7.
- Milunsky 1996** *{published data only}*  
Milunsky A, Nebiolo L. Maternal serum triple analyte screening and adverse pregnancy outcome. *Fetal Diagnosis and Therapy* 1996;**11**(4):249–53.
- Minobe 2002** *{published data only}*  
Minobe S. [A study on the screening of prenatal trisomy 21 using the fucosylated alpha-fetoprotein ratio measured by a liquid-phase binding assay]. [Japanese]. *Hokkaido Igaku Zasshi - Hokkaido Journal of Medical Science* 2002;**77**(6): 527–32.
- Miron 2008** *{published data only}*  
Miron P, Cote YP, Lambert J. Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy. *Prenatal Diagnosis* 2008; **28**(3):180–5.
- Miron 2009** *{published data only}*  
Miron P, Cote YP, Lambert J. Nuchal translucency thresholds in prenatal screening for Down syndrome and trisomy 18. *Journal of Obstetrics & Gynaecology Canada: JOGC* 2009;**31**(3):227–35.
- Miron 2010** *{published data only}*  
Miron P, Lambert J, Marcil A, Cowans NJ, Stamatopoulou A, Spencer K. Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome. *Prenatal Diagnosis* 2010;**30**(3):224–8.
- Miyamura 1999** *{published data only}*  
Miyamura T, Saito N, Touno A, Nagata S, Hidaki T, Ishimaru T, et al. Multicenter study for maternal serum triple markers to establish Japanese standards: Maternal serum marker study group, Japan Association of Prenatal Diagnostics. *Acta Obstetrica et Gynaecologica Japonica* 1999; **51**(11):1042–8.
- Moghadam 1998** *{published data only}*  
Moghadam S, Engel W, Bougoussa M, Hennen G, Igout A, Sancken U. Maternal serum placental growth hormone and insulinlike growth factor binding proteins 1 and 3 in pregnancies affected by fetal aneuploidy and other abnormalities: implications for prenatal diagnosis of trisomy 21. *Fetal Diagnosis and Therapy* 1998;**13**(5):291–7.
- Monni 2000** *{published data only}*  
Monni G, Zoppi MA, Ibba RM, Putzolu M, Floris M. Nuchal translucency in multiple pregnancies. *Croatian Medical Journal* 2000;**41**(3):266–9.
- Monni 2002** *{published data only}*  
Monni G, Zoppi MA. New ultrasonographic markers of aneuploidies: nasal bones. *Ultrasound Review of Obstetrics and Gynecology* 2002;**2**(4):229–34.
- Mooney 1994** *{published data only}*  
Mooney RA, Peterson CJ, French CA, Saller DN Jr, Arvan DA. Effectiveness of combining maternal serum alpha-fetoprotein and hCG in a second-trimester screening program for Down syndrome. *Obstetrics and Gynecology* 1994;**84**(2):298–303.
- Muhcu 2008** *{published data only}*  
Muhcu M, Mungen E, Atay V, Ipcioglu OM, Dundar O, Ergur R, et al. First trimester screening for Down syndrome in rhesus negative women. *Prenatal Diagnosis* 2008;**28**(5): 404–7.
- Muller 1994** *{published data only}*  
Muller F, Bussieres L, Pelissier MC, Oury JF, Boue C, Uzan S, et al. Do racial differences exist in second-trimester maternal hCG levels? A study of 23,369 women. *Prenatal Diagnosis* 1994;**14**(7):633–6.
- Muller 1996** *{published data only}*  
Muller F, Dommergues M, Bussieres L, Aegerter P, Le Fiblec B, Uzan S, et al. Prenatal screening for Down syndrome: should first trimester ultrasound replace maternal serum screening?. *Early Human Development* 1996;**47** Suppl: S37–S39.
- Muller 1999** *{published data only}*  
Muller F, Ngo S, Rebiffe M, Oury JF, Uzan S, Satge D. Maternal serum s100b protein is ineffective for Down syndrome screening. *Prenatal Diagnosis* 1999;**19**(11):1086.
- Muller 2002a** *{published data only}*  
Muller F, Dreux S, Oury JF, Luton D, Uzan S, Uzan M, et al. Down syndrome maternal serum marker screening after 18 weeks' gestation. *Prenatal Diagnosis* 2002;**22**(11): 1001–4.
- Muller 2002b** *{published data only}*  
Muller F, Forestier F, Dineon B, for the ABA Study Group. Second trimester trisomy 21 maternal serum

- marker screening. Results of a countrywide study of 854, 902 women. *Prenatal Diagnosis* 2002;**22**(10):925–9.
- Muller 2003b** *{published data only}*  
Muller F, Dreux S, Lemeur A, Sault C, Desgres J, Bernard MA, et al. Medically assisted reproduction and second-trimester maternal serum marker screening for Down syndrome. *Prenatal Diagnosis* 2003;**23**(13):1073–6.
- Murta 2002** *{published data only}*  
Murta CG, Moron AF, Avila MA, Weiner CP. Application of ductus venosus Doppler velocimetry for the detection of fetal aneuploidy in the first trimester of pregnancy. *Fetal Diagnosis and Therapy* 2002;**17**(5):308–14.
- Musone 2000** *{published data only}*  
Musone R, Bonafiglia R, Menditto A, Paccone M, Cassese E, Russo G, et al. Fetuses with cystic hygroma. A retrospective study. *Panminerva Medica* 2000;**42**(1):39–43.
- Musto 1986** *{published data only}*  
Musto JD, Pizzolante JM, Chesaroni VP, Sassi AM, Sane R. Alpha-fetoprotein: an enhanced-sensitivity assay for neural tube defect and Down syndrome evaluation. *Clinical Chemistry* 1986;**32**(7):1412.
- Myrick 1990** *{published data only}*  
Myrick JE, Caudill SP, Hubert IL, Robinson MK, Adams MJ Jr, Pueschel SM. Identification of haptoglobin alpha-2FF variants in mid-trimester maternal serum as potential markers for Down syndrome. *Applied and Theoretical Electrophoresis* 1990;**1**(5):233–41.
- Naidoo 2008** *{published data only}*  
Naidoo P, Erasmus I, Jeeboddh J, Nicolaou E, van Gelderen CJ. Nuchal translucency as a method of first-trimester screening for aneuploidy. *South African Medical Journal* 2008;**Suid-Afrikaanse Tydskrif Vir Geneeskunde**. **98**(4):295–9.
- Nau 2009a** *{published data only}*  
Nau JY. [Screening for trisomy 21 in France]. [French]. *Revue Medicale Suisse* 2009;**5**(211):1531.
- Nau 2009b** *{published data only}*  
Nau JY. [Trisomy 21, after a half century]. [French]. *Revue Medicale Suisse* 2009;**5**(190):380.
- Neveux 1996a** *{published data only}*  
Neveux LM, Palomaki GE, Larrivee DA, Knight GJ, Haddow JE. Refinements in managing maternal weight adjustment for interpreting prenatal screening results. *Prenatal Diagnosis* 1996;**16**(12):1115–9.
- Neveux 1996b** *{published data only}*  
Neveux LM, Palomaki GE, Knight GJ, Haddow JE. Multiple marker screening for Down syndrome in twin pregnancies. *Prenatal Diagnosis* 1996;**16**(1):29–34.
- Ng 2004** *{published data only}*  
Ng EK, El-Sheikhah A, Chiu RW, Chan KC, Hogg M, Bindra R, et al. Evaluation of human chorionic gonadotropin  $\beta$ -subunit mRNA concentrations in maternal serum in aneuploid pregnancies: a feasibility study. *Clinical Chemistry* 2004;**50**(6):1055–7.
- Nicolaides 1992** *{published data only}*  
Nicolaidis KH, ZAR G, Snijders RJM, Gosden CM. Fetal nuchal oedema associated malformations and chromosomal defects. *Fetal Diagnosis and Therapy* 1992;**7**(2):123–31.
- Nicolaides 2000** *{published data only}*  
Nicolaidis KH, Cicero S, Liao AW. One-stop clinic for assessment of risk of chromosomal defects at 12 weeks of gestation. *Prenatal and Neonatal Medicine* 2000;**5**(3):145–54.
- Nicolaides 2004** *{published data only}*  
Nicolaidis KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *American Journal of Obstetrics and Gynecology* 2004;**191**(1):45–67.
- Nicolaides 2005a** *{published data only}*  
Nicolaidis KH, Wegrzyn P. [First trimester diagnosis of chromosomal defects][Polish]. *Ginekologia Polska* 2005;**76**(1):1–8.
- Nicolaides 2005b** *{published data only}*  
Nicolaidis KH, Wegrzyn P. [Sonographic features of chromosomal defects at 11(+0) to 13(+6) weeks of gestation][Polish]. *Ginekologia Polska* 2005;**76**(6):423–30.
- Nicolaides 2005c** *{published data only}*  
Nicolaidis KH, Wegrzyn P. [Increased nuchal translucency with normal karyotype]. [Polish]. *Ginekologia Polska* 2005;**76**(8):593–601.
- Nicolaides 2005d** *{published data only}*  
Nicolaidis KH, Wegrzyn P. [Fetal nuchal translucency]. [Polish]. *Ginekologia Polska* 2005;**76**(3):179–86.
- Nicolaides 2005e** *{published data only}*  
Nicolaidis KH, Wegrzyn P. [Fetal nuchal translucency thickness and risk for chromosomal defects]. [Polish]. *Ginekologia Polska* 2005;**76**(4):257–63.
- Nicolaides 2005f** *{published data only}*  
Nicolaidis Kypros H. First-trimester screening for chromosomal abnormalities. *Seminars in Perinatology (Philadelphia)* 2005;**29**(4):190–4.
- Niemimaa 2001b** *{published data only}*  
Niemimaa M, Heinonen S, Seppala M, Hippelainen M, Martikainen H, Ryyanen M. First-trimester screening for Down's syndrome in in vitro fertilization pregnancies. *Fertility and Sterility* 2001;**76**(6):1282–3.
- Niemimaa 2002** *{published data only}*  
Niemimaa M, Suonpaa M, Heinonen S, Seppala M, Bloigu R, Ryyanen M. Maternal serum human chorionic gonadotrophin and pregnancy-associated plasma protein A in twin pregnancies in the first trimester. *Prenatal Diagnosis* 2002;**22**(3):183–5.
- Niemimaa 2003** *{published data only}*  
Niemimaa M, Heinonen S, Seppala M, Ryyanen M. The influence of smoking on the pregnancy-associated plasma protein A, free  $\beta$  human chorionic gonadotrophin and nuchal translucency. *BJOG: an international journal of obstetrics and gynaecology* 2003;**110**(7):664–7.

- Noble 1997a** *{published data only}*  
Noble PL, Sniijders RJ, Abraha HD, Sherwood RA, Nicolaides KH. Maternal serum free  $\beta$ -hCG at 10 to 14 weeks of gestation in trisomic twin pregnancies. *British Journal of Obstetrics and Gynaecology* 1997;**104**(6):741–3.
- Norgaard 1990** *{published data only}*  
Norgaard Pedersen B, Larsen SO, Arends J, Svenstrup B, Tabor A. Maternal serum markers in screening for Down syndrome. *Clinical Genetics* 1990;**37**(1):35–43.
- Norton 1992** *{published data only}*  
Norton ME, Golbus MS. Maternal serum CA 125 for aneuploidy detection in early pregnancy. *Prenatal Diagnosis* 1992;**12**(9):779–81.
- Novakov-Mikic 2007** *{published data only}*  
Novakov-Mikic A, Potic Z, Pjevic A. [Ultrasound screening program for chromosomal abnormalities—the first 2000 women]. [Serbian]. *Medicinski Pregled* 2007;**60**(1-2):66–70.
- O'Brien 1997a** *{published data only}*  
O'Brien JE, Dvorin E, Yaron Y, Ayoub M, Johnson MP, Hume RF Jr, et al. Differential increases in AFP, hCG, and uE3 in twin pregnancies: Impact on attempts to quantify Down syndrome screening calculations. *American Journal of Medical Genetics* 1997;**73**(2):109–12.
- O'Brien 1997b** *{published data only}*  
O'Brien JE, Dvorin E, Drugan A, Johnson MP, Yaron Y, Evans MI. Race-ethnicity-specific variation in multiple-marker biochemical screening: Alpha-fetoprotein, hCG, and estriol. *Obstetrics and Gynecology* 1997;**89**(3):355–8.
- Odibo 2004** *{published data only}*  
Odibo AO, Sehdev HM, Dunn L, McDonald R, Macones GA. The association between fetal nasal bone hypoplasia and aneuploidy. *Obstetrics and Gynecology* 2004;**104**(6):1229–33.
- Odibo 2007** *{published data only}*  
Odibo AO, Sehdev HM, Stamilio DM, Cahill A, Dunn L, Macones GA. Defining nasal bone hypoplasia in second-trimester Down syndrome screening: does the use of multiples of the median improve screening efficacy?. *American Journal of Obstetrics and Gynecology* 2007;**197**(4):361–4.
- Odibo 2008** *{published data only}*  
Odibo AO, Sehdev HM, Gerkowicz S, Stamilio DM, Macones GA. Comparison of the efficiency of second-trimester nasal bone hypoplasia and increased nuchal fold in Down syndrome screening. *American Journal of Obstetrics and Gynecology* 2008;**199**(3):281–5.
- Odibo 2009** *{published data only}*  
Odibo AO, Schoenborn JA, Haas K, Macones GA. Does the combination of fronto-maxillary facial angle and nasal bone evaluation improve the detection of Down syndrome in the second trimester?. *Prenatal Diagnosis* 2009;**29**(10):947–51.
- Offerdal 2008** *{published data only}*  
Offerdal K, Blaas HG, Eik-Nes SH. Prenatal detection of trisomy 21 by second-trimester ultrasound examination and maternal age in a non-selected population of 49 314 births in Norway. *Ultrasound in Obstetrics & Gynecology* 2008;**32**(4):493–500.
- Ognibene 1999** *{published data only}*  
Ognibene A, Ciuti R, Tozzi P, Messeri G. Maternal serum superoxide dismutase (SOD): a possible marker for screening Down syndrome affected pregnancies.[see comment]. *Prenatal Diagnosis* 1999;**19**(11):1058–60.
- Oh 2007** *{published data only}*  
Oh C, Harman C, Baschat AA. Abnormal first-trimester ductus venosus blood flow: a risk factor for adverse outcome in fetuses with normal nuchal translucency. *Ultrasound in Obstetrics & Gynecology* 2007;**30**(2):192–6.
- Olajide 1989** *{published data only}*  
Olajide F, Kitau MJ, Chard T. Maternal serum AFP levels in the first trimester of pregnancy. *European Journal of Obstetrics, Gynecology, & Reproductive Biology* 1989;**30**(2):123–8.
- Onda 1996** *{published data only}*  
Onda T, Kitagawa M, Takeda O, Sago H, Kubonoya K, Iinuma K, et al. Triple marker screening in native Japanese women. *Prenatal Diagnosis* 1996;**16**(8):713–7.
- Onda 1998** *{published data only}*  
Onda T, Tanaka T, Takeda O, Kitagawa M, Kuwabara Y, Yamamoto H, et al. Agreement between predicted risk and prevalence of Down syndrome in second-trimester triple-marker screening in Japan. *Prenatal Diagnosis* 1998;**18**(9):956–8.
- Onda 2000** *{published data only}*  
Onda T, Tanaka T, Yoshida K, Nakamura Y, Kudo R, Yamamoto H, et al. Triple marker screening for trisomy 21, trisomy 18 and open neural tube defects in singleton pregnancies of native Japanese pregnant women. *Journal of Obstetrics and Gynaecology Research* 2000;**26**(6):441–7.
- Orlandi 2002** *{published data only}*  
Orlandi F, Rossi C, Allegra A, Krantz D, Hallahan T, Orlandi E, et al. First trimester screening with free  $\beta$ -hCG, PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction. *Prenatal Diagnosis* 2002;**22**(8):718–21.
- Ozkaya 2010** *{published data only}*  
Ozkaya O, Sezik M, Ozbasar D, Kaya H. Abnormal ductus venosus flow and tricuspid regurgitation at 11-14 weeks' gestation have high positive predictive values for increased risk in first-trimester combined screening test: results of a pilot study. *Taiwanese Journal of Obstetrics & Gynecology* 2010;**49**(2):145–50.
- Páez 2004** *{published data only}*  
Páez L, Peña E, González F, Bello F, Bellorín J, Espinoza F, et al. Plasma protein "A" and chorionic gonadotropin at first trimester pregnancy. *Informe Medico* 2004;**6**(2):99–109.
- Paladini 2007** *{published data only}*  
Paladini D, Sglavo G, Penner I, Pastore G, Nappi C. Fetuses with Down syndrome have an enlarged anterior fontanelle in the second trimester of pregnancy. *Ultrasound in Obstetrics & Gynecology* 2007;**30**(6):824–9.



- Palka 1998** *{published data only}*  
Palka G, Guanciali Franchi P, Papponetti M, Marcuccitti J, Morizio E, Calabrese G, et al. Prenatal diagnosis using the triple test. *Minerva Ginecologica* 1998;**50**(10):411–5.
- Palomaki 1989** *{published data only}*  
Palomaki GE, Williams J, Haddow JE. Combining maternal serum alpha-fetoprotein measurements and age to screen for Down syndrome in pregnant women under age 35. *American Journal of Obstetrics and Gynecology* 1989;**160**(3): 575–81.
- Palomaki 1993** *{published data only}*  
Palomaki GE, Knight GJ, Haddow JE, Canick JA, Wald NJ, Kennard A. Cigarette smoking and levels of maternal serum alpha-fetoprotein, unconjugated estriol, and hCG: Impact on Down syndrome screening. *Obstetrics and Gynecology* 1993;**81**(5):675–8.
- Palomaki 1994** *{published data only}*  
Palomaki GE, Knight GJ, Haddow JE. Human chorionic gonadotropin and unconjugated oestriol measurements in insulin-dependent diabetic pregnant women being screened for fetal Down syndrome. *Prenatal Diagnosis* 1994;**14**(1): 65–8.
- Palomaki 1996** *{published data only}*  
Palomaki GE, Neveux LM, Haddow JE. Can reliable Down's syndrome detection rates be determined from prenatal screening intervention trials?. *Journal of Medical Screening* 1996;**3**(1):12–7.
- Palomaki 2005** *{published data only}*  
Palomaki GE, Knight GJ, Neveux LM, Pandian R, Haddow JE. Maternal serum invasive trophoblast antigen and first-trimester Down syndrome screening. *Clinical Chemistry* 2005;**51**(8):1499–504.
- Panburana 2001** *{published data only}*  
Panburana P, Ajjimakorn S, Tungkajiwangoon P. First trimester Down Syndrome screening by nuchal translucency in a Thai population. *International Journal of Gynaecology and Obstetrics* 2001;**75**(3):311–2.
- Pandya 1994** *{published data only}*  
Pandya PP, Brizot ML, Kuhn P, Snijders RJ, Nicolaides KH. First-trimester fetal nuchal translucency thickness and risk for trisomies. *Obstetrics and Gynecology* 1994;**84**(3):420–3.
- Pandya 1995** *{published data only}*  
Pandya PP, Santiago C, Snijders RJM, Nicolaides KH. First trimester fetal nuchal translucency. *Current Opinion in Obstetrics and Gynecology* 1995;**7**(2):95–102.
- Papadopoulou 2008** *{published data only}*  
Papadopoulou E, Sifakis S, Giahnakis E, Fragouli Y, Karkavitsas N, Koumantakis E, et al. Human placental growth hormone is increased in maternal serum in pregnancies affected by Down syndrome. *Fetal Diagnosis and Therapy* 2008;**23**(3):211–6.
- Parra-Cordero 2007** *{published data only}*  
Parra-Cordero M, Quiroz L, Rencoret G, Pedraza D, Munoz H, Soto-Chacon E, et al. Screening for trisomy 21 during the routine second-trimester ultrasound examination in an unselected Chilean population. *Ultrasound in Obstetrics & Gynecology* 2007;**30**(7):946–51.
- Paterlini-Brechot 2007** *{published data only}*  
Paterlini-Brechot P. [Non invasive prenatal diagnosis of trisomy 21: dream or reality?]. [French]. *Medecine Sciences : M/S* 2007;**23**(6-7):592–4.
- Paul 2001** *{published data only}*  
Paul C, Krampl E, Skentou C, Jurkovic D, Nicolaides KH. Measurement of fetal nuchal translucency thickness by three-dimensional ultrasound. *Ultrasound in Obstetrics & Gynecology* 2001;**18**(5):481–4.
- Peralta 2005** *{published data only}*  
Peralta CF, Falcon O, Wegrzyn P, Faro C, Nicolaides KH. Assessment of the gap between the fetal nasal bones at 11 to 13 + 6 weeks of gestation by three-dimensional ultrasound. *Ultrasound in Obstetrics & Gynecology* 2005;**25**(5):464–7.
- Perenc 1998** *{published data only}*  
Perenc M, Dudarewicz L, Kaluzewski B. Analysis of triple test results in 27 cases of twin pregnancies. *Acta Geneticae Medicae et Gemellologiae* 1998;**47**(3-4):249–54.
- Perheentupa 2002** *{published data only}*  
Perheentupa A, Ruokonen A, Tuomivaara L, Ryyänen M, Martikainen H. Maternal serum (β)-HCG and (alpha)-fetoprotein concentrations in singleton pregnancies following assisted reproduction. *Human Reproduction* 2002;**17**(3):794–7.
- Perona 1998** *{published data only}*  
Perona M, Mancini G, Dall'Amico D, Guaraldo V, Carbonara A. Influence of smoking habits on Down's syndrome risk evaluation at mid-trimester through biochemical screening. *International Journal of Clinical & Laboratory Research* 1998;**28**(3):179–82.
- Persico 2008** *{published data only}*  
Persico N, Borenstein M, Molina F, Azumendi G, Nicolaides KH. Prenatal thickness in trisomy-21 fetuses at 16-24 weeks of gestation. *Ultrasound in Obstetrics & Gynecology* 2008;**32**(6):751–4.
- Petervari 2000** *{published data only}*  
Petervari L, Varga A, Tanko A, Szabo L, Godo G. [Significance of nuchal edema in fetuses of pregnant women under 35 years of age]. [Hungarian]. *Orvosi Hetilap* 2000;**141**(8):399–402.
- Petrocik 1989** *{published data only}*  
Petrocik E, Wassman ER, Kelly JC. Prenatal screening for Down syndrome with maternal serum human chorionic gonadotropin levels.[see comment]. *American Journal of Obstetrics and Gynecology* 1989;**161**(5):1168–73.
- Phillips 1992** *{published data only}*  
Phillips OP, Elias S, Shulman LP, Andersen RN, Morgan CD, Simpson JL. Maternal serum screening for fetal Down syndrome in women less than 35 years of age using alpha-fetoprotein, hCG, and unconjugated estriol: a prospective 2-year study. *Obstetrics and Gynecology* 1992;**80**(3):353–8.

- Phillips 1993** *{published data only}*  
Phillips OP, Shulman LP, Elias S, Simpson JL. Maternal serum screening for fetal Down syndrome using alpha-fetoprotein, human chorionic gonadotrophin, and unconjugated estriol in adolescents. *Adolescent and Pediatric Gynecology* 1993;**6**(2):91–4.
- Pihl 2008** *{published data only}*  
Pihl K, Larsen T, Jonsson L, Hougaard D, Krebs L, Norgaard-Pedersen B, et al. [Quality control of prenatal screening]. [Danish]. *Ugeskrift for Læger* 2008;**170**(35):2691–5.
- Pinette 2003** *{published data only}*  
Pinette MG, Egan JF, Wax JR, Blackstone J, Cartin A, Benn PA. Combined sonographic and biochemical markers for Down syndrome screening. *Journal of Ultrasound in Medicine* 2003;**22**(11):1185–90.
- Platt 2004** *{published data only}*  
Platt LD, Greene N, Johnson A, Zachary J, Thom E, Krantz D, et al. Sequential pathways of testing after first-trimester screening for trisomy 21. *Obstetrics and Gynecology* 2004;**104**(4):661–6.
- Podobnik 1995** *{published data only}*  
Podobnik M, Singer Z, Podobnik-Sarkanji S, Bulic M. First trimester diagnosis of cystic hygromata using transvaginal ultrasound and cytogenetic evaluation. *Journal of Perinatal Medicine* 1995;**23**(4):283–91.
- Poon 2009** *{published data only}*  
Poon LC, Chelemen T, Minekawa R, Frisova V, Nicolaides KH. Maternal serum ADAM12 (A disintegrin and metalloprotease) in chromosomally abnormal pregnancy at 11–13 weeks. *American Journal of Obstetrics and Gynecology* 2009;**200**(5):508–6.
- Prefumo 2002** *{published data only}*  
Prefumo F, Thilaganathan B. Agreement between predicted risk and prevalence of Down syndrome in first trimester nuchal translucency screening. *Prenatal Diagnosis* 2002;**22**(10):917–8.
- Prefumo 2004** *{published data only}*  
Prefumo F, Sairam S, Bhide A, Penna L, Hollis B, Thilaganathan B. Maternal ethnic origin and fetal nasal bones at 11–14 weeks of gestation. *BJOG: an international journal of obstetrics and gynaecology*. 2004;**111**(2):109–12.
- Price 1998** *{published data only}*  
Price KM, Van Lith JM, Silman R, Mantingh A, Grudzinskas JG. First trimester maternal serum concentrations of fetal antigen 2 in normal pregnancies and those affected by trisomy 21. *Human Reproduction* 1998;**13**(6):1706–8.
- Raty 2000** *{published data only}*  
Raty R, Virtanen A, Koskinen P, Laitinen P, Forsstrom J, Salonen R, et al. Maternal midtrimester serum AFP and free  $\beta$ -hCG levels in in vitro fertilization twin pregnancies. *Prenatal Diagnosis* 2000;**20**(3):221–3.
- Rätty 2002** *{published data only}*  
Rätty R, Virtanen A, Koskinen P, Anttila L, Forsström J, Laitinen P, et al. Serum free ( $\beta$ )-HCG and alpha-fetoprotein levels in IVF, ICSI and frozen embryo transfer pregnancies in maternal mid-trimester serum screening for Down's syndrome. *Human Reproduction* 2002;**17**(2):481–4.
- Rembouskos 2004** *{published data only}*  
Rembouskos G, Cicero S, Longo D, Vandecruys H, Nicolaides KH. Assessment of the fetal nasal bone at 11–14 weeks of gestation by three-dimensional ultrasound. *Ultrasound in Obstetrics & Gynecology* 2004;**23**(3):232–6.
- Ren 1992** *{published data only}*  
Ren S-G, Braunstein GD. Human chorionic gonadotropin. *Seminars in Reproductive Endocrinology* 1992;**10**(2):95–105.
- Renier 1998** *{published data only}*  
Renier MA, Vereecken A, Van Herck E, Straetmans D, Ramaekers P, Buytaert P. Second trimester maternal dimeric inhibin-A in the multiple-marker screening test for Down's syndrome. *Human Reproduction* 1998;**13**(3):744–8.
- Resta 1990** *{published data only}*  
Resta RG, Nyberg D. The role of ultrasound in screening for Down syndrome. *Birth Defects: Original Article Series* 1990;**26**(3):104.
- Reynders 1997** *{published data only}*  
Reynders CS, Pauker SP, Benacerraf BR. First trimester isolated fetal nuchal lucency: significance and outcome. *Journal of Ultrasound in Medicine* 1997;**16**(2):101–5.
- Reynolds 1989** *{published data only}*  
Reynolds TM, Penney MD. The mathematical basis of multivariate risk screening: with special reference to screening for Down's syndrome associated pregnancy. *Annals of Clinical Biochemistry* 1989;**27**(5):452–8.
- Reynolds 1999** *{published data only}*  
Reynolds TM, Schaeffer HJ, Schlensker S. Estimation of Down's syndrome risks in the first trimester of pregnancy: Experience of testing with PAPP-A, total hCG and free  $\beta$ -hCG levels in maternal blood samples in a German population. *Clinical Laboratory* 1999;**45**(1-2):49–53.
- Reynolds 2008** *{published data only}*  
Reynolds TM, Aldis J. Median parameters for Down's syndrome screening should be calculated using a moving time-window method. *Annals of Clinical Biochemistry* 2008;**45**(Pt 6):567–70.
- Ribbert 1996** *{published data only}*  
Ribbert LS, Kornman LH, de Wolf BT, Simons AH, Jansen CA, Beekhuis JR, et al. Maternal serum screening for fetal Down syndrome in IVF pregnancies. *Prenatal Diagnosis* 1996;**16**(1):35–8.
- Rice 2005** *{published data only}*  
Rice JD, McIntosh SF, Halstead AC. Second-trimester maternal serum screening for Down syndrome in in vitro fertilization pregnancies. *Prenatal Diagnosis* 2005;**25**(3):234–8.
- Rich 1991** *{published data only}*  
Rich N, Boots L, Davis R, Finley S. Efficiency of maternal serum hCG AFP and free estriol in the identification of trisomy 21 and other complications of pregnancy. *Journal of the Alabama Academy of Science* 1991;**62**(2-3):135.

- Roberts 1995** *{published data only}*  
Roberts LJ, Bewley S, Mackinson AM, Rodeck CH. First trimester fetal nuchal translucency: problems with screening the general population. 1. *British Journal of Obstetrics and Gynaecology* 1995;**102**(5):381–5.
- Robertson 1991** *{published data only}*  
Robertson EF. Maternal serum screening for neural tube defects and Down's syndrome.[see comment]. *Medical Journal of Australia* 1991;**155**(2):67–8.
- Rode 2003** *{published data only}*  
Rode L, Wojdemann KR, Shalmi AC, Larsen SO, Sundberg K, Norgaard-Pedersen B, et al. Combined first- and second-trimester screening for Down syndrome: an evaluation of proMBP as a marker. *Prenatal Diagnosis* 2003;**23**(7):593–8.
- Ronge 2006** *{published data only}*  
Ronge R. Combined first trimester screening for Down's syndrome is superior to quadruple test. *Geburtshilfe und Frauenheilkunde* 2006;**66**(4):332.
- Rose 1995** *{published data only}*  
Rose NC, Mennuti MT. Multiple marker screening for women 35 and older. *Contemporary OB/GYN* 1995;**40**(9):55–6.
- Ross 1997** *{published data only}*  
Ross HL, Elias S. Maternal serum screening for fetal genetic disorders. *Obstetrics & Gynecology Clinics of North America* 1997;**24**(1):33–47.
- Rotmensch 1996** *{published data only}*  
Rotmensch S, Liberati M, Kardana A, Copel JA, Ben-Rafael Z, Cole LA. Nicked free  $\beta$ -subunit of human chorionic gonadotropin: A potential new marker for Down syndrome screening. *American Journal of Obstetrics and Gynecology* 1996;**174**(2):609–11.
- Rotmensch 1999** *{published data only}*  
Rotmensch S, Celentano C, Shalev J, Vishne TH, Lipitz S, Ben-Rafael Z, et al. Midtrimester maternal serum screening after multifetal pregnancy reduction in pregnancies conceived by in vitro fertilization. *Journal of Assisted Reproduction and Genetics* 1999;**16**(1):8–12.
- Rozenberg 2006** *{published data only}*  
Rozenberg P, Bussieres L, Chevret S, Bernard JP, Malagrida L, Cuckle H, et al. Screening for Down syndrome using first-trimester combined screening followed by second-trimester ultrasound examination in an unselected population. *American Journal of Obstetrics and Gynecology* 2006;**195**(5):1379–87.
- Rudnicka 2002** *{published data only}*  
Rudnicka AR, Wald NJ, Huttly W, Hackshaw AK. Influence of maternal smoking on the birth prevalence of Down syndrome and on second trimester screening performance. *Prenatal Diagnosis* 2002;**22**(10):893–7.
- Ryall 1992** *{published data only}*  
Ryall RG, Staples AJ, Robertson EF, Pollard AC. Improved performance in a prenatal screening programme for Down's syndrome incorporating serum-free hCG subunit analyses. *Prenatal Diagnosis* 1992;**12**(4):251–61.
- Ryall 2001** *{published data only}*  
Ryall RG, Callen D, Cocciolone R, Duvnjak A, Esca R, Frantzis N, et al. Karyotypes found in the population declared at increased risk of Down syndrome following maternal serum screening. *Prenatal Diagnosis* 2001;**21**(7):553–7.
- Sabriá 2002** *{published data only}*  
Sabriá J, Cabrero D, Bach C. Aneuploidy screening: ultrasound versus biochemistry. *Ultrasound Review of Obstetrics and Gynecology* 2002;**2**(4):221–8.
- Sacchini 2003** *{published data only}*  
Sacchini C, El-Sheikhah A, Cicero S, Rembouskos G, Nicolaides KH. Ear length in trisomy 21 fetuses at 11-14 weeks of gestation. *Ultrasound in Obstetrics & Gynecology* 2003;**22**(5):460–3.
- Sahota 2009** *{published data only}*  
Sahota DS, Leung TY, Chan LW, Law LW, Fung TY, Chan OK, et al. First-trimester fetal nasal bone length in an ethnic Chinese population. *Ultrasound in Obstetrics & Gynecology* 2009;**34**(1):33–7.
- Sahota 2010a** *{published data only}*  
Sahota DS, Leung TY, Chen M, Chan LW, Fung TY, Lau TK. Comparison of likelihood ratios of first-trimester nuchal translucency measurements: multiples of median, delta or mixture. *Ultrasound in Obstetrics & Gynecology* 2010;**36**(1):15–9.
- Salazar 2007** *{published data only}*  
Salazar López R, Ibarra Gallardo AL, Iduma Meléndrez M, Leyva Bojórquez R. [Specificity of biochemical markers of pregnancy second trimester]. [Spanish]. *Ginecología y Obstetricia de Mexico* 2007;**75**(10):608–14.
- Salazar 2008** *{published data only}*  
Salazar López R, Ibarra Gallardo AL, Iduma Meléndrez M, Leyva R. [Evaluation of plasmatic A protein as only marker during first trimester of pregnancy]. [Spanish]. *Ginecología y Obstetricia de Mexico* 2008;**76**(10):576–81.
- Saller 1997** *{published data only}*  
Saller DN Jr, Canick JA, Kellner LH, Rose NC, Garza J, French CA, et al. Maternal serum analyte levels in pregnancies with fetal Down syndrome resulting from translocations. *American Journal of Obstetrics and Gynecology* 1997;**177**(4):879–81.
- Salomon 2001** *{published data only}*  
Salomon LJ, Bernard JP, Taupin P, Benard C, Ville Y. Relationship between nuchal translucency at 11-14 weeks and nuchal fold at 20-24 weeks of gestation. *Ultrasound in Obstetrics & Gynecology* 2001;**18**(6):636–7.
- Salonen 1997** *{published data only}*  
Salonen R, Turpeinen U, Kurki L, Lappalainen M, Ammala P, Hiilesmaa V, et al. Maternal serum screening for Down's syndrome on population basis. *Acta Obstetrica et Gynecologica Scandinavica* 1997;**76**(9):817–21.
- Saltvedt 2005** *{published data only}*  
Saltvedt S, Almstrom H, Kublickas M, Valentin L, Bottinga R, Bui TH, et al. Screening for Down syndrome based on

- maternal age or fetal nuchal translucency: a randomized controlled trial in 39,572 pregnancies. *Ultrasound in Obstetrics & Gynecology* 2005;**25**(6):537–45.
- Saridogan 1996** *{published data only}*  
Saridogan E, Djahanbakhch O, Naftalin AA. Screening for Down's syndrome: experience in an inner city health district. *British Journal of Obstetrics and Gynaecology* 1996;**103**(12):1205–11.
- Savoldelli 1993** *{published data only}*  
Savoldelli G, Binkert F, Achermann J, Schmid W. Ultrasound screening for chromosomal anomalies in the first trimester of pregnancy. *Prenatal Diagnosis* 1993;**13**(6): 513–8.
- Schielen 2009** *{published data only}*  
Schielen PC, Wildschut HI, Loeber JG. Down syndrome screening: determining the cutoff level of risk for invasive testing. *Prenatal Diagnosis* 2009;**29**(2):190–2.
- Schiott 2006** *{published data only}*  
Schiott KM, Christiansen M, Petersen OB, Sorensen TL, Uldbjerg N. The "Consecutive Combined Test"--using double test from week 8 + 0 and nuchal translucency scan, for first trimester screening for Down syndrome. *Prenatal Diagnosis* 2006;**26**(12):1105–9.
- Schmidt 2007a** *{published data only}*  
Schmidt P, Rom J, Maul H, Vaske B, Hillemanns P, Scharf A. Advanced first trimester screening (AFS): an improved test strategy for the individual risk assessment of fetal aneuploidies and malformations. *Archives of Gynecology and Obstetrics* 2007;**276**(2):159–66.
- Schmidt 2007b** *{published data only}*  
Schmidt P, Staboulidou I, Soergel P, Wustemann M, Hillemanns P, Scharf A. Comparison of Nicolaides' risk evaluation for Down's syndrome with a novel software: an analysis of 1,463 cases. *Archives of Gynecology and Obstetrics* 2007;**275**(6):469–74.
- Schmidt 2007c** *{published data only}*  
Schmidt P, Pruggmayer M, Steinborn A, Schippert C, Staboulidou I, Hillemanns P, et al. Are nuchal translucency, pregnancy associated plasma protein-A or free-beta-human chorionic gonadotropin depending on maternal age? A multicenter study of 8,116 pregnancies. *Archives of Gynecology and Obstetrics* 2007;**276**(3):259–62.
- Schmidt 2008a** *{published data only}*  
Schmidt P, Hormansdorfer C, Pruggmayer M, Schutte C, Neumann A, Gerritzen A, et al. Improved prenatal aneuploidy screening using the novel advanced first-trimester screening algorithm: a multicenter study of 10, 017 pregnancies. *Journal of Clinical Ultrasound* 2008;**36**(7): 397–402.
- Schmidt 2008b** *{published data only}*  
Schmidt P, Staboulidou I, Elsasser M, Vaske B, Hillemanns P, Scharf A. How imprecise may the measurement of fetal nuchal translucency be without worsening first-trimester screening?. *Fetal Diagnosis and Therapy* 2008;**24**(3):291–5.
- Schmidt 2008c** *{published data only}*  
Schmidt P, Hormansdorfer C, Oehler K, Hartel H, Hillemanns P, Scharf A. [Three-dimensional scatter plot analysis to estimate the risk of foetal aneuloidy]. [German]. *Zeitschrift für Geburtshilfe und Neonatologie* 2008;**212**(4): 127–35.
- Schmidt 2010** *{published data only}*  
Schmidt P, Hormansdorfer C, Golatta M, Scharf A. Analysis of the distribution shift of detected aneuploidies by age independent first trimester screening. *Archives of Gynecology and Obstetrics* 2010;**281**(3):393–9.
- Schuchter 1998** *{published data only}*  
Schuchter K, Wald N, Hackshaw AK, Hafner E, Liebhart E. The distribution of nuchal translucency at 10-13 weeks of pregnancy. *Prenatal Diagnosis* 1998;**18**(3):281–6.
- Scott 1995** *{published data only}*  
Scott F, Boogert A, Smart S, Anderson J. Maternal serum screening and routine 18-week ultrasound in the detection of all chromosomal abnormalities. *Australian & New Zealand Journal of Obstetrics & Gynaecology* 1995;**35**(2): 165–8.
- Seeds 1990** *{published data only}*  
Seeds JW, Watson WJ. Ultrasound and maternal serum alpha-fetoprotein screening: a complementary relationship. *Ultrasound Quarterly* 1990;**8**(2):145–66.
- Seki 1995** *{published data only}*  
Seki K, Mitsui C, Nagata I. Measurement of urinary free  $\beta$ -human chorionic gonadotropin by immunoradiometric assay. *Gynecologic and Obstetric Investigation* 1995;**40**(3): 162–7.
- Shenhav 2003** *{published data only}*  
Shenhav S, Gemer O, Sherman DJ, Peled R, Segal S. Midtrimester triple-test levels in women with chronic hypertension and altered renal function. *Prenatal Diagnosis* 2003;**23**(2):166–7.
- Shintaku 1989** *{published data only}*  
Shintaku Y, Takabayashi T, Sasaki H, Ozawa N, Shinkawa O, Hamazaki Y, et al. [Screening for chromosomal anomalies with maternal serum alpha-fetoprotein]. [Japanese]. *Nippon Sanka Fujinka Gakkai Zasshi - Acta Obstetrica et Gynaecologica Japonica* 1989;**41**(2):185–90.
- Shulman 2003** *{published data only}*  
Shulman A, Maymon R. Mid-gestation Down syndrome screening test and pregnancy outcome among unstimulated assisted-conception pregnancies. *Prenatal Diagnosis* 2003;**23**(8):625–8.
- Sieroszewski 2008** *{published data only}*  
Sieroszewski P, Perenc M, Budecka EB, Sobala W, Deutinger J. Sonographical integrated test for detection of chromosomal aberrations. *Ultraschall in der Medizin* 2008;**29**(2):190–6.
- Simon-Bouy 1999** *{published data only}*  
Simon-Bouy B. [Markers for trisomy 21][French]. *Fertilité Contraception Sexualité* 1999;**27**(9):289–91.

- Simpson 1986** *{published data only}*  
Simpson JL, Baum LD, Marder R, Elias S, Ober C, Martin AO. Maternal serum alpha-fetoprotein screening: low and high values for detection of genetic abnormalities. *American Journal of Obstetrics and Gynecology* 1986;**155**(3):593–7.
- Smith 1990** *{published data only}*  
Smith C, Grube GL, Wilson S. Maternal serum alpha-fetoprotein screening and the role of ultrasound. *Journal of Diagnostic Medical Sonography* 1990;**6**(6):312–6.
- Smith 1996** *{published data only}*  
Smith ER, Petersen J, Okorodudu AO, Bissell MG. Does the addition of unconjugated estriol in maternal serum screening improve the detection of trisomy 21? A meta-analysis. *Clinical Laboratory Management Review* 1996;**10**(2):176–81.
- Smith 1999** *{published data only}*  
Smith NC, Hau C. A six year study of the antenatal detection of fetal abnormality in six Scottish health boards. *British Journal of Obstetrics and Gynaecology* 1999;**106**(3):206–12.
- Smith-Bindman 2001** *{published data only}*  
Smith-Bindman R, Hosmer W, Feldstein VA, Deeks JJ, Goldberg JD. Second-trimester ultrasound to detect fetuses with Down syndrome: a meta-analysis.[see comment]. *JAMA* 2001;**285**(8):1044–55.
- Smith-Bindman 2003** *{published data only}*  
Smith-Bindman R, Chu P, Bacchetti P, Waters JJ, Mutton D, Alberman E. Prenatal screening for Down syndrome in England and Wales and population-based birth outcomes. *American Journal of Obstetrics and Gynecology* 2003;**187**(4):980–5.
- Snijders 1995** *{published data only}*  
Snijders RJM, Sebire NJ, Nicolaides KH. Maternal age and gestational age-specific risk for chromosomal defects. *Fetal Diagnosis and Therapy* 1995;**10**(6):356–67.
- Snijders 1999** *{published data only}*  
Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. *Ultrasound in Obstetrics and Gynecology* 1999;**13**(3):167–70.
- Soergel 2006** *{published data only}*  
Soergel P, Pruggmayer M, Schwerdtfeger R, Muhlhaus K, Scharf A. Screening for trisomy 21 with maternal age, fetal nuchal translucency and maternal serum biochemistry at 11–14 weeks: a regional experience from Germany. *Fetal Diagnosis and Therapy* 2006;**21**(3):264–8.
- Sokol 1998** *{published data only}*  
Sokol AL, Kramer RL, Yaron Y, O'Brien JE, Muller F, Johnson MP, et al. Age-specific variation in aneuploidy incidence among biochemical screening programs. *American Journal of Obstetrics and Gynecology* 1998;**179**(4):971–3.
- Sonek 2003** *{published data only}*  
Sonek JD. Nasal bone evaluation with ultrasonography: a marker for fetal aneuploidy. *Ultrasound in Obstetrics and Gynecology* 2003;**22**(1):11–5.
- Sonek 2007** *{published data only}*  
Sonek J, Borenstein M, Downing C, McKenna D, Neiger R, Croom C, et al. Frontomaxillary facial angles in screening for trisomy 21 at 14–23 weeks' gestation. *American Journal of Obstetrics and Gynecology* 2007;**197**(2):160–5.
- Sood 2010** *{published data only}*  
Sood M, Rochelson B, Krantz D, Ravens R, Tam Tam H, Vohra N, et al. Are second-trimester minor sonographic markers for Down syndrome useful in patients who have undergone first-trimester combined screening?. *American Journal of Obstetrics and Gynecology* 2010;**203**(4):408–4.
- Sooklim 2010** *{published data only}*  
Sooklim R, Manotaya S. Fetal facial sonographic markers for second trimester Down syndrome screening in a Thai population. *International Journal of Gynaecology and Obstetrics* 2010;**111**(2):144–7.
- Spencer 1985** *{published data only}*  
Spencer K, Carpenter P. Screening for Down's syndrome using serum alpha fetoprotein: a retrospective study indicating caution. *BMJ (Clinical Research Ed.)* 1985;**290**(6486):1940–3.
- Spencer 1991a** *{published data only}*  
Spencer K. Evaluation of an assay of the free  $\beta$ -subunit of choriogonadotropin and its potential value in screening for Down's syndrome. *Clinical Chemistry* 1991;**37**(6):809–14.
- Spencer 1991b** *{published data only}*  
Spencer K. Maternal serum CA125 is not a second trimester marker for Down's syndrome. *Annals of Clinical Biochemistry* 1991;**28**(3):299–300.
- Spencer 1992** *{published data only}*  
Spencer K, Coombes EJ, Mallard AS, Ward AM. Free  $\beta$  human choriogonadotropin in Down's syndrome screening: a multicentre study of its role compared with other biochemical markers.[see comment]. *Annals of Clinical Biochemistry* 1992;**29**(5):506–18.
- Spencer 1993a** *{published data only}*  
Spencer K, Carpenter P. Prospective study of prenatal screening for Down's syndrome with free  $\beta$  human chorionic gonadotrophin.[see comment]. *BMJ* 1993;**307**(6907):764–9.
- Spencer 1993b** *{published data only}*  
Spencer K, Macri JN, Carpenter P, Anderson R, Krantz DA. Stability of intact chorionic gonadotropin (hCG) in serum, liquid whole blood, and dried whole-blood filter-paper spots: impact on screening for Down syndrome by measurement of free  $\beta$ -hCG subunit. *Clinical Chemistry* 1993;**39**(6):1064–8.
- Spencer 1993c** *{published data only}*  
Spencer K, Wood PJ, Anthony FW. Elevated levels of maternal serum inhibin immunoreactivity in second trimester pregnancies affected by Down's syndrome. *Annals of Clinical Biochemistry* 1993;**30**(Pt 2):219–20.
- Spencer 1993d** *{published data only}*  
Spencer K, Macri JN, Anderson RW, Aitken DA, Berry E, Crossley JA, et al. Dual analyte immunoassay in neural

- tube defect and Down's syndrome screening: results of a multicentre clinical trial. *Annals of Clinical Biochemistry* 1993;**30**(4):394–401.
- Spencer 1993e** *{published data only}*  
Spencer K. Free alpha-subunit of human chorionic gonadotropin in Down syndrome. *American Journal of Obstetrics and Gynecology* 1993;**168**(1):132–5.
- Spencer 1995** *{published data only}*  
Spencer K. The influence of gravidity on Down's syndrome screening with free  $\beta$  hCG. *Prenatal Diagnosis* 1995;**15**(1): 87–9.
- Spencer 1996** *{published data only}*  
Spencer K, Wallace EM, Ritoe S. Second-trimester dimeric inhibin-A in Down's syndrome screening. *Prenatal Diagnosis* 1996;**16**(12):1101–10.
- Spencer 1997** *{published data only}*  
Spencer K, Noble P, Snijders RJ, Nicolaides KH. First-trimester urine free  $\beta$  hCG,  $\beta$  core, and total oestriol in pregnancies affected by Down's syndrome: implications for first-trimester screening with nuchal translucency and serum free  $\beta$  hCG. *Prenatal Diagnosis* 1997;**17**(6):525–38.
- Spencer 1998a** *{published data only}*  
Spencer K. The influence of smoking on maternal serum AFP and free  $\beta$  hCG levels and the impact on screening for Down syndrome. *Prenatal Diagnosis* 1998;**18**(3):225–34.
- Spencer 1998b** *{published data only}*  
Spencer K, Carpenter P. Is prostate-specific antigen a marker for pregnancies affected by Down syndrome?. *Clinical Chemistry* 1998;**44**(11):2362–5.
- Spencer 1999b** *{published data only}*  
Spencer K. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free  $\beta$  hCG: a seven year review. *British Journal of Obstetrics and Gynaecology* 1999;**106**(12):1287–93.
- Spencer 1999c** *{published data only}*  
Spencer K. Accuracy of Down's syndrome risks produced in a prenatal screening program. *Annals of Clinical Biochemistry* 1999;**36**(1):101–3.
- Spencer 2000a** *{published data only}*  
Spencer K, Berry E, Crossley JA, Aitken DA, Nicolaides KH. Is maternal serum total hCG a marker of trisomy 21 in the first trimester of pregnancy?. *Prenatal Diagnosis* 2000;**20**(4):311–7.
- Spencer 2000b** *{published data only}*  
Spencer K. Screening for trisomy 21 in twin pregnancies in the first trimester using free  $\beta$ -hCG and PAPP-A, combined with fetal nuchal translucency thickness. *Prenatal Diagnosis* 2000;**20**(2):91–5.
- Spencer 2000c** *{published data only}*  
Spencer K. The influence of smoking on maternal serum PAPP-A and free  $\beta$  hCG levels in the first trimester of pregnancy. *Prenatal Diagnosis* 1999;**19**(11):1065–6.
- Spencer 2000d** *{published data only}*  
Spencer K, Ong CY, Liao AW, Nicolaides KH. The influence of parity and gravidity on first trimester markers of chromosomal abnormality. *Prenatal Diagnosis* 2000;**20** (10):792–4.
- Spencer 2000e** *{published data only}*  
Spencer K. The influence of fetal sex in screening for Down syndrome in the second trimester using AFP and free  $\beta$ -hCG. *Prenatal Diagnosis* 2000;**20**(8):648–51.
- Spencer 2000f** *{published data only}*  
Spencer K, Ong CY, Liao AW, Nicolaides KH. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenatal Diagnosis* 2000;**20**(6):491–4.
- Spencer 2000g** *{published data only}*  
Spencer K, Tul N, Nicolaides KH. Maternal serum free  $\beta$ -hCG and PAPP-A in fetal sex chromosome defects in the first trimester. *Prenatal Diagnosis* 2000;**20**(5):390–4.
- Spencer 2000h** *{published data only}*  
Spencer K. Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome. *Prenatal Diagnosis* 2000;**20**(8):652–6.
- Spencer 2000i** *{published data only}*  
Spencer K, Ong CY, Liao AW, Papademetriou D, Nicolaides KH. The influence of fetal sex in screening for trisomy 21 by fetal nuchal translucency, maternal serum free  $\beta$ -hCG and PAPP-A at 10-14 weeks of gestation. *Prenatal Diagnosis* 2000;**20**(8):673–5.
- Spencer 2001a** *{published data only}*  
Spencer K. Age related detection and false positive rates when screening for Down's syndrome in the first trimester using fetal nuchal translucency and maternal serum free  $\beta$ hCG and PAPP-A. *BJOG: an international journal of obstetrics and gynaecology* 2001;**108**(10):1043–6.
- Spencer 2001b** *{published data only}*  
Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH. First trimester maternal serum placenta growth factor (PIGF) concentrations in pregnancies with fetal trisomy 21 or trisomy 18. *Prenatal Diagnosis* 2001;**21**(9):718–22.
- Spencer 2001c** *{published data only}*  
Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH. Maternal serum levels of dimeric Inhibin A in pregnancies affected by trisomy 21 in the first trimester. *Prenatal Diagnosis* 2001;**21**(6):441–4.
- Spencer 2001d** *{published data only}*  
Spencer K, Liao AW, Skentou H, Ong CY, Nicolaides KH. Maternal serum levels of total activin-A in first-trimester trisomy 21 pregnancies. *Prenatal Diagnosis* 2001;**21**(4): 270–3.
- Spencer 2001e** *{published data only}*  
Spencer K. Screening for trisomy 21 in twin pregnancies in the first trimester: does chorionicity impact on maternal serum free  $\beta$ -hCG or PAPP-A levels?. *Prenatal Diagnosis* 2001;**21**(9):715–7.
- Spencer 2002b** *{published data only}*  
Spencer K, Nicolaides KH. A first trimester trisomy 13/trisomy 18 risk algorithm combining fetal nuchal

- translucency thickness, maternal serum free  $\beta$ -hCG and PAPP-A. *Prenatal Diagnosis* 2002;**22**(10):877–9.
- Spencer 2002c** *{published data only}*  
Spencer K. Accuracy of Down syndrome risks produced in a first-trimester screening programme incorporating fetal nuchal translucency thickness and maternal serum biochemistry. *Prenatal Diagnosis* 2002;**22**(3):244–6.
- Spencer 2002d** *{published data only}*  
Spencer K, Cuckle HS. Screening for chromosomal anomalies in the first trimester: does repeat maternal serum screening improve detection rates?. *Prenatal Diagnosis* 2002;**22**(10):903–6.
- Spencer 2002e** *{published data only}*  
Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy. *Annals of Clinical Biochemistry* 2002;**39**(6):567–76.
- Spencer 2003a** *{published data only}*  
Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. The effect of temporal variation in biochemical markers of trisomy 21 across the first and second trimesters of pregnancy on the estimation of individual patient-specific risks and detection rates for Down's syndrome. *Annals of Clinical Biochemistry* 2003;**40**(3):219–31.
- Spencer 2003b** *{published data only}*  
Spencer K. The influence of different sample collection types on the levels of markers used for Down's syndrome screening as measured by the Kryptor Immunosassay system. *Annals of Clinical Biochemistry* 2003;**40**(2):166–8.
- Spencer 2003c** *{published data only}*  
Spencer K, Bindra R, Nicolaides KH. Maternal weight correction of maternal serum PAPP-A and free  $\beta$ -hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. *Prenatal Diagnosis* 2003;**23**(10):851–5.
- Spencer 2003d** *{published data only}*  
Spencer K, Nicolaides KH. Screening for trisomy 21 in twins using first trimester ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years experience. *BJOG: an international journal of obstetrics and gynaecology* 2003;**110**(3):279–80.
- Spencer 2004** *{published data only}*  
Spencer K, Bindra R, Cacho AM, Nicolaides KH. The impact of correcting for smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. *Prenatal Diagnosis* 2004;**24**(3):169–73.
- Spencer 2005a** *{published data only}*  
Spencer K, Cicero S, Atzei A, Otigbah C, Nicolaides KH. The influence of maternal insulin-dependent diabetes on fetal nuchal translucency thickness and first-trimester maternal serum biochemical markers of aneuploidy. *Prenatal Diagnosis* 2005;**25**(10):927–9.
- Spencer 2005b** *{published data only}*  
Spencer K, Heath V, El-Sheikhah A, Ong CY, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. *Prenatal Diagnosis* 2005;**25**(5):365–9.
- Spencer 2005c** *{published data only}*  
Spencer K. First trimester maternal serum screening for Down's syndrome: an evaluation of the DPC Immulite 2000 free  $\beta$ -hCG and pregnancy-associated plasma protein-A assays.[see comment]. *Annals of Clinical Biochemistry* 2005;**42**(1):30–40.
- Spencer 2008** *{published data only}*  
Spencer K, Cowans NJ, Uldbjerg N, Vereecken A, Torring N. First trimester intact hCG as an early marker of trisomy 21: a promise unrecognised?. *Prenatal Diagnosis* 2008;**28**(12):1156–9.
- Spong 1999** *{published data only}*  
Spong CY, Ghidini A, Stanley-Christian H, Meck JM, Seydel FD, Pezzullo JC. Risk of abnormal triple screen for Down syndrome is significantly higher in women with female fetuses. *Prenatal Diagnosis* 1999;**19**(4):337–9.
- Staboulidou 2009** *{published data only}*  
Staboulidou I, Galindo A, Maiz N, Karagiannis G, Nicolaides KH. First-trimester uterine artery Doppler and serum pregnancy-associated plasma protein-a in preeclampsia and chromosomal defects. *Fetal Diagnosis and Therapy* 2009;**25**(3):336–9.
- Stevens 1998** *{published data only}*  
Stevens SL. The use of nuchal lucency as a screening tool in first trimester sonography. *Journal of Diagnostic Medical Sonography* 1998;**14**(6):251–4.
- Stoll 1992** *{published data only}*  
Stoll C. A new approach of prenatal prevention of constitutional disabilities - the study of markers of maternal serum. *Journal de Medecine de Strasbourg* 1992;**23**(1):25–7.
- Stressig 2011** *{published data only}*  
Stressig R, Kozlowski P, Froehlich S, Siegmann HJ, Hammer R, Blumenstock G, et al. Assessment of the ductus venosus, tricuspid blood flow and the nasal bone in second-trimester screening for trisomy 21. *Ultrasound in Obstetrics & Gynecology* 2011;**37**(4):444–9.
- Su 2002** *{published data only}*  
Su YN, Hsu JJ, Lee CN, Cheng WF, Kung CC, Hsieh FJ. Raised maternal serum placenta growth factor concentration during the second trimester is associated with Down syndrome. *Prenatal Diagnosis* 2002;**22**(1):8–12.
- Suchet 1995** *{published data only}*  
Suchet IB. Ultrasonography of the fetal neck in the first and second trimesters. Part 2. Anomalies of the posterior nuchal region. *Canadian Association of Radiologists Journal* 1995;**46**(5):344–52.

- Suchy 1990** *{published data only}*  
Suchy SF, Yeager MT. Down syndrome screening in women under 35 with maternal serum hCG. *Obstetrics and Gynecology* 1990;**76**(1):20–4.
- Summers 2003a** *{published data only}*  
Summers AM, Farrell SA, Huang T, Meier C, Wyatt PR. Maternal serum screening in Ontario using the triple marker test. *Journal of Medical Screening* 2003;**10**(3):107–11.
- Summers 2003b** *{published data only}*  
Summers AM, Huang T, Meier C, Wyatt PR. The implications of a false positive second-trimester serum screen for Down syndrome. *Obstetrics and Gynecology* 2003;**101**(6):1301–6.
- Suntharasaj 2005** *{published data only}*  
Suntharasaj T, Ratanasiri T, Chanprapaph P, Kengpol C, Kor-anantakul O, Leetanaporn R, et al. Variability of nuchal translucency measurement: a multicenter study in Thailand. *Gynecologic and Obstetric Investigation* 2005;**60**(4):201–5.
- Susman 2010** *{published data only}*  
Susman MR, Amor DJ, Muggli E, Jaques AM, Halliday J. Using population-based data to predict the impact of introducing noninvasive prenatal diagnosis for Down syndrome. *Genetics in Medicine* 2010;**12**(5):298–303.
- Sutton 2004** *{published data only}*  
Sutton JM, Cole LA. Sialic acid-deficient invasive trophoblast antigen (sd-ITA): a new urinary variant for gestational Down syndrome screening. *Prenatal Diagnosis* 2004;**24**(3):194–7.
- Suzuki 1998** *{published data only}*  
Suzuki Y, Takada J, Iwaki T, Isaka K, Takayama M. Screening for trisomy 21 in the first trimester by measurement of serum PAPP-A and free  $\beta$ -hCG. *Acta Obstetrica et Gynaecologica Japonica* 1998;**50**(1):37–40.
- Tabor 1987** *{published data only}*  
Tabor A, Larsen SO, Nielsen J, Philip J, Pilgaard B, et al. Screening for Down's syndrome using an iso-risk curve based on maternal age and serum alpha-fetoprotein level. *British Journal of Obstetrics and Gynaecology* 1987;**94**(7):636–42.
- Tanski 1999** *{published data only}*  
Tanski S, Rosengren SS, Benn PA. Predictive value of the triple screening test for the phenotype of Down syndrome. *American Journal of Medical Genetics* 1999;**85**(2):123–6.
- Thilaganathan 1998** *{published data only}*  
Thilaganathan B, Khare M, Williams B, Wathen NC. Influence of ethnic origin on nuchal translucency screening for Down's syndrome. *Ultrasound in Obstetrics & Gynecology* 1998;**12**(2):112–4.
- Thilaganathan 1999** *{published data only}*  
Thilaganathan B. First-trimester nuchal translucency and maternal serum biochemical screening for Down's syndrome: a happy union?. *Ultrasound in Obstetrics and Gynecology* 1999;**13**(4):229–30.
- Tislaric 2002** *{published data only}*  
Tislaric D, Brajenovic-Milic B, Ristic S, Latin V, Zuvic-Butorac M, Bacic J, et al. The influence of smoking and parity on serum markers for Down's syndrome screening. *Fetal Diagnosis and Therapy* 2002;**17**(1):17–21.
- Torok 1997** *{published data only}*  
Torok O, Veress L, Szabo M, Zsupan I, Buczko Z, Bolodar A, et al. [Biochemical and ultrasonic screening of chromosomal aneuploidies in the second trimester of pregnancy]. [Hungarian]. *Orvosi Hetilap* 1997;**138**(3):123–7.
- Torrington 2009** *{published data only}*  
Torrington N. Performance of first-trimester screening between gestational weeks 7 and 13. *Clinical Chemistry* 2009;**55**(8):1564–7.
- Trninc-Pjevic 2007** *{published data only}*  
Trninc-Pjevic A, Novakov-Mikic A. [First trimester ultrasound screening of chromosomal abnormalities]. [Serbian]. *Srpski Arhiv Za Celokupno Lekarstvo* 2007;**135**(3-4):153–6.
- Tsai 2001** *{published data only}*  
Tsai MS, Huang YY, Hwa KY, Cheng CC, Lee FK. Combined measurement of fetal nuchal translucency, maternal serum free  $\beta$ -hCG, and pregnancy-associated plasma protein A for first-trimester Down's syndrome screening. *Journal of the Formosan Medical Association* 2001;**100**(5):319–25.
- Valerio 1996** *{published data only}*  
Valerio D, Aiello R, Altieri V, Fagnoni P. Maternal serum screening of fetal chromosomal abnormalities by AFP, UE3, hCG and free- $\beta$  hCG. Prospective and retrospective results. *Minerva Ginecologica* 1996;**48**(5):169–73.
- Van Blerk 1992** *{published data only}*  
Van Blerk M, Smitz J, De Catte L, Kumps C, Van der Elst J, Van Steirteghem AC. Second-trimester cancer antigen 125 and Down's syndrome.[see comment]. *Prenatal Diagnosis* 1992;**12**(12):1062–6.
- Van Dyke 2007** *{published data only}*  
Van Dyke DL, Ebrahim SA, Al Saadi AA, Powell SA, Zenger-Hain JL, Micale MA, et al. The impact of maternal serum screening programs for Down syndrome in southeast Michigan, 1988-2003. *Prenatal Diagnosis* 2007;**27**(6):583–4.
- Van Heesch, 2006** *{published data only}*  
Van Heesch PN, Schielen PC, Wildhagen MF, Den Hollander K, Steegers EA, Wildschut HI. Combined first trimester screening for trisomy 21: Lack of agreement between risk calculation methods. *Journal of Perinatal Medicine* 2006;**34**(2):162–5.
- Van Lith 1991** *{published data only}*  
Van Lith JM, Mantingh A, Beekhuis JR, De Bruijn HW, Breed AS. First trimester CA 125 and Down's syndrome. *British Journal of Obstetrics and Gynaecology* 1991;**98**(5):493–4.



- Van Lith 1993** *{published data only}*  
Van Lith JM, Mantingh A, De Bruijn HW. Maternal serum CA 125 levels in pregnancies with chromosomally-normal and -abnormal fetuses. Dutch Working Party on Prenatal Diagnosis. *Prenatal Diagnosis* 1993;**13**(12):1123–31.
- Van Lith 1994** *{published data only}*  
Van Lith JM, Mantingh A, Pratt JJ. First-trimester maternal serum immunoreactive inhibin in chromosomally normal and abnormal pregnancies. Dutch Working Party on Prenatal Diagnosis. *Obstetrics and Gynecology* 1994;**83**(5 Pt 1):661–4.
- Veress 1986** *{published data only}*  
Veress L, Szabo M, Horvath K, Polgar K, Papp Z. [Low maternal serum alpha-fetoprotein concentration and Down syndrome]. [Hungarian]. *Orvosi Hetilap* 1986;**127**(20):1232–3.
- Veress 1988** *{published data only}*  
Veress L, Szabo M, Polgar K, Takacs L, Papp Z. [Prenatal screening for Down's syndrome by measuring the AFP concentration in the maternal serum]. [Hungarian]. *Orvosi Hetilap* 1988;**129**(31):1677.
- Vergani 2008** *{published data only}*  
Vergani P, Ghidini A, Weiner S, Locatelli A, Pozzi E, Biffi A. Risk assessment for Down syndrome with genetic sonogram in women at risk. *Prenatal Diagnosis* 2008;**28**(12):1144–8.
- Vintzileos 2003** *{published data only}*  
Vintzileos A, Walters C, Yeo L. Absent nasal bone in the prenatal detection of fetuses with trisomy 21 in a high-risk population. *Obstetrics and Gynecology* 2003;**101**(5):905–8.
- Wald 1988a** *{published data only}*  
Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T, et al. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* 1988;**297**(6653):883–7.
- Wald 1988b** *{published data only}*  
Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Canick JA, Haddow JE, et al. Maternal serum unconjugated oestriol as an antenatal screening test for Down's syndrome. *British Journal of Obstetrics and Gynaecology* 1988;**95**(4):334–41.
- Wald 1991** *{published data only}*  
Wald N, Cuckle H, Wu TS, George L. Maternal serum unconjugated oestriol and human chorionic gonadotrophin levels in twin pregnancies: implications for screening for Down's syndrome. *British Journal of Obstetrics and Gynaecology* 1991;**98**(9):905–8.
- Wald 1992a** *{published data only}*  
Wald NJ, Kennard A, Densem JW, Cuckle HS, Chard T, Butler L. Antenatal maternal serum screening for Down's syndrome: results of a demonstration project.[see comment]. *BMJ* 1992;**305**(6850):391–4.
- Wald 1992b** *{published data only}*  
Wald NJ, Cuckle HS, Densem JW, Stone RB. Maternal serum unconjugated oestriol and human chorionic gonadotrophin levels in pregnancies with insulin-dependent diabetes: implications for screening for Down's syndrome. *British Journal of Obstetrics and Gynaecology* 1992;**99**(1):51–3.
- Wald 1992c** *{published data only}*  
Wald NJ, Cuckle HS, Densem JW, Kennard A, Smith D. Maternal serum screening for Down's syndrome: the effect of routine ultrasound scan determination of gestational age and adjustment for maternal weight.[see comment]. *British Journal of Obstetrics and Gynaecology* 1992;**99**(2):144–9.
- Wald 1993** *{published data only}*  
Wald N, Densem J, Stone R, Cheng R. The use of free  $\beta$ -hCG in antenatal screening for Down's syndrome.[see comment]. *British Journal of Obstetrics and Gynaecology* 1993;**100**(6):550–7.
- Wald 1994** *{published data only}*  
Wald NJ, Densem JW. Maternal serum free alpha-human chorionic gonadotrophin levels in twin pregnancies: implications for screening for Down's syndrome. *Prenatal Diagnosis* 1994;**14**(8):717–9.
- Wald 1994a** *{published data only}*  
Wald NJ, Watt HC. Choice of serum markers in antenatal screening for Down's syndrome. *Journal of Medical Screening* 1994;**1**(2):117–20.
- Wald 1996a** *{published data only}*  
Wald NJ, Watt HC. Serum markers for Down's syndrome in relation to number of previous births and maternal age. *Prenatal Diagnosis* 1996;**16**(8):699–703.
- Wald 1996b** *{published data only}*  
Wald NJ, George L, Smith D, Densem JW, Petterson K. Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy. International Prenatal Screening Research Group.[see comment]. *British Journal of Obstetrics and Gynaecology*. 1996;**103**(5):407–12.
- Wald 1996c** *{published data only}*  
Wald NJ, Watt HC, George L. Maternal serum inhibin-A in pregnancies with insulin-dependent diabetes mellitus: implications for screening for Down's syndrome. *Prenatal Diagnosis* 1996;**16**(10):923–6.
- Wald 1996d** *{published data only}*  
Wald NJ, Densem JW, George L, Muttukrishna S, Knight PG. Prenatal screening for Down's syndrome using inhibin-A as a serum marker. *Prenatal Diagnosis* 1996;**16**(2):143–53.
- Wald 1997** *{published data only}*  
Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome.[see comment]. *Prenatal Diagnosis* 1997;**17**(9):821–9.
- Wald 1998** *{published data only}*  
Wald NJ, Watt HC, Haddow JE, Knight GJ. Screening for Down syndrome at 14 weeks of pregnancy. *Prenatal Diagnosis* 1998;**18**(3):291–3.
- Wald 1999a** *{published data only}*  
Wald NJ, Hackshaw AK, Diamandis EP, Melegos DN. Maternal serum prostate-specific antigen and Down

- syndrome in the first and second trimesters of pregnancy. *Prenatal Diagnosis* 1999;**19**(7):674–6.
- Wald 1999b** *{published data only}*  
Wald NJ, Watt HC, Norgaard-Pederson B, Christiansen M. SP1 in pregnancies with Down syndrome in the first trimester of pregnancy. *Prenatal Diagnosis* 1999;**19**(6): 517–20.
- Wald 1999c** *{published data only}*  
Wald NJ, White N, Morris JK, Huttly WJ, Canick JA. Serum markers for Down's syndrome in women who have had in vitro fertilisation: implications for antenatal screening. *British Journal of Obstetrics and Gynaecology* 1999;**106**(12):1304–6.
- Wald 1999d** *{published data only}*  
Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters.[see comment]. *New England Journal of Medicine* 1999;**341**(7):461–7.
- Wald 2003b** *{published data only}*  
Wald NJ, Rish S, Hackshaw AK. Combining nuchal translucency and serum markers in prenatal screening for Down syndrome in twin pregnancies. *Prenatal Diagnosis* 2003;**23**(7):588–92.
- Wald 2003c** *{published data only}*  
Wald NJ, Huttly WJ, Hackshaw AK. Antenatal screening for Down's syndrome with the quadruple test.[see comment]. *Lancet* 2003;**361**(9360):835–6.
- Wald 2006** *{published data only}*  
Wald NJ, Rudnicka AR, Bestwick JP. Sequential and contingent prenatal screening for Down syndrome. *Prenatal Diagnosis* 2006;**26**(9):769–77.
- Wallace 1994** *{published data only}*  
Wallace EM, Harkness LM, Burns S, Liston WA. Evaluation of maternal serum immunoreactive Inhibin A as a first trimester marker of Down's syndrome. *Clinical Endocrinology* 1994;**41**(4):483–6.
- Wallace 1997** *{published data only}*  
Wallace EM, Crossley JA, Ritoe SC, Groome NP, Aitken DA. Maternal serum inhibin-A in pregnancies complicated by insulin dependent diabetes mellitus. *British Journal of Obstetrics and Gynaecology* 1997;**104**(8):946–8.
- Wang 2010** *{published data only}*  
Wang E, Chen C, Glimco E, Grobman W. The performance of second trimester long bone ratios for Down syndrome screening is influenced by gestational age. *Journal of Maternal-fetal & Neonatal Medicine* 2010;**23**(7):642–5.
- Ward 2005** *{published data only}*  
Ward A. Nuchal translucency measurement. Synergy (<http://www.highbeam.com/doc/1P3-866108421.html>) (accessed 2007) 2005.
- Watt 1996a** *{published data only}*  
Watt HC, Wald NJ, Smith D, Kennard A, Densem J. Effect of allowing for ethnic group in prenatal screening for Down's syndrome. *Prenatal Diagnosis* 1996;**16**(8):691–8.
- Watt 1996b** *{published data only}*  
Watt HC, Wald NJ, George L. Maternal serum inhibin-A levels in twin pregnancies: implications for screening for Down's syndrome. *Prenatal Diagnosis* 1996;**16**(10):927–9.
- Wax 2007** *{published data only}*  
Wax JR, Pinette MG, Cartin A, Blackstone J. Optimal crown-rump length for measuring the nuchal translucency. *Journal of Clinical Ultrasound* 2007;**35**(6):302–4.
- Weinans 2001** *{published data only}*  
Weinans MJN, Pratt JJ, De Wolf, Mantingh A. First-trimester maternal serum human thyroid-stimulating hormone in chromosomally normal and Down syndrome pregnancies. *Prenatal Diagnosis* 2001;**21**(9):723–5.
- Weinans 2004** *{published data only}*  
Weinans MJ, Kooij L, Müller MA, Bilardo KM, Van Lith JM, Tymstra T. A comparison of the impact of screen-positive results obtained from ultrasound and biochemical screening for Down syndrome in the first trimester: a pilot study. *Prenatal Diagnosis* 2004;**24**(5):347–51.
- Weisz 2007** *{published data only}*  
Weisz B, Pandya P, Chitty L, Jones P, Huttly W, Rodeck C. Practical issues drawn from the implementation of the integrated test for Down syndrome screening into routine clinical practice. *BJOG: an international journal of obstetrics and gynaecology* 2007;**114**(4):493–7.
- Welborn 1994** *{published data only}*  
Welborn JL, Timm NS. Trisomy 21 and cystic hygromas in early gestational age fetuses. *American Journal of Perinatology* 1994;**11**(1):19–20.
- Wenstrom 1993** *{published data only}*  
Wenstrom KD, Williamson RA, Grant SS, Hudson JD, Getchell JP. Evaluation of multiple-marker screening for Down syndrome in a statewide population. *American Journal of Obstetrics and Gynecology* 1993;**169**(4):793–7.
- Wenstrom 1995a** *{published data only}*  
Wenstrom KD, Owen J, Boots L, Ethier M. The influence of maternal weight on human chorionic gonadotropin in the multiple-marker screening test for fetal Down syndrome. *American Journal of Obstetrics and Gynecology* 1995;**173**(4): 1297–300.
- Wenstrom 1995b** *{published data only}*  
Wenstrom KD, Desai R, Owen J, Dubard MB, Boots L. Comparison of multiple-marker screening with amniocentesis for the detection of fetal aneuploidy in women greater than or equal 35 years old. *American Journal of Obstetrics and Gynecology* 1995;**173**(4):1287–92.
- Wetta 2011** *{published data only}*  
Wetta L, Biggio JJ, Owen J. Use of ethnic-specific medians for Hispanic patients reduces ethnic disparities in multiple marker screening. *Prenatal Diagnosis* 2011;**31**(4):331–3.
- Whitlow 1998a** *{published data only}*  
Whitlow BJ, Lazanakis ML, Kadir RA, Chatzipapas I, Economides DL. The significance of choroid plexus cysts, echogenic heart foci and renal pyelectasis in the first

- trimester. *Ultrasound in Obstetrics & Gynecology* 1998;**12**(6):385–90.
- Whitlow 1998b** *{published data only}*  
Whitlow BJ, Economides DL. First trimester detection of fetal abnormalities in an unselected population. *Contemporary Reviews in Obstetrics and Gynaecology* 1998;**10**(4):245–53.
- Whitlow 1999** *{published data only}*  
Whitlow BJ, Chatzipapas IK, Lazanakis ML, Kadir RA, Economides DL. The value of sonography in early pregnancy for the detection of fetal abnormalities in an unselected population. *British Journal of Obstetrics and Gynaecology* 1999;**106**(9):929–36.
- Williamson 1994** *{published data only}*  
Williamson R. Expanded maternal serum alpha fetoprotein screening. *Iowa Medicine* 1994;**84**(9):397–400.
- Wilson 2000** *{published data only}*  
Wilson K. New first-trimester prenatal screening for down syndrome. *Laboratory Medicine* 2000;**31**(11):591.
- Wojdemann 2001** *{published data only}*  
Wojdemann KR, Larsen SO, Shalmi A, Sundberg K, Christiansen M, Tabor A. First trimester screening for Down syndrome and assisted reproduction: no basis for concern. *Prenatal Diagnosis* 2001;**21**(7):563–5.
- Wong 2003** *{published data only}*  
Wong SF, Choi H, Ho LC. Nasal bone hypoplasia: is it a common finding amongst chromosomally normal fetuses of southern Chinese women?. *Gynecologic and Obstetric Investigation* 2003;**56**(2):99–101.
- Wright 2006** *{published data only}*  
Wright D, Bradbury I, Cuckle H, Gardosi J, Tonks A, Standing S, et al. Three-stage contingent screening for Down syndrome. *Prenatal Diagnosis* 2006;**26**(6):528–34.
- Wright 2007** *{published data only}*  
Wright D, Spencer K, Nix B. First trimester screening for Down syndrome using free beta hCG, total hCG and PAPP-A: an exploratory study. *Prenatal Diagnosis* 2007;**27**(12):1118–22.
- Xie 2010** *{published data only}*  
Xie Z, Lu S, Li H. Contingent triple-screening for Down syndrome in the second trimester: a feasibility study in Mainland Chinese population. *Prenatal Diagnosis* 2010;**30**(1):74–6.
- Yagel 1998** *{published data only}*  
Yagel S, Anteby EY, Hochner-Celnikier D, Ariel I, Chaap T, Ben Neriah Z. The role of midtrimester targeted fetal organ screening combined with the “triple test” and maternal age in the diagnosis of trisomy 21: a retrospective study. *American Journal of Obstetrics and Gynecology* 1998;**178**(1):40–4.
- Yamamoto 2001a** *{published data only}*  
Yamamoto R, Azuma M, Kishida T, Yamada H, Satomura S, Fujimoto S. Total alpha-fetoprotein and Lens culinaris agglutinin-reactive alpha-fetoprotein in fetal chromosomal abnormalities. *BJOG: an international journal of obstetrics and gynaecology* 2001;**108**(11):1154–8.
- Yamamoto 2001b** *{published data only}*  
Yamamoto R, Azuma M, Hoshi N, Kishida T, Satomura S, Fujimoto S. Lens culinaris agglutinin-reactive alpha-fetoprotein, an alternative variant to alpha-fetoprotein in prenatal screening for Down's syndrome. *Human Reproduction* 2001;**16**(11):2438–44.
- Yamamoto 2001c** *{published data only}*  
Yamamoto R, Azuma M, Wakui Y, Kishida T, Yamada H, Okuyama K, et al. Alpha-fetoprotein microheterogeneity: a potential biochemical marker for Down's syndrome. *Clinica Chimica Acta* 2001;**304**(1-2):137–41.
- Yaron 2001** *{published data only}*  
Yaron Y, Wolman I, Kupferminc MJ, Ochshorn Y, Many A, Orr-Urtreger A. Effect of fetal gender on first trimester markers and on Down syndrome screening. *Prenatal Diagnosis* 2001;**21**(12):1027–30.
- Ye 1995** *{published data only}*  
Ye G, Liao S, Zhao X. The possibility of prenatal screening for fetal abnormalities in second-trimester pregnancies by measuring AFP,  $\beta$ -HCG and uE-3 levels. *Xi'an Yike Daxue Xuebao* 1995;**16**(4):408–11.
- Yoshida 2000** *{published data only}*  
Yoshida K, Kuwabara Y, Tanaka T, Onda T, Kudo R, Yamamoto H, et al. Dimeric Inhibin A as a fourth marker for Down's syndrome maternal serum screening in native Japanese women. *Journal of Obstetrics and Gynecology Research* 2000;**26**(3):171–4.
- Zalel 2008** *{published data only}*  
Zalel Y, Achiron R, Yagel S, Kivilevitch Z. Fetal aberrant right subclavian artery in normal and Down syndrome fetuses. *Ultrasound in Obstetrics & Gynecology* 2008;**31**(1):25–9.
- Zeitune 1991** *{published data only}*  
Zeitune M, Aitken DA, Crossley JA, Yates JR, Cooke A, Ferguson-Smith MA. Estimating the risk of a fetal autosomal trisomy at mid-trimester using maternal serum alpha-fetoprotein and age: A retrospective study of 142 pregnancies. *Prenatal Diagnosis* 1991;**11**(11):847–57.
- Zelop 2005** *{published data only}*  
Zelop CM, Milewski E, Brault K, Benn P, Borgida AF, Egan JFX. Variation of fetal nasal bone length in second-trimester fetuses according to race and ethnicity. *Journal of Ultrasound in Medicine* 2005;**24**(11):1487–9.
- Zhang 2011** *{published data only}*  
Zhang J, Lambert-Messerlian G, Palomaki GE, Canick JA. Impact of smoking on maternal serum markers and prenatal screening in the first and second trimesters. *Prenatal Diagnosis* 2011;**31**(6):583–8.
- Zhao 1998** *{published data only}*  
Zhao Xiaolan, Ye Guoling, Liu Qi. Using maternal serum PAPP-A and other pregnancy-associated proteins in screening for fetal abnormalities. *Xi'an Yike Daxue Xuebao* 1998;**19**(1):94–6, 110.

**Zhong 2011** {published data only}

Zhong Y, Longman R, Bradshaw R, Odibo AO. The genetic sonogram: comparing the use of likelihood ratios versus logistic regression coefficients for Down syndrome screening. *Journal of Ultrasound in Medicine* 2011;**30**(4): 463–9.

**Zoppi 2003** {published data only}

Zoppi MA, Ibba RM, Floris M, Manca F, Axiana C, Monni G. Changes in nuchal translucency thickness in normal and abnormal karyotype fetuses. *BJOG: an international journal of obstetrics and gynaecology*. 2003;**110**(6):584–8.

**Additional references****Alfirevic 2003**

Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane Database of Systematic Reviews* 2003, Issue 3. [DOI: 10.1002/14651858.CD003252]

**Alfirevic 2004**

Alfirevic Z, Neilson JP. Antenatal screening for Down's syndrome. *BMJ* 2004;**9**(329(7470)):811–2.

**Alldred 2010**

Alldred SK, Deeks JJ, Neilson JP, Alfirevic Z. Antenatal screening for Down's syndrome: generic protocol. *Cochrane Database of Systematic Reviews* 2010, Issue 4. [DOI: 10.1002/14651858.CD007384.pub2]

**Alldred 2012**

Alldred SK, Deeks JJ, Guo B, Neilson JP, Alfirevic Z. Second trimester serum tests for Down's Syndrome screening. *Cochrane Database of Systematic Reviews* 2012, Issue 6. [DOI: 10.1002/14651858.CD009925]

**Bersinger 1995**

Bersinger NA, Zakher A, Huber U, Pescia G, Schneider H. A sensitive enzyme immunoassay for pregnancy-associated plasma protein A (PAPP-A): a possible first trimester method of screening for Down syndrome and other trisomies. *Archives of Gynecology and Obstetrics* 1995; **256**(4):185–92.

**Bogart 1987**

Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with

fetal chromosome abnormalities. *Prenatal Diagnosis* 1987;**7**(9):623–30.

**Cuckle 1995**

Cuckle HS, Holding S, Jones R, Wallace EM, Groome NP. Maternal serum dimeric inhibin A in second-trimester Down's syndrome pregnancies. *Prenatal Diagnosis* 1995; Vol. 15, issue 4:385–6.

**Macri 1990**

Macri JN, Kasturi RV, Krantz DA, Cook EJ, Moore ND, Young JA, et al. Maternal serum Down syndrome screening: free beta-protein is a more effective marker than human chorionic gonadotropin. *American Journal of Obstetrics and Gynecology* 1990;**163**(4 Pt 1):1248–53.

**Macri 1993**

Macri JN, Spencer K, Aitken D, Garver K, Buchanan PD, Muller F, et al. First-trimester free beta (hCG) screening for Down syndrome. *Prenatal Diagnosis* 1993;**13**(7):557–62.

**Mol 1999**

Mol BW, Lijmer JG, van der Meulen J, Pajkrt E, Bilardo CM, Bossuyt PM. Effect of study design on the association between nuchal translucency measurement and Down syndrome. *Obstetrics and Gynecology* 1999;**94**(5 Pt 2): 864–9.

**Penrose 1933**

Penrose LS. The relative effects of parental and maternal age in mongolism. *Journal of Genetics* 1933;**27**:219–24.

**Steele 1966**

Steele MW, Breg WR. Chromosome analysis of human amniotic-fluid cells. *Lancet* 1966;**i**:383–5.

**Vaklenti 1968**

Vaklenti C, Schutta EJ, Kehaty T. Prenatal diagnosis of Down's syndrome. *Lancet* 1968;**ii**:220.

**Whiting 2003**

Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Medical Research Methodology* 2003;**3**:25.

\* Indicates the major publication for the study

## CHARACTERISTICS OF STUDIES

### Characteristics of included studies *[ordered by study ID]*

#### Baviera 2010

Clinical features and settings	Routine screening.
Participants	579 participants: 17 cases and 562 controls matched for gestational age Italy - single centre. December 2006-May 2009. Pregnant women. Mean maternal age 35.3 years (cases) and 30.4 years (controls) Singleton pregnancies. 7-10 and 14-17 weeks' gestation.
Study design	Case- control study.
Target condition and reference standard(s)	Down's syndrome: 17 cases (14 identified by amniocentesis, 3 from follow-up to birth) Reference standards: amniocentesis or follow-up to birth.
Index and comparator tests	Frozen serum samples tested for: First trimester and second trimester ADAM12s (time resolved fluorescence immunoassay, DELFIA assay kit, Perkin Elmer Life and Analytical Sciences) First trimester PAPP-A (details not reported). Second trimester AFP, uE3 and hCG (details not reported).
Follow-up	Details of follow-up not reported.
Aim of study	To demonstrate the potential value of repeated measures of ADAM12s for the screening of Down's syndrome
Notes	

#### *Table of Methodological Quality*

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.

**Baviera 2010** (Continued)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Benattar 1999**

Clinical features and settings	Routine screening.
Participants	1656 participants. France - single centre. January to December 1995. Singleton pregnancies. Pregnant women. Mean age 32 years (16-46 years). Enrolled before 13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 5 cases. Reference standards: amniocentesis due to maternal age > 38 years (6.1% of women) . Karyotyping encouraged for women with positive result on 1 or more index test. No details of reference standard for index test negative women
Index and comparator tests	Maternal age. NT at 12-14 weeks (Toshiba SSA 270), cut-point 1/250. First trimester (12-14 weeks) serum AFP and free $\beta$ hCG (Elsa AFP and Elsa free $\beta$ hCG; Cis-Bio International) Second trimester (15-18 weeks) serum AFP and total hCG (AFP-2T and hCG-60; Ortho-Clinical Diagnostics)
Follow-up	Details of follow-up not stated. Unclear whether women were followed up to birth
Aim of study	To evaluate the sequential combination of ultrasound screening for fetal aneuploidy at 11-14 weeks with maternal biochemistry at 12-14 and 15-18 weeks of gestation

**Benattar 1999** (Continued)

Notes		
<i>Table of Methodological Quality</i>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	Yes	12 patients were lost to follow-up due to miscarriages.

**Biagiotti 1995**

Clinical features and settings	High-risk referral for invasive testing.
Participants	287 participants: 41 cases and 246 controls matched for maternal and gestational age, and duration of sample storage (from cohort of 4452 participants undergoing invasive testing) Italy - single centre. Dates not specified. Pregnant women. Singleton pregnancies. 8-12 weeks' gestation.

**Biagiotti 1995** (Continued)

Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 41 cases. Reference standards: amniocentesis or CVS.
Index and comparator tests	Maternal age. Frozen samples tested for: First trimester AFP - AFP-M-K S Kit. First trimester uE3 - Amersham Amerlex M. First trimester Intact hCG - Hybritech tandem. First trimester free $\beta$ hCG - ELSA Free beta hCG CIS.
Follow-up	100% karyotyping.
Aim of study	Evaluate first trimester maternal serum AFP, uE3 and hCG to assess the efficacy of different combinations of these markers on a screening test for Down's syndrome in the first trimester
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had invasive testing.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (lab analysis blinded)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice



**Biagiotti 1995** (Continued)

Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Biagiotti 1998**

Clinical features and settings	High-risk referral for invasive testing.	
Participants	232 participants: 32 cases and 200 randomly selected controls (selected from series of 3731 women) Italy - single centre. July 1993 to December 1996. Pregnant women. Singleton pregnancies. 10 to 13 weeks' gestation.	
Study design	Retrospective case-control study.	
Target condition and reference standard(s)	Down's syndrome: 32 cases. Reference standards: amniocentesis or CVS.	
Index and comparator tests	Maternal age. First trimester NT (in longitudinal section of the fetus with caliper measurements to the nearest 0.1 mm) First trimester serum PAPP-A (Amerlex-M PAPP-A IRMA, Ortho-Clinical Diagnostics) First trimester serum free $\beta$ hCG (Elsa9free B-hCG CIS).	
Follow-up	100% karyotyping.	
Aim of study	To evaluate the potential effectiveness of maternal serum PAPP-A and free beta hCG in combination with NT measurement in the first trimester of pregnancy	
Notes		

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.

**Biagiotti 1998** (Continued)

Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Unclear	No details of withdrawals given.

**Brambati 1993**

Clinical features and settings	High-risk referral for invasive testing.
Participants	522 participants. Italy. Dates not specified. Pregnant women. Median age 38 years (20-47 years). Singleton pregnancies. 6-11 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 14 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen blood sample tested for: First trimester serum PAPP-A (radio-immunoassay).
Follow-up	100% karyotyping. 47 women who miscarried prior to CVS were excluded from the study
Aim of study	To assess the relationship between maternal serum PAPP-A in the first trimester and the outcome of pregnancy by karyotype

**Brambati 1993** (Continued)

Notes		
<i>Table of Methodological Quality</i>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Brambati 1994**

Clinical features and settings	High-risk referral for invasive testing.
Participants	102 participants: 13 case and 89 randomly selected controls matched for gestational age Italy. Dates not specified. Pregnant women. 8-12 weeks' gestation.
Study design	Case-control study.

**Brambati 1994** (Continued)

Target condition and reference standard(s)	Down's syndrome: 13 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester PAPP-A (radioimmunoassay, as described in Sinosich 1982) First trimester free $\beta$ hCG (radioimmunoassay, CIS, UK).
Follow-up	100% karyotyping.
Aim of study	To report the results for the combined measurement of serum PAPP-A and free- $\beta$ hCG in women attending for prenatal diagnosis
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical testing conducted blind to karyotype results)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements

**Brambati 1994** (Continued)

Withdrawals explained? All tests	No	No details of withdrawals given.
-------------------------------------	----	----------------------------------

**Brameld 2008**

Clinical features and settings	Routine screening.
Participants	22,280 participants with complete screening results and outcome data August 2001-October 2003. Australia - State-wide screening programme evaluation. Pregnant women. Median maternal age 31 years (range 14-47 years), 20% ≥ 35 years Singleton pregnancies. 10-14 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 60 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester PAPP-A, free βhCG and NT (details not reported) Risk cut-point 1:300.
Follow-up	Data on outcome from the Western Australia Midwives data collection, Birth Defects Registry and hospital morbidity and mortality data
Aim of study	To identify first trimester indicators of adverse pregnancy outcomes
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.

**Brameld 2008** (Continued)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Brizot 1994**

Clinical features and settings	High-risk referral for invasive testing.
Participants	393 participants: 45 cases of Down's syndrome and 348 controls matched for crown rump length, maternal age and storage time UK. Dates not specified. Pregnant women. Median age 38 years (22-45 years). Singleton pregnancies. 10-13 weeks' gestation.
Study design	Retrospective case-control study.
Target condition and reference standard(s)	Down's syndrome: 45 cases. Reference standard: fetal karyotyping.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First Trimester PAPP-A (Double sandwich time resolved immunofluorometric assay with chelated europium as a label. Antibody to PAPP-A binding Ig was polyclonal rabbit IgG in stabilised solution)
Follow-up	100% karyotyping
Aim of study	To determine if the risk for fetal trisomies during the first trimester of pregnancy can be derived by combining data from maternal serum PAPP-A and fetal NT thickness
Notes	Cases were pre-selected for increased NT thickness.

<i>Table of Methodological Quality</i>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical testing conducted blind to karyotype results)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Casals 1996**

Clinical features and settings	High-risk referral for invasive testing.
Participants	1138 participants. Spain. 1990-1993. Pregnant women. 94.4% of women aged > 35 years. Singleton pregnancies. 10-13 weeks' gestation.
Study design	Retrospective case-control study.

Target condition and reference standard(s)	Down's syndrome: 19 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester serum free $\beta$ hCG (IMx microparticle enzyme immunoassay technology) (19 case and > 80 control samples) First trimester serum AFP (Stratus fluorometric enzyme immunoassay) (19 case and > 80 control samples) First trimester serum PAPP-A (radioimmunoassay) (only for 16 case and 80 control samples)
Follow-up	100% karyotyping.
Aim of study	To examine the value of $\beta$ hCG, AFP and PAPP-A in biochemical screening for Down's syndrome in 19 women carrying Down's syndrome versus normal pregnancies
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index test interpreted without knowledge of reference standard results (PAPP-A testing conducted blind to CVS results but presence of blinding is not stated for free $\beta$ hCG and AFP)



**Casals 1996** (Continued)

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Christiansen 1999**

Clinical features and settings	High-risk referral for invasive testing.	
Participants	181 participants (for first trimester serum samples): 25 cases and 156 controls matched for length of storage Denmark. Dates not specified. Pregnant women. 5-20 weeks' gestation.	
Study design	Case-control study.	
Target condition and reference standard(s)	Down's syndrome: 25 cases. Reference standard: karyotyping.	
Index and comparator tests	Maternal age. Frozen serum samples tested for: First and second trimester ProMBP (2 site immunoradiometric assay samples reduced and alkylated and added to microtitre wells coated with monoclonal antibody J13 6B6)	
Follow-up	100% karyotyping.	
Aim of study	To examine whether the maternal serum concentration of ProMBP was influenced by the presence of a Down's syndrome fetus. To evaluate its potential as a screening marker for Down's syndrome in the first and second trimester of pregnancy. To examine the performance characteristics of a serum screening programme using ProMBP in combination with age as risk markers	
Notes		

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.

**Christiansen 1999** (Continued)

Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Christiansen 2004**

Clinical features and settings	Routine screening.
Participants	334 participants: 156 cases, 546 control samples (348 control women, 198 of these were sampled from the same women in first and second trimesters) Denmark. Dates not specified. Pregnant women. Singleton pregnancies. 4-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 156 cases. Reference standard: CVS (for 120 of cases of Down's) or follow-up to birth (for 36 of cases of Down's)
Index and comparator tests	Maternal age. Frozen serum samples tested for: First/second trimester hCG and AFP (AutoDELFIA analytical system)

	First/second trimester PAPP-A and SP1 (in-house sandwich immunoassays) First/second trimester ProMBP (2 site immunoradiometric assay (IRMA)) First/second trimester free $\beta$ hCG and some AFP (dual label kit)
Follow-up	The Danish Cytogenetic Central Registry was routinely used to ascertain that none of the controls were pregnancies with a chromosomally diseased fetus
Aim of study	To evaluate 6 markers of fetal Down's syndrome pregnancies (includes first trimester markers)
Notes	Unclear criteria for the selection of controls.

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Christiansen 2005**

Clinical features and settings	Screening programmes for syphilis and Down's syndrome.
Participants	108 participants: 27 cases of Down's syndrome and 81 controls Denmark - Statens Serum Institute. Dates not specified. 5-11 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 27 cases (18 diagnosed in 2nd trimester, 9 at birth) Reference standard: karyotyping.
Index and comparator tests	Maternal age. First trimester (week 11-14) NT. Frozen samples tested for: First trimester (week 5-11) 1T Inhibin A (dimer assay kit MCA 950KZZ, Serotec) First trimester (week 5-11) $\beta$ hCG (available for some samples) First trimester (week 5-11) PAPP-A (available for some samples) (combined PAPP-A and B-hCG TrIFMA assay) Risk cut-points of 1:100, 1:250 and 1:400. Performance assessed with SPlus algorithm.
Follow-up	All diagnosis were verified by karyotyping.
Aim of study	To investigate whether first trimester Inhibin A can be used in the first trimester for Down's syndrome screening
Notes	Identified through the Danish central cytogenetic registry as part of quality assurance programme

***Table of Methodological Quality***

<b>Item</b>	<b>Authors' judgement</b>	<b>Description</b>
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women had a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

**Christiansen 2005** (Continued)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of NT results.
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Christiansen 2007a**

Clinical features and settings	Routine screening.
Participants	183 participants: 47 cases and 136 controls matched for gestational age Dates not reported. Denmark - Statens Serum Institute. Pregnant women. Singleton pregnancies. Median age cases 37.7 years (24-48 years) and controls 36.4 years (22-44 years) 8-14 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 47 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. Fresh serum samples tested for: First trimester PAPP-A and free $\beta$ hCG (AutoDelfia, PerkinElmer, Turku or Kryptor, Brahms) Frozen serum samples tested for: First trimester human placental lactogen (hPL) (hPL ELISA, enzyme immunoassay (EIA) -1283, DRG Instruments GmbH)
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry
Aim of study	To examine the potential of human placental lactogen as a first trimester maternal serum screening marker for fetal Down's syndrome
Notes	

Christiansen 2007a (Continued)

<i>Table of Methodological Quality</i>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Christiansen 2009**

Clinical features and settings	Routine screening.
Participants	335 participants: 74 cases and 261 controls matched for length of sample storage and maternal age Denmark - screening programme. Dates not reported. Pregnant women. Singleton pregnancies. Median maternal age cases 37.5 years and controls 36.4 years 8-13 weeks' gestation.
Study design	Case-control study.

Target condition and reference standard(s)	Down's syndrome: 74 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (details not reported). Fresh serum samples tested for: First trimester PAPP-A and free $\beta$ hCG (AutoDelfia, PerkinElmer, Turku or Kryptor, Brahms) Frozen serum samples tested for: First trimester placental growth hormone (PGH) (double monoclonal ELISA, DSL-10-19 200, Diagnostic Systems Laboratory Inc) First trimester growth hormone binding protein (GHBP) (Enzyme-amplified ELISA, DSL-10-48 100, Diagnostic Systems Laboratory Inc)
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry
Aim of study	To examine the potential of placental growth hormone and growth hormone binding protein as maternal serum screening markers for Down's syndrome
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice

**Christiansen 2009** (Continued)

Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Christiansen 2010**

Clinical features and settings	Routine screening.	
Participants	531 participants: 28 cases and 503 controls. Denmark - screening programme. Dates not specified. Pregnant women. Singleton pregnancies. Median age cases 36 years (range 25-44 years) and controls 29 years (range 17-45 years) 8-14 weeks' gestation.	
Study design	Case-control study.	
Target condition and reference standard(s)	Down's syndrome: 28 cases. Reference standards: karyotyping or follow-up to birth.	
Index and comparator tests	Maternal age. First trimester NT (details not reported). First trimester PAPP-A and free $\beta$ hCG (details not reported). First trimester ADAM12s (AutoDELFIA/Delfia ADAM12 Research kit 4025-0010, PerkinElmer Life and Analytical Sciences, on the 1235 AutoDELFIA automatic immunoassay system)	
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry	
Aim of study	To examine the efficiency of a second generation assay for ADAM12	
Notes		

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.



**Christiansen 2010** (Continued)

Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Cowans 2010**

Clinical features and settings	Routine screening.
Participants	445 participants: 70 cases and 375 controls matched for storage time and gestational age January 2007-October 2008. UK. Pregnant women. Singleton pregnancies. Mean maternal age cases 37.0 years (IQR 32.9-40.5 years) and controls 32.4 years (IQR 29.0-35.9 years) 11-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 70 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF certified sonographers). Fresh serum samples tested for: First trimester PAPP-A and free $\beta$ hCG (Kryptor analyser, Brahms) Frozen serum samples tested for: First trimester placental growth factor (PIGF) (Solid-phase, two-site fluoroimmuno-metric research assay (4083-0010) on 6000 DELFIA Xpress random access platform, PerkinElmer) Modelled on UK 2002 population data.

Follow-up	Karyotype and results for pregnancy outcome were received from cytogenetics laboratories and maternity units where deliveries took place
Aim of study	To examine placental growth factor levels in first trimester maternal serum in trisomy 21 pregnancies and to investigate the potential value of PIGF in a first trimester screening test
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Crandall 1993**

Clinical features and settings	High-risk referral for invasive testing.
Participants	893 participants. USA - 3 centres. Dates not specified. Pregnant women, 90% > 35 years. 11-15 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 11 cases. Reference standard: amniocentesis.
Index and comparator tests	Maternal age. Frozen samples tested for: First trimester serum AFP (Tandem E kit). First trimester serum uE3 (Amerlex M). First trimester serum hCG (Hybritech tandem E kit).
Follow-up	100% karyotyping.
Aim of study	To investigate whether hCG is a useful predictor of Down's syndrome between 11 and 15 weeks' gestation
Notes	Unclear criteria for the selection of controls.

***Table of Methodological Quality***

<b>Item</b>	<b>Authors' judgement</b>	<b>Description</b>
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Amniocentesis.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results

**Crandall 1993** (Continued)

Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	33 samples excluded because out of the date range or insufficient sample volume or information
Withdrawals explained? All tests	No	No details of withdrawals given.

**Crossley 2002a**

Clinical features and settings	Routine screening.
Participants	17,229 participants. UK - 15 centres. Dates not specified. Pregnant women with median age 29.9 years, 15.4% ≥ 35 years. 10-14 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 45 cases. Reference standards: CVS offered where women had high NT measurements, amniocentesis or follow-up to birth
Index and comparator tests	Maternal age. NT (FMF method) in 73% of patients. Clotted blood samples tested for: Free βhCG and PAPP-A (Kryptor analyser) in 98.4% of patients
Follow-up	Reported that the outcome of all pregnancies was followed up
Aim of study	To evaluate the use of ultrasound measurements of fetal NT obtained in a routine antenatal clinic setting in combination with appropriate biochemical markers as a first trimester screening test for Down's syndrome
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
------	--------------------	-------------

**Crossley 2002a** (Continued)

Representative spectrum? All tests	Yes	Routine screening of typical pregnant population .
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	Report average success rate of NT (72.9%).
Withdrawals explained? All tests	Yes	Numbers of patients not undergoing NT and biochemical testing given

**De Graaf 1999a**

Clinical features and settings	High-risk referral for invasive testing.
Participants	292 participants (207 participants before 14 weeks' gestation): 37 cases and 255 controls matched for maternal age (within 2 years), gestational age (within 2 weeks) and duration of sample storage (within 2 months) The Netherlands - single centre. 1994-1997. Pregnant women. 9-15 weeks' gestation (in a few cases, blood samples for serum testing taken at 15-19 weeks)
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 37 cases (30 affected pregnancies in women with serum testing enrolled before 14 weeks' gestation) Reference standards: CVS or amniocentesis.

Index and comparator tests	Maternal age. First trimester NT (FMF methods) with cut off > 3 mm. Frozen serum samples tested for: First trimester free $\beta$ hCG and AFP (DELFI A dual labelled time resolved fluorescent assay) First trimester serum PAPP-A (DELFI A research assay (CR61-105)) First trimester serum AFP.
Follow-up	100% karyotyping.
Aim of study	To determine the expected detection rate and false positive rate for Down's syndrome achievable by early pregnancy screening with combined measurements of serum PAPP-A, free beta hCG and fetal NT, with the addition of AFP
Notes	

*Table of Methodological Quality*

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women had a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	Yes	Failed to measure NT in 11 control women.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Forest 1995**

Clinical features and settings	Routine screening.
Participants	1023 participants (512 first trimester participants). Canada - 6 centres. June 1989-October 1993. Pregnant women. 23 cases of Down's syndrome (12 in of women recruited first trimester and 11 in second trimester) 1000 control samples (100 for each gestational week from 9-18) matched to the age of the original cohort in which Down's cases were detected (n = 14,612) Mean maternal age 29.1 (SD 4.7) years, 10.7% aged $\geq$ 35 years Singleton pregnancies. 9-13 (first trimester) and 14-18 (second trimester) weeks' gestation
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 12 cases (first trimester). Reference standards: follow-up to birth.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester AFP and total hCG (Enzymum-Test enzyme immunoassay analyser, ES-300 analyser) First trimester uE3 (ultra sensitive radioimmunometric assay) First trimester free $\alpha$ hCG and free $\beta$ hCG (radioimmunometric assays) 3 different models used for risk calculation (Wald, Spencer and Ryall)
Follow-up	Review of maternal and neonatal charts in each centre.
Aim of study	Evaluate the impact of risk estimation parameters for screening for Down's syndrome during the first and second trimesters of pregnancy
Notes	3 different models used for risk calculation (Wald, Spencer and Ryall)

***Table of Methodological Quality***

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if women received different reference standards.

**Forest 1995** (Continued)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Forest 1997**

Clinical features and settings	Routine screening.
Participants	518 participants. Canada - 6 centres. June 1989-January 1995. Pregnant women. 18 cases of Down's syndrome. 500 control samples (representative of the cohort from which they were taken: n = 10, 160) 100 for each gestational week from 9-13 weeks. Mean maternal age 27.9 years, 10.7% aged ≥ 35 years. Singleton pregnancies. 9-13 weeks' gestation at enrolment.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 18 cases. Reference standards: follow-up to birth.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester PAPP-A (fluorescence-linked immunosorbent assay) First trimester free βhCG (radioimmunoassay, Bioclone Australia) Risk cut-point 1:384.
Follow-up	Review of maternal and neonatal charts in each centre and consulting the database of the local cytogenetic laboratories



**Forest 1997** (Continued)

Aim of study	To confirm the usefulness of free $\beta$ hCG and AFP as first trimester screening markers in a prospective study
Notes	Exclusion of cases of babies that died before 20 weeks' gestation. Unclear criteria (apart from age) for the selection of controls

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if women received different reference standards.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Gyselaers 2005**

Clinical features and settings	Routine screening.
Participants	13,267 participants (13,207 participant received both NT test and serum testing) Belgium - multicentre study (35 centres). First Jan 2004-First April 2004 (data added to previous database from before 2003) Pregnant women. Singleton pregnancies.

Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 26 cases. Reference standards: amniocentesis, CVS and postnatal karyotyping
Index and comparator tests	Maternal age. First trimester serum PAPP-A (ELISA 2397, DRG International Inc) First trimester serum free $\beta$ hCG (free $\beta$ hCG IRMA K1P1001, BioSource Europe SA) Second trimester PAPP-A and free $\beta$ hCG. First trimester NT. Risk cut-points of 1:200 and 1:300.
Follow-up	Follow-up to birth reported by mail by obstetricians. Non-responding obstetricians contacted personally to obtain missing data. Cases of miscarriages (n = 49) and other fetal chromosomal abnormalities excluded from the study. Unclear if other patients were lost to follow-up
Aim of study	To evaluate the performance of a first trimester fetal aneuploidy screening programme
Notes	Women with miscarriages or cases of other chromosomal defects were excluded from the study. 9 live births of babies with Down's syndrome

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results

**Gyselaers 2005** (Continued)

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	Yes	Numbers of women excluded due to miscarriage or other chromosomal defects and numbers not undergoing NT and biochemical testing reported

**Haddow 1998**

Clinical features and settings	High-risk referral for invasive testing.	
Participants	3217 participants. USA - 16 prenatal diagnostic centres. June 1994 to November 1996. Pregnant women aged 15-51 years (median 37 years). Singleton pregnancies. 9-14 weeks' gestation.	
Study design	Prospective cohort study.	
Target condition and reference standard(s)	Down's syndrome: 48 cases. Reference standards: amniocentesis or CVS.	
Index and comparator tests	Maternal age. Fresh serum sample tested for: First trimester serum hCG (hCG MAIA clone assay). First trimester serum PAPP-A (enzyme-linked immunosorbent assay, Dako) First trimester free $\beta$ hCG and AFP - Fluoroimmunoassay (DELFLIA hAFP/Free beta hCG dual kit) First trimester uE3 (Ultrasensitive uE3 kit).	
Follow-up	100% karyotyping.	
Aim of study	To further examine the efficacy of serum and ultrasound screening for Down's syndrome in the first trimester and the possible advantages and disadvantages of screening at this time rather than in the second trimester	
Notes		

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.

**Haddow 1998** (Continued)

Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Kagan 2009**

Clinical features and settings	Routine screening.
Participants	56,954 participants with available outcome data. UK - multicentre study. July 1999 - April 2007. Pregnant women. Singleton pregnancies. Mean maternal age 35.4 years (14.1-52.2 years). 11-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 395 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT. First trimester fetal heart rate (pulsed-wave Doppler). First trimester nasal bone (FMF certified sonographers).

	<p>First trimester ductus venous flow (FMF certified sonographers)</p> <p>First trimester flow across tricuspid valve (FMF certified sonographers)</p> <p>First trimester PAPP-A and free <math>\beta</math>hCG (Kryptor, Brahms AG or Delfia Express, Perkin Elmer)</p> <p>Multiple publications with different test evaluations.</p>
Follow-up	Karyotype results and details of pregnancy outcome were added to databases as they became available. Women without complete screening and outcome data (n = 3053, 5.1%) were excluded from the study
Aim of study	<p>To examine the performance of first-trimester screening for trisomies 21, 18 and 13 by maternal age, fetal NT thickness, fetal heart rate and maternal serum free <math>\beta</math>-hCG and PAPP-A</p> <p>Other objectives in related publications.</p>
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements

**Kagan 2009** (Continued)

Withdrawals explained? All tests	No	No details of withdrawals given.
-------------------------------------	----	----------------------------------

**Kornman 1998**

Clinical features and settings	High-risk referral for invasive testing.
Participants	The Netherlands - antenatal diagnosis unit. October 1990-February 1994. Pregnant women. 15 cases of Down's syndrome. 97 control samples (matched on gestational age, sample storage time and maternal age) Singleton pregnancies. 8-12 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 15 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester serum SP1 (modified commercial radioimmunoassay, RIA-gnost SP1))
Follow-up	100% karyotyping.
Aim of study	To compare SP1 levels in Down's syndrome versus normal pregnancies
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

**Kornman 1998** (Continued)

Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Kozlowski 2007 GC**

Clinical features and settings	Routine referral.
Participants	6906 participants with complete outcome data. Germany - gynaecologists practices. January 2000-December 2003. Pregnant women. Median maternal age 32 years (15-48 years), 26.4% ≥ 35 years 11-14 weeks' gestation.
Study design	Cohort study.
Target condition and reference standard(s)	Down's syndrome: 19 cases. Reference standard: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF certified gynaecologists). First trimester free βhCG and PAPP-A (Kryptor analyser, Brahms) Risk cut-point 1:300.
Follow-up	Data on pregnancy outcome were obtained by contacting the patient or their general gynaecologist. Women without complete outcome data (36%) were excluded from the study
Aim of study	To evaluate and compare the screening performance for fetal trisomy 21 in the first trimester of pregnancy in general gynaecologists practices and specialised centres for prenatal care in Germany
Notes	

**Table of Methodological Quality**

**Kozlowski 2007 GC** (Continued)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	146 women (including 11 with Down's syndrome) excluded as results could not be assigned to gynaecologists or prenatal centre group.

**Kozlowski 2007 PC**

Clinical features and settings	Routine referral.
Participants	3862 participants with complete outcome data. Germany - tertiary level prenatal centres. January 2000-December 2003. Pregnant women. Median maternal age 34 years (14-46 years), 43.2% ≥ 35 years 11-14 weeks' gestation.
Study design	Cohort study.
Target condition and reference standard(s)	Down's syndrome: 26 cases. Reference standard: karyotyping or follow-up to birth.



Index and comparator tests	Maternal age. First trimester NT (FMF certified sonographers). First trimester free $\beta$ hCG and PAPP-A (Kryptor analyser, Brahms) Risk cut-point 1:300.
Follow-up	Data on pregnancy outcome were obtained by contacting the patient or their general gynaecologist. Women without complete outcome data (8%) were excluded from the study
Aim of study	To evaluate and compare the screening performance for fetal trisomy 21 in the first trimester of pregnancy in general gynaecologists practices and specialised centres for prenatal care in Germany
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	146 women (including 11 with Down's syndrome) excluded as results could not be assigned to gynaecologists or prenatal centre group.

**Krantz 2000**

Clinical features and settings	Routine screening.
Participants	10,251 participants. USA. September 1995 to June 1998. Pregnant women. Singleton pregnancies. Maternal age 34.7% $\geq$ 35 years. No diabetes. 9-13 weeks' gestation.
Study design	Prospective cohort..
Target condition and reference standard(s)	Down's syndrome: 50 cases (33 had undergone biochemical testing) Reference standard: not reported.
Index and comparator tests	Maternal age. Dried blood samples tested for: First trimester NT in 5809 patients (FMF methods). First trimester free $\beta$ hCG and PAPP-A in 10,251 patients (enzyme-linked immunosorbent assay procedures)
Follow-up	No details of follow-up reported.
Aim of study	To assess the effectiveness of free $\beta$ hCG, PAPP-A and NT for first-trimester screening for Down's syndrome and trisomy 18
Notes	

***Table of Methodological Quality***

<b>Item</b>	<b>Authors' judgement</b>	<b>Description</b>
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear reference standard.
Partial verification avoided? All tests	Unclear	Unclear if all patients had a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if choice of reference depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

**Krantz 2000** (Continued)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Kratzer 1991**

Clinical features and settings	High-risk referral for invasive testing.
Participants	141 participants. USA. Dates not stated. Pregnant women. Controls matched for maternal age. Singleton pregnancies. 9-12 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 17 cases Reference standard: CVS
Index and comparator tests	Frozen serum samples tested for: First trimester hCG and free $\beta$ hCG (double antibody radio-immunoassay) First trimester free $\alpha$ hCG (radio-immunoassay, monoclonal antibody, Biomerica Inc) First trimester progesterone (radio-immunoassay).
Follow-up	100% karyotyping.
Aim of study	To present evidence on the value of first trimester serum assays as an early, non-invasive screen for aneuploidy
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
------	--------------------	-------------

**Kratzer 1991** (Continued)

Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index tests interpreted without knowledge of reference standard results (index tests conducted blind to outcome)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Laigaard 2003**

Clinical features and settings	Routine screening.
Participants	172 participants (18 cases of Down's syndrome and 154 controls) Denmark - University hospital Dates not specified. Pregnant women. Singleton pregnancies. 8-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 18 cases. Reference standards: karyotyping, unclear reference standard for controls

**Laigaard 2003** (Continued)

Index and comparator tests	Frozen serum tested for: First trimester ADAM12 (ELISA, 6E6 and 8F8 antibodies).
Follow-up	No details of follow-up reported.
Aim of study	To determine whether ADAM12 concentration is a useful indicator of fetal health
Notes	

***Table of Methodological Quality***

<b>Item</b>	<b>Authors' judgement</b>	<b>Description</b>
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear reference standard in controls.
Partial verification avoided? All tests	Unclear	Unclear if all women had a reference standard.
Differential verification avoided? All tests	No	Different reference standards used.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Macintosh 1993**

Clinical features and settings	High-risk referral for invasive testing.
Participants	692 participants. UK and Italy. Dates not specified. Pregnant women. Median maternal age 38 years (27-40 years). Singleton pregnancies. 6-12 weeks' gestation.
Study design	Retrospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 14 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum tested for: First trimester serum SP1 (Radioimmunoassay).
Follow-up	100% karyotyping.
Aim of study	To examine the relationship between first trimester maternal serum SP1 and the karyotype of the pregnancy and to quantify its potential use as a screening test
Notes	

***Table of Methodological Quality***

<b>Item</b>	<b>Authors' judgement</b>	<b>Description</b>
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results

**Macintosh 1993** (Continued)

Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Muller 2003a**

Clinical features and settings	Routine screening.
Participants	5694 pregnant women who had first trimester NT and biochemical testing France - 9 centres serving 12 maternity units. January 1998-June 2001. Singleton pregnancies. Maternal age not reported. 11-13 weeks' gestation.
Study design	Retrospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 26 cases. Reference standards: invasive testing (offered to women with high NT measurement) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT in 98% of patients (methods not specified. 60 sonographers - 2 trained by FME, who trained 30 in turn. 8 externally trained in France. 20 were self-taught. Machines not specified) Frozen serum tested for: First trimester PAPP-A (99% of patients), free $\beta$ hCG 99% of patients and AFP (93% of patients) (time-resolved fluorescent assay, Perkin-Elmer Life sciences) Risk cut-point 1:250.
Follow-up	Data from the French national screening programme used for follow-up at birth. 211 women (3.7%) who did not return after NT or were found to be > 14 weeks were excluded. It is unclear how many patients had follow-up to birth
Aim of study	Prospective study of NT and retrospective evaluation of serum (in same patient population) to evaluate whether or not to move the national French Down's screening programme to a first trimester programme
Notes	FME methods - some self-taught sonographers.

<i>Table of Methodological Quality</i>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice .
Uninterpretable results reported? All tests	Yes	Women with NT too small to measure assumed to have NT of < 0.5 mm
Withdrawals explained? All tests	Yes	Women failing to return or who more than 14 weeks' pregnant were excluded (214)

**Nebiolo 1990**

Clinical features and settings	High-risk referral for invasive testing.
Participants	492 participants. Italy. Dates not specified. Pregnant women, approximately 75% were aged $\geq 35$ years. Singleton pregnancies. 8-12 weeks' gestation.
Study design	Retrospective cohort study.



Target condition and reference standard(s)	Down's syndrome: 9 cases. Reference standard: CVS.
Index and comparator tests	Frozen serum tested for: First trimester serum AFP and beta/alpha hCG ratio (simultaneous sandwich monoclonal based radioimmunoassay)
Follow-up	100% karyotyping.
Aim of study	To determine the efficacy of combined maternal serum AFP and hCG screening in detecting chromosome defects in the first trimester of pregnancy
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical tests conducted blind to pregnancy outcome)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	Yes	Samples from 48 patients were either no longer available or had been stored at 4°C and were discarded.

**Nebiolo 1990** (Continued)

Withdrawals explained? All tests	No	No details of withdrawals given.
-------------------------------------	----	----------------------------------

**Niemimaa 2001a**

Clinical features and settings	Routine screening.
Participants	2515 participants. Finland - primary care centres and maternity clinics of hospitals During 1999. Pregnant women, 17.5% $\geq$ 35 years. 10-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 8 cases. Reference standards: invasive testing (patients considered high risk based on NT screening) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT ( $\geq$ 3 mm) (64% of women) (method not described) Fresh serum tested for: First trimester free $\beta$ hCG and PAPP-A (Wallac analytes and 1st trimester risk calculation programme Maternal weight correction) Risk cut-point 1:250.
Follow-up	Follow-up data from maternity clinics and the National Research and Development Centre for Welfare and Health. Test negative patients followed up by contacting all maternity clinics and the National Research and Development Centre for Welfare and Health. Unclear if follow-up information was obtained in all cases
Aim of study	To evaluate efficacy of combining first trimester maternal serum and fetal NT measurement in screening for Down's syndrome in Finland
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.

**Niemimaa 2001a** (Continued)

Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Noble 1995**

Clinical features and settings	Routine screening in a high-risk population.
Participants	2529 participants. UK. October 1994 to April 1995. Singleton pregnancies. Pregnant women. Median maternal age 34 years (15-47 years), 47% ≥ 35 years. 10-14 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 61 cases. Reference standards: karyotyping performed (27% of women) due to increased NT (14%), advanced maternal age (10%), previous chromosomally abnormal child (0.5%) or parental anxiety (2%). Ultrasound examination at 20 weeks (65% of patients). Follow-up to birth (9% of women)
Index and comparator tests	Maternal age. First trimester NT (methods not stated). Fresh serum (or serum frozen over a weekend) tested for: First trimester free βhCG (immunoradiometric assay, CIS).
Follow-up	Pregnancy outcome obtained from maternity units or the patients themselves. Follow-up information only appears to have been obtained in 9% of cases (second trimester

**Noble 1995** (Continued)

	ultrasound used as reference standard for other women)
Aim of study	To measure the contribution of maternal serum free $\beta$ hCG in a screening programme for fetal trisomy 21 based on fetal NT in the first trimester of pregnancy
Notes	No proper results, data are presented for this study.

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	No	Invasive testing, ultrasound at 20 weeks or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Noble 1997**

Clinical features and settings	Routine screening, women self-referred for first trimester NT
Participants	876 participants. UK - Research Centre for Fetal Medicine. Dates not stated.

	76 cases of Down's syndrome. 800 controls matched for maternal and gestational age. Pregnant women. Median maternal age 34 years (15-47 years). Singleton pregnancies. 10-14 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 76 cases. Reference standards: CVS, follow-up to birth not reported.
Index and comparator tests	Maternal age. Frozem serum tested for: First trimester serum Inhibin A (ELISA). Fresh serum (or serum stored over weekend) tested for: First trimester serum free $\beta$ hCG (immunoradiometric assay, CIS France)
Follow-up	Details of methods of follow-up not reported.
Aim of study	To determine the relationship between maternal serum first trimester Inhibin A and free $\beta$ hCG concentrations in chromosomally normal pregnancies and to compare 2 biochemical markers for their sensitivity in identifying trisomy 21 pregnancies
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear what the reference standard was.
Partial verification avoided? All tests	Unclear	Unclear if all women had a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if the reference standard differed between women.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results

**Noble 1997** (Continued)

Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical tests conducted blind to pregnancy outcome).
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**O'Leary 2006**

Clinical features and settings	Routine screening.
Participants	22,340 participants. Australia - 13 ultrasound practices. August 2001 to October 2003. Singleton pregnancies. Pregnant women aged 14-47 years (median 31 years), 20% ≥ 35 years 11-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 60 cases. Reference standards: CVS or amniocentesis (women assessed to be high risk on screening), or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (FMF methods). First trimester free βhCG and PAPP-A (machine not stated). All study participants underwent all tests. Risk cut-point 1:300.
Follow-up	Follow-up data obtained by review of the Midwives Notification System and the Birth Defects Registry. 415 patients (1.8%) excluded due to no follow-up data. Patients with multiple pregnancies or incomplete screens (n = 3946) were also excluded from the study
Aim of study	To assess fetal outcomes for pregnancies identified at increase risk for Down's syndrome by first trimester combined ultrasound examination and maternal serum biochemistry
Notes	Appears likely that patients with miscarriages and terminations excluded

*Table of Methodological Quality*

**O'Leary 2006** (Continued)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Unclear	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	Yes	Details given of patients excluded due to incomplete screening data or loss to follow-up

**Orlandi 1997**

Clinical features and settings	Routine screening of general- and high-risk women.
Participants	2,010 participants (744 in subgroup undergoing NT testing). Italy. Dates not reported. Recruited through private physician or genetic counselling program for women of advanced maternal age Pregnant women aged 15-46 years, 35% > 35 years. Singleton pregnancies. 9-13 weeks' gestation.
Study design	Cohort.

Target condition and reference standard(s)	Down's syndrome: 11 cases (7 in subgroup with NT testing). Reference standards: not reported.
Index and comparator tests	Maternal age. First trimester NT (37% of patients) (FMF methods, Toshiba SSA 250A or Acuson XP 10) First trimester free $\beta$ hCG and PAPP-A (all patients) (dried blood samples, enzyme-linked immunosorbent assays) Risk cut-point 1:380.
Follow-up	Not reported.
Aim of study	To evaluate first trimester combined screening for Down's syndrome
Notes	Unclear as to what reference standard (if any) was used. All cases of Down's syndrome identified had been picked up by screening

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Reference standard not reported.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if the choice of reference standard depended on screening results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements



**Orlandi 1997** (Continued)

Withdrawals explained? All tests	Yes	Details given of women undergoing NT but not biochemical testing
-------------------------------------	-----	--

**Palomaki 2007**

Clinical features and settings	Routine screening.
Participants	10,775 participants. Canada - General Hospital. October 2003-November 2004. Pregnant women. Mean maternal age 32.3 years (SD 4.6 years). 10-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 23 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (encouraged to only accept measurements from sonographers with FMF certification) First trimester PAPP-A (AutoDELFIA, PerkinElmer). First trimester hyperglycosylated-hCG (Nichols Advantage Specialty system, Nichols Institute Diagnostics)
Follow-up	From electronic record searches of local patient and cytogenetic records and case finding of local and regional birth records
Aim of study	To validate Down's syndrome screening protocols that include hyperglycosylated-hCG measurements
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.

**Palomaki 2007** (Continued)

Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Qin 1997**

Clinical features and settings	Routine screening.
Participants	702 participants. Copenhagen. Dates not specified. Pregnant women. 156 cases of Down's syndrome (25 in weeks 3-9 and 131 in weeks 10-20 gestation) 546 controls (260 in weeks 3-9 and 286 in weeks 10-20 gestation) 5-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 25 cases (3-9 weeks' gestation). Reference standards: CVS, amniocentesis, karyotyping at birth, unclear reference standard for controls
Index and comparator tests	Frozen serum tested for: First trimester schwangerschaftsprotein 1 (SP1) (non-competitive time-resolved immunofluorometric assay, A131, DAKO A/S))
Follow-up	No details of follow-up reported.
Aim of study	To assess the potential of the maternal concentration of schwangerschaftsprotein 1 as a marker for Down's syndrome pregnancies

Notes		
<b>Table of Methodological Quality</b>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Sahota 2010**

Clinical features and settings	Routine screening.
Participants	10,854 pregnancies with complete outcome data. China - University Hospital. January 2005-May 2008. Pregnant women. Singleton pregnancies. Median maternal age 33.1 years, 30.1% of women aged $\geq$ 35 years 10-13 weeks' gestation.
Study design	Retrospective cohort.

Target condition and reference standard(s)	Down's syndrome: 32 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF accredited sonographers, HDI 5000, Philips Medical System) First trimester PAPP-A and free $\beta$ hCG (kryptor analyser, Brahms Diagnostica GmbH)
Follow-up	Fetal karyotypes were entered into a database when information was available. Data on pregnancy outcomes were obtained from either a local maternity database (for those who delivered in the unit) or via telephone calls to patients
Aim of study	To assess the relative performance of a multi-stage first trimester screening protocol for fetal Down's syndrome
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Schaelike 2009**

Clinical features and settings	Routine screening.
Participants	10,668 participants with complete outcome data. Germany - Private centre. Pregnant women. November 2000-December 2006. Singleton pregnancies. Maternal age $\geq 35$ years in 31.0% of women. 11-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 59 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF certified physicians). First trimester PAPP-A and free $\beta$ hCG (Kryptor analyser, Brahms GmbH) Cut-point 1:300.
Follow-up	Information provided by either obstetric departments or obstetricians. Results obtained from CVS and amniocentesis, as well as karyotypes from aborted fetal tissue or postnatal investigations. 3.9% of women were lost to follow-up and were excluded from the study
Aim of study	To assess the performance of a combined first trimester screening concept for trisomies 21, 18 and 13 applied to a low- and high-risk patient sample in a specialised private centre for prenatal medicine.
Notes	

***Table of Methodological Quality***

<b>Item</b>	<b>Authors' judgement</b>	<b>Description</b>
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

**Schaelike 2009** (Continued)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Scott 2004**

Clinical features and settings	Routine screening.
Participants	2053 participants. Australia - Private practice (Sydney Ultrasound for Women). July 2000 to May 2002. Pregnant women 15-44 years (median 32 years). Singleton pregnancies. 11-14 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 5 cases. Reference standards: invasive testing or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF methods, sagittal plane, ATL 5000; Philips) First trimester free $\beta$ hCG and PAPP-A (kryptor analyser, Brahms Diagnostics) All participants had all tests. Risk cut-point 1:300.
Follow-up	Data obtained from referring doctors or patients via letter, phone or completed feedback form given at the time of consultation. Only cases of known outcome included in the study. 68 (1.3%) lost to follow-up, largely due to miscarriage (n = 20) and loss to follow-up (n = 40)
Aim of study	To report the sensitivity of combined first trimester biochemistry and ultrasound screening for Down's syndrome in an Australian private practice specialising in obstetric ultrasound
Notes	Only women having biochemical testing before NT were included in the study. This was done to avoid bias from women declining biochemical testing following negative NT

<i>Table of Methodological Quality</i>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	No details of withdrawals given.

**Spencer 1999a**

Clinical features and settings	Women referred for invasive testing or self-referred for screening
Participants	1156 participants. UK - Fetal medicine research centre. Dates not specified. 210 cases of Down's syndrome, maternal age 19-46 (median 38 years) 946 controls matched for gestational and maternal age, maternal age 15-47 years (median age 36 years) 10-14 weeks' gestation.
Study design	Case-control study.

Spencer 1999a (Continued)

Target condition and reference standard(s)	Down's syndrome: 210 cases. Reference standards: invasive testing (high-risk women) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (methods not reported). First trimester free $\beta$ hCG and PAPP-A (Kryptor analyser, time resolved amplified cryptate emission (TRACE))
Follow-up	Details of methods for follow-up to birth not reported.
Aim of study	To examine the potential impact of combining maternal age with fetal NT thickness and maternal serum free $\beta$ hCG and PAPP-A in screening for trisomy 21 at 10-14 weeks of gestation
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.



**Spencer 1999a** (Continued)

Withdrawals explained? All tests	No	No details of withdrawals given.
-------------------------------------	----	----------------------------------

**Spencer 2002a**

Clinical features and settings	Routine screening.
Participants	278 participants. UK - Single hosp1T ITAI study (OSCAR screening program). Samples collected since 1998. 54 cases of Down's syndrome, maternal age 20-44 years, median 36 years 224 controls (no details of selection), maternal age 16-41 years, median 30 years 11-13 weeks' gestation..
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 54 cases. Reference standards: no description given.
Index and comparator tests	Maternal age. First trimester NT (FMF methods). First trimester free $\beta$ hCG, PAPP-A and ThCG (Kryptor Analyser (TRACE) and automated immunofluorescent assays) All women underwent all tests.
Follow-up	Methods for follow-up to birth not reported.
Aim of study	To assess serum hyperglycosylated hCG for use in the first trimester of pregnancy as a marker of Down's syndrome
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth (Nicolaidis ref (OSCAR)).
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.

**Spencer 2002a** (Continued)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear of all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Torring 2010**

Clinical features and settings	Routine screening.
Participants	691 participants: 46 cases and 645 controls. Denmark - nationwide screening programme. Dates not reported. Pregnant women. Singleton pregnancies. Mean maternal age cases 35 years, controls 31 years. 8-11 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 46 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (11-13 weeks' gestation) (FMF certified sonographers) Fresh serum tested for: First trimester PAPP-A and free $\beta$ hCG (8-11 weeks' gestation) (Kryptor analyser, Brahms) Frozen serum tested for: First trimester ADAM12s (8-11 weeks' gestation) (Kryptor analyser, assay by Cezanne SAS, TRACE technology)
Follow-up	Details not reported.
Aim of study	To determine whether ADAM12s is a useful serum marker for fetal trisomy 21 using the mixture model

**Torring 2010** (Continued)

Notes		
<b>Table of Methodological Quality</b>		
Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Tsukerman 1999**

Clinical features and settings	Routine screening.
Participants	1595 participants. Belarus. Started January 1996. Pregnant women. 1,564 controls matched for gestational age and duration of storage 8-13 weeks' gestation.
Study design	Case-control study.

Target condition and reference standard(s)	Down's syndrome: 31 cases. Reference standards: karyotyping, karyotyping at birth, follow-up to birth not reported
Index and comparator tests	Frozen or fresh serum tested for: First trimester free $\beta$ hCG, AFP and PAPP-A (DELFI, EG&G Wallac Oy)
Follow-up	No details of follow-up reported.
Aim of study	To report results of a large population study looking at AFP, free $\beta$ hCG and PAPP-A in the first trimester of pregnancy among women having routine ultrasound dating as part of NT assessment
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Women received different reference standards.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Valinen 2007**

Clinical features and settings	Routine screening
Participants	7534 participants. Finland - screening programme. 2002-2004. Pregnant women. Singleton pregnancies. Mean maternal age 29.6 years, 18.6% $\geq$ 35 years. 10-12 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 30 cases (24 underwent NT as well as biochemical testing) Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (trained nurses, midwives and doctors) (4765 women) First trimester PAPP-A and free $\beta$ hCG (details not reported) (7534 women) Cut-point 1:250.
Follow-up	Contacted chromosome laboratory at the department of clinical genetics in the Oulu university clinic and the Finish Register of Congenital Malformation and the National Research and Development Centre for Welfare and Health
Aim of study	To compare the efficacy of both separate and combined maternal serum testing and fetal NT measurement in the first trimester screening for Down's syndrome in northern Finland
Notes	

***Table of Methodological Quality***

<b>Item</b>	<b>Authors' judgement</b>	<b>Description</b>
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

**Valinen 2007** (Continued)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Valinen 2009**

Clinical features and settings	Routine screening.
Participants	279 participants: 53 cases and 226 controls matched for maternal and gestational age and sample storage time Finland - screening programme. May 2002-December 2007. Pregnant women. Maternal age not reported. 9-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 53 cases (in 5 cases, NT not measured). Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. Fresh serum tested for: First trimester PAPP-A and free $\beta$ hCG (details not reported). Frozen serum tested for: First trimester ADAM12 (DELFI/AutoDELFI ADAM12 research kit, PerkinElmer Wallac) Cut-point 1:250.
Follow-up	Details not reported.
Aim of study	To investigate whether incorporating the measurement of ADAM12 in the risk calculation program LifeCycle can improve Down's syndrome screening in the first trimester
Notes	

*Table of Methodological Quality*

**Valinen 2009** (Continued)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

**Van Lith 1992**

Clinical features and settings	High-risk referral for invasive testing.
Participants	1372 participants (24 cases and 1348 controls, criteria for matching not reported) The Netherlands - 6 prenatal diagnostic centres. Dates not stated. Pregnant women. Less than 13 weeks' gestation.
Study design	Case-control study (changed from cohort).
Target condition and reference standard(s)	Down's syndrome: 24 cases. Reference standard: CVS.
Index and comparator tests	Frozen serum rested for: Total hCG (IMx hCG assay, Abbott).

Follow-up	100% karyotyping.
Aim of study	To assess the value of MS-hCG in the first trimester of pregnancy in screening for Down's syndrome
Notes	

*Table of Methodological Quality*

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical measurement conducted blind to pregnancy outcome)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.



**Wald 2003a**

Clinical features and settings	Routine screening.
Participants	606 participants. UK and Austria - multicentre trial. September 1996 to April 2000. Pregnant women: 101 cases, 505 controls matched for gestation, duration of storage and centre 9-13 and 14-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 101 cases. Reference standards: invasive testing (following second trimester screening) or follow-up to birth
Index and comparator tests	First trimester NT (midsagittal section, optimal magnification of thickness of translucent space between inner skin surface and fascia covering cervical spine (white black interface (outer) - black white interface (inner), 41 models of ultrasound machine, 20 minutes allotted scanning time) First and second trimester serum AFP, hCG, uE3, PAPP-A, free $\beta$ hCG (time resolved fluoroimmunoassay, AutoDELFIA) First and second trimester inhibin A (Sandwich enzyme linked immunosorbent assay, Oxford bioinnovation) First and second trimester urinary beta core fragment, total hCG, ITA and free $\beta$ hCG (ITA and beta core fragment, Quest diagnostics USA)
Follow-up	Follow-up by: 1) Staff at local hospitals completed a study outcome form at, or just after delivery, 2) Study records of CVS, amniocentesis or karyotype at birth linked to information from cytogenic laboratories, 3) Study records linked to records of cases of Down's syndrome from the National Down's Syndrome Cytogenetic Register, 4) Information obtained from local obstetrical outcome records, 5) Forms sent to all women with a request to return details of the outcome of their pregnancy, 6) individual searches in respect of women whose outcomes of pregnancy had not been obtained by any of the previous methods. 96% birth/karyotype full outcome documentation obtained
Aim of study	To identify the most effective, safe and cost-effective strategy for antenatal screening for Down's syndrome using NT, maternal serum and urine markers in the first and second trimesters of pregnancy and maternal age in various combinations
Notes	Performance of screening assessed at 17 weeks' gestation. Study tried to be non-interventional in the first trimester - second trimester testing was aimed to be used as the basis for any referral for invasive testing

***Table of Methodological Quality***

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.

**Wald 2003a** (Continued)

Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	Rates of NT failure on average 9%. pre-10 weeks' gestation, > 33% failure rate, declined to 7% at 12 weeks
Withdrawals explained? All tests	No	No details of withdrawals given.

**Wallace 1995**

Clinical features and settings	Routine screening.
Participants	112 participants. UK. Dates not stated. Pregnant women. 23 cases (maternal age 22-44 years, mean 32 years). 89 controls matched for gestational age and duration of sample storage (maternal age 19-38 years, mean 28 years) 11-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 23 cases. Reference standard: not reported.
Index and comparator tests	Frozen serum tested for dimeric first trimester Inhibin A (enzyme-linked two-site immunoassay)

**Wallace 1995** (Continued)

Follow-up	Methods of follow-up not reported.
Aim of study	To evaluate dimeric first trimester inhibin A as a possible first trimester screening marker for Down's syndrome screening
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear reference standard.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if the reference standard differed between women.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Wapner 2003**

Clinical features and settings	Routine screening.
Participants	8216 participants. USA - multicentre study (12 prenatal diagnostic centres). Dates not specified. Singleton pregnancies.

	Pregnant women with mean age 35 years (SD 4.6), 50% $\geq$ 35 years 11 to 14 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 61 cases. Reference standards: invasive testing, miscarriage with cytogenetic testing, follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (FMF methods). Dried blood samples tested for: First trimester free $\beta$ hCG and PAPP-A (dried blood samples, enzyme-linked immunoadsorbent assay as previously described) Risk cut-point 1:270.
Follow-up	Follow-up to birth by directly following up women and reviewing delivery records. An effort was also made to obtain information on terminated or miscarried pregnancies. 196 (2.3%) of patients without follow-up information were excluded
Aim of study	To evaluate the use of combined first trimester markers for aneuploidy in clinical practice
Notes	16 live Down's syndrome births.

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results

**Wapner 2003** (Continued)

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	No details of withdrawals given.

**Weinans 2005**

Clinical features and settings	High-risk referral for invasive testing.	
Participants	344 participants. The Netherlands - antenatal diagnosis unit. 1999-2002. Pregnant women with mean age 38 years (SD 2.7 years) for cases and 37 years (SD 3.0) for controls 24 cases, 320 controls matched for maternal and gestational age and length of storage Singleton pregnancies. 9 to 11 weeks' gestation.	
Study design	Case-control study.	
Target condition and reference standard(s)	Down's syndrome: 24 cases. Reference standard; CVS.	
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester serum free $\beta$ hCG and PAPP-A (fluoroimmunoassay, Auto Delfia, Perkin Elmer) First trimester maternal serum ITA (immunochemiluminometric assay, Nichols Advantage platform)	
Follow-up	100% karyotyping.	
Aim of study	To investigate Down's syndrome screening performance of serum ITA before 12 weeks' gestation and compare it with performance of PAPP-A and free $\beta$ hCG in the same sample set	
Notes		

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.

**Weinans 2005** (Continued)

Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Wojdemann 2005**

Clinical features and settings	Referrals for screening.
Participants	8622 participants (6441 with serum screening). Denmark - 3 obstetrics departments. March 1998 to June 2001 Pregnant women with mean age 29 years, 10.8% $\geq$ 35 years. Singleton pregnancies. 11 to 14 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 12 cases. Reference standards: invasive testing (in cases of increased risk) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (all patients) (FMF methods, Logic 700 MR machine) First trimester free $\beta$ hCG (AFP/ $\beta$ hCG Auto Delfia kit) and PAPP-A (In-house ELISA (Sandwich)) in 6,441 patients (75%) Risk cut-point 1:250.

Follow-up	Cross-checking with all the chromosome laboratories in Denmark. Follow-up in 96.2% of pregnancies through patients records
Aim of study	To determine the performance of screening for Down's syndrome and other major chromosomal abnormalities using NT, free $\beta$ hCG and PAPP-A in a prospective study of a non-selected population
Notes	Uptake of screening was 73% (9941 accepted out of 13,621 offered screening) Women with miscarriages excluded from the study. 3 live Down's syndrome births.

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	NT could not be measured in 2.5% of cases.
Withdrawals explained? All tests	No	No details of withdrawals given.

**Zaragoza 2009**

Clinical features and settings	Routine screening.
Participants	699 participants: 90 cases and 609 controls matched for length of storage UK - single centre. Dates not reported. Pregnant women. Singleton pregnancies. Median maternal age cases 37.9 years (19.1-46.5 years), controls 32.7 years (16.1-45.2 years) 11-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 90 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. Fresh samples tested for: First trimester PAPP-A and free $\beta$ hCG (Delfia Express system, PerkinElmer, Waltham) Frozen samples tested for: First trimester PIGF (ELISA, Quantikine human PIGF immunoassay, R&D systems Europe Ltd)
Follow-up	Karyotype results and details on pregnancy outcome were added to database as soon as they became available
Aim of study	To investigate the potential value of maternal serum placental growth factor (PIGF) in first trimester screening from trisomy 21 and other major chromosomal abnormalities
Notes	

**Table of Methodological Quality**

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.



Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

AFP: alpha-fetoprotein

$\alpha$ hCG: alpha human chorionic gonadotrophin

$\beta$ hCG: beta human chorionic gonadotrophin

CVS: chorionic villus sampling

ELISA: enzyme-linked immunosorbent assay

FMF: Fetal Medicine Foundation

GHBP: growth hormone binding protein

hCG: human chorionic gonadotrophin

ITA: invasive trophoblast antigen

IQR: interquartile range

NT: nuchal translucency

PAPP-A: Pregnancy-associated plasma protein A

PGH: placental growth hormone

PIGF: placental growth factor

PROMBP: proform of eosinophil major basic protein

SD: standard deviation

SP 1: Schwangerschafts protein 1

uE3: unconjugated oestriol

### Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Abbas 1995	Unable to extract useful data.
Abdul-Hamid 2004	No Down's syndrome pregnancies.
Abraha 1999	Unable to extract useful data.
Abu-Rustum 2010	Not Down's syndrome specific.

(Continued)

Achiron 2010	Study only includes cases of Down's syndrome.
Adekunle 1999	Unable to extract useful information.
Agaard-Tillery 2010	Results presented in another study.
Aitken 1993	Unable to extract useful data.
Aitken 1996a	Fewer than 80% of pregnancies had gestational age confirmed by USS
Aitken 1996b	Fewer than 80% of pregnancies had gestational age confirmed by USS
Ajayi 2011	No diagnostic data.
Akbas 2001	Less than 5 Down's syndrome pregnancies.
Alexioly 2009	Study only includes test positives.
Allingham-Hawkins 2011	Quantitative fluorescent polymerase chain reaction study.
American College 2009	Discussion article.
Antona 1998	Likely fewer than 80% of pregnancies dated by USS.
Antsaklis 1999	Women screened at greater than 24 weeks' gestation.
Anuwutnavin 2009	Second trimester ultrasound.
Ashwood 1987	Unable to extract useful data.
Asrani 2005	Review article.
Audibert 2001	Unable to ascertain whether part of screening population in Rozenberg et al. No response from authors, therefore excluded to reduce risk of data replication
Axt-Fleidner 2006	Unable to extract useful data.
Azuma 2002	Unable to extract useful data.
Baghagho 2004	Unable to obtain paper.
Bahado-Singh 1995	USS markers greater than 14 weeks' gestation.
Bahado-Singh 1996	USS markers greater than 14 weeks' gestation.
Bahado-Singh 1999	USS markers greater than 14 weeks' gestation.

(Continued)

Bahado-Singh 2002	USS markers greater than 14 weeks' gestation.
Bahado-Singh 2003	Review article.
Ball 2007	Data from the FASTER trial.
Bar-Hava 2001	No Down's pregnancies in study population.
Barkai 1996	No Down's pregnancies in study population.
Barnabei 1995	No Down's pregnancies in study population.
Bartels 1988	Unable to extract useful data.
Bartels 1993	No Down's pregnancies in study population.
Barth 1991	Second trimester ultrasound study.
Bas-Budecka 2007	No diagnostic data.
Baviera 2004	Unclear method of confirmation of gestational age.
Bazzett 1998	Male versus female fetuses.
Beke 2008	Results are not specific to Down's syndrome.
Bellver 2005	No Down's syndrome pregnancies in study.
Benn 1995	Less than 80% follow-up.
Benn 1996	Less than 80% follow-up.
Benn 1997	No Down's pregnancies in study population.
Benn 1998	Less than 80% follow-up.
Benn 2001	Statistical modelling (computer simulation).
Benn 2002	Modelled data.
Benn 2003a	Less than 80% of pregnancies dated by USS.
Benn 2003b	Editorial.
Benn 2005a	No Down's pregnancies included.
Benn 2005b	Mathematical model.

(Continued)

Benn 2007	No follow-up information.
Berry 1995	Less than 80% of pregnancies USS dated.
Berry 1997	Less than 80% of pregnancies USS dated.
Bersinger 1994	Gestational age not USS estimated.
Bersinger 2000	Unable to extract useful data.
Bersinger 2001	No Down's syndrome pregnancies in study population.
Bersinger 2003	Unable to extract useful data.
Bersinger 2004	No Down's syndrome pregnancies in study population.
Bersinger 2005	No Down's syndrome pregnancies in study population.
Bestwick 2008	All healthy pregnancies.
Biggio 2004	Cost-effectiveness analysis.
Bilardo 2011	Not a proper sample - most had elevated NT.
Bindra 2002	Review article.
Blundell 1999	Unable to extract useful data.
Boormans 2010	Study of testing on amniocentesis samples.
Boots 1989	Population risk factor calculations.
Bornstein 2009a	No diagnostic data.
Bornstein 2009b	No diagnostic data.
Bornstein 2010	No diagnostic data.
Borowski 2007	No diagnostic data.
Borrell 2007	No follow-up data.
Borruto 2002	Unable to extract useful data.
Bottalico 2009	Second trimester ultrasound.
Boue 1990	Review article.

(Continued)

Bradley 1994	Screen negative population gestations not confirmed by ultrasound
Braithwaite 1996	Review article.
Brambati 1995	USS screening inclusive of women greater than 14 weeks' gestation
Brambati 1996	Review article.
Brizot 1995a	Unable to extract useful data.
Brizot 1995b	Unable to extract useful data.
Brizzi 1989	Second trimester ultrasound.
Brock 1990	Unable to extract useful data.
Calda 2010	No data for false positive rates.
Campogrande 2001	Unable to extract useful data.
Canick 1988	Unable to extract useful data.
Canick 1995	Unable to extract useful data.
Canini 2002	No Down's syndrome pregnancies in study population.
Cans 1998	Second trimester ultrasound.
Carreras 1991	Second trimester ultrasound.
Caughey 2007	No diagnostic data.
Cebesoy 2008	No diagnostic data.
Chelli 2008	No follow-up for false negatives.
Chen 1999	Review article.
Chen 2002	No Down's syndrome pregnancies in study population.
Chen 2004	Less than 5 Down's cases in study population.
Chen 2005	Unable to extract useful data.
Chen 2008	No diagnostic data.
Cheng 1993	Likely that fewer than 80% of gestational age confirmed by USS

(Continued)

Cheng 1999	Case series. No Down's syndrome pregnancies in study population
Cheng 2004a	No Down's syndrome pregnancies in study population.
Cheng 2004b	No Down's syndrome pregnancies in study population.
Chitayat 2002	Less than 5 Down's cases in study population.
Chiu 2011	Study of maternal DNA testing.
Cho 2009	Study of testing amniotic fluid.
Chou 2009	Not possible to calculate specificity.
Christiansen 2002	Unable to extract useful data.
Christiansen 2007b	Unable to extract useful data.
Christiansen 2008	No diagnostic data.
Chung 2000	Less than 5 Down's syndrome pregnancies in study population.
CNGOF 1996	Unable to obtain translation.
Cole 1996	Review article.
Comas 2001	USS at greater than 14 weeks.
Comas 2002a	USS at greater than 14 weeks.
Comas 2002b	USS at greater than 14 weeks.
Comstock 2006	Unable to extract useful data.
Conde 1998	Review article.
Cowans 2011	No diagnostic data.
Crossley 1991	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1993	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1996	No Down's syndrome pregnancies in study population.
Crossley 2002b	Adjustment factors for smokers.
Cuckle 1984	Gestational age not confirmed by USS.

(Continued)

Cuckle 1987a	Gestational age not confirmed by USS.
Cuckle 1987b	No gestational age limits given.
Cuckle 1990	Paper presenting adjustment factors.
Cuckle 1996	Data modelled on 4 meta-analysed studies.
Cuckle 1999a	Unable to extract useful data.
Cuckle 1999b	Review article.
Cullen 1990	Abnormal scans only in study population.
Cusick 2004	Less than 5 Down's syndrome pregnancies in study population.
Cusick 2007	Second trimester ultrasound.
D'Ottavio 1997	Second trimester USS.
Dancoine 2001	No Down's syndrome pregnancies in study population.
Dane 2008	Not specific to Down's syndrome.
De Biasio 2000	Unable to extract useful information.
De Biasio, 1999	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Biasio, 2001	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Graaf 1991	Unable to extract useful data.
De Graaf 1999b	Modelled data.
Del Carmen Saucedo 2009	No follow-up information.
DeVore 2001	Second trimester ultrasound.
Dhaifalah 2007a	Unable to obtain translation.
Dhaifalah 2007b	Unable to obtain translation.
Dhallan 2007	DNA testing of blood samples from parents.
Dickerson 1994	Comment.

(Continued)

Dimaio 1987	Gestational age by USS only in screen-positive population.
Doran 1986	Ultrasound confirmation of gestational age performed in screen positive women only
Dreux 2008	No information for specificity.
Drugan 1996a	Second trimester ultrasound.
Drugan 1996b	Unable to extract useful data.
Drysdale 2002	Fewer than 5 Down's syndrome pregnancies in population.
Dugoff 2008	Not specific to Down's syndrome.
Ebell 1999	Review article.
Economides 1998	Unable to extract useful data.
Erickson 2004	No Down's syndrome pregnancies in population.
Evans 1996	No Down's syndrome pregnancies in population.
Evans 2007	Data previously presented in another study.
Falcon 2005	Unable to extract useful data.
Falcon 2006	Unable to extract useful data.
Ford 1998	Audit.
Frishman 1997	No Down's syndrome pregnancies in population.
Fukada 2000	Unable to extract useful data.
Gaudry 2009	Study of karyotyping.
Gebb 2009	Study only examines screen positives.
Geerts 2008	Study only examines abnormal fetuses.
Geipel 2010	Second trimester ultrasound.
Gekas 2009	Diagnostic data from other studies.
Gekas 2011a	Diagnostic data from other studies.
Gekas 2011b	Diagnostic parameters from other studies.



(Continued)

Gerovassili 2007	No diagnostic data.
Ghidini 1998	Comparison of male versus female fetuses.
Goetzing 2010	Second trimester ultrasound.
Goldie 1995	Fewer than 80% of study population and gestational age confirmed by USS
Gollo 2008	Only one case of Down's syndrome.
Gonçalves 2004	Greater than 14 weeks USS screening.
Goodburn 1994	Likely that fewer than 80% of pregnancies had gestational age estimated by USS
Gorduz 2007	Study of FISH technique.
Grace 2010	Second trimester ultrasound.
Grati 2010	No diagnostic data.
Gray 2009	Second trimester ultrasound.
Gregor 2007	Unable to obtain translation.
Gregor 2009	Unable to obtain translation.
Grether 2009	Systematic review and guidelines.
Grozdea 2002	Unable to extract useful data.
Guo 2010	Study of fetal samples.
Gyselaers 2004a	Less than 80% follow-up.
Gyselaers 2004b	Less than 80% follow-up.
Gyselaers 2006a	Unaffected pregnancies only.
Gyselaers 2006b	Unable to extract useful data.
Hackshaw 1995	No Down's syndrome pregnancies in population.
Hackshaw 2001	No Down's syndrome pregnancies in population
Haddow 1992	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Hadzsiev 2007	Study of FISH technique.

(Continued)

Hafner 1995	Less than 5 Down's pregnancies in study population.
Hallahan 1998	Gestational age greater than 24 weeks.
Han 2008	Study of findings on amniocentesis.
Harper 2010	Second trimester ultrasound.
Harrison 2006	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Harry 2006	Editorial.
Hayashi 1995	Unable to extract useful data.
Hayashi 1996	Less than 5 Down's pregnancies in study population.
Heikkila 1997	Fewer than 80% of pregnancies had gestational age confirmed by USS
Heinig 2007	No Down's syndrome data.
Heinonen 1996	No Down's syndrome pregnancies in population.
Herman 2000	No Down's syndrome pregnancies in study population.
Herman 2003	Correlation between markers, not evaluation of screening tests
Herrou 1992	Unable to extract useful data.
Hershey 1985	Gestation unclear.
Hershey 1986	Gestation based on LMP.
Hewitt 1993	Unable to extract useful data.
Hills 2010	Study of testing on CVS and amniocentesis samples.
Ho 2010	Study of FISH diagnosis.
Hogdall 1992	Unclear method of determination of gestational age. Unable to extract useful data
Hong Kong Practitioner	CME.
Hoogendoorn 2008	Diagnostic data from other studies used.
Howe 2000	Second trimester ultrasound scans.
Hsiao 1991	Unable to obtain translation.

(Continued)

Hsieh 1999	No Down's syndrome pregnancies in study population.
Hsu 1997	Adjustment factors.
Hsu 1998	No Down's syndrome pregnancies in study population.
Hsu 1999	No Down's pregnancies.
Hu 2007	Same data as <a href="#">Liu 2010</a> .
Huang 2003	No Down's syndrome pregnancies in study population.
Huang 2007a	Not possible to obtain detection rate.
Huang 2007b	No diagnostic data.
Huggon 2004	Study of cardiac function in pregnancies with normal and abnormal NT results
Hui 2003	No Down's syndrome pregnancies in population.
Hui 2005	No Down's syndrome pregnancies in population.
Hultén 2004	Editorial/commentary.
Hung 2003	Modelling.
Hung 2008	Second trimester ultrasound.
Hurley 1993	Unable to extract useful data.
Huttly 2004	No Down's syndrome pregnancies in population.
Hwa 2004	Less than 5 Down's pregnancies in population.
Iles 1996	Review.
Ind 1994	Unable to extract useful data.
Ivorra-Deleuze 2010	No diagnostic data.
Jakobsen 2011	Not Down's syndrome specific.
Jean-Pierre 2005	Review article.
Johnson 1991	Gestational age estimated by USS in fewer than 80% of cases.
Johnson 1993	Normal pregnancies only.

(Continued)

Jorgensen 1999	Gestation greater than 14 weeks for USS.
Jorgez 2007	Study of DNA testing on maternal blood.
Josefsson 1998	No Down's syndrome pregnancies in study population.
Jou 2001	Less than 5 Down's syndrome pregnancies in study population.
Jung 2007	Second trimester ultrasound.
Kagan 2006	Screen positive pregnancies only.
Kagan 2007	No diagnostic data.
Kagan 2008	Not Down's syndrome detection.
Kalelioglu 2007	Second trimester ultrasound.
Kautzmann 1995	Fewer than 80% pregnancies had gestational age estimated by USS
Kazerouni 2009	Not possible to obtain complete diagnostic data.
Keith 1992	Summary article.
Kelekci 2004	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995a	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995b	Less than 80% follow-up. Unable to ascertain proportion of population with gestational age confirmed by USS
Kellner 1997	Assumption of normal karyotype without reference standard in significant proportion of control pregnancies
Kirkegaard 2008	False positive rate only calculated for subset of the cohort
Kjaergaard 2008	Unable to obtain translation.
Knight 1990	Review article.
Knight 2001	Validation of a specific assay.
Knight 2005	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Koos 2006	Review article.
Kornman 1996	Less than 5 Down's syndrome pregnancies in population.

(Continued)

Kornman 1997	Unable to extract useful information.
Kotaska 2007	No new data.
Kramer 1998	No Down's syndrome pregnancies in study population.
Krantz 1996	Modelled data.
Krantz 2005	Adjustment factor.
Krantz 2007	Uses data from other published studies.
Kulch 1993	No Down's cases in population.
Lai 1998	Modelled population.
Lai 2003	No Down's syndrome pregnancies in study population.
Laigaard 2006a	Unable to extract useful data.
Laigaard 2006b	Simulation.
Lam 1997	Unable to extract useful data.
Lam 1998	Fewer than 80% pregnancies had gestational age estimated by USS
Lam 1999a	No Down's syndrome pregnancies in population.
Lam 1999b	Unable to extract useful data.
Lam 2000	Study of women's decisions about screening.
Lam 2001	Male versus female fetuses.
Lambert-Messerlian 1996	Fewer than 80% of pregnancies USS dated.
Lambert-Messerlian 1998	Unable to extract useful data.
Lauria 2007	No diagnostic data.
Lehavi 2005	Down's syndrome pregnancies only.
Leung 2006	Unable to separate twins from singletons therefore unable to extract useful data
Leymarie 1993	Appears to be a review article (French).
Li 1998	Unable to obtain translation.

(Continued)

Li 1999	Unable to obtain translation.
Li 2010	No diagnostic data.
Liao 1997	Unable to obtain translation.
Liao 2001	Unable to extract useful data.
Lim 2002	Second trimester ultrasound.
Lippman 1987	Editorial.
Liu 2003	Unable to obtain translation.
Liu 2010	Not possible to separate out data for cases of Down's syndrome
Lo 2010	Pooled test results.
Lustig 1988	Gestational age by LMP only.
Luthgens 2008	False positive rate and detection rate obtained from different cohorts
MacDonald 1991	Fewer than 80% of gestational ages estimated by USS.
Macintosh 1994	Unable to extract useful data.
Macintosh 1997	Unable to extract useful data.
MacRae 2010	Pooled test results.
Macri 1994	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Macri 1996	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Malone 1998	Review article.
Malone 2003	Review article.
Mandryka-Stankewycz 2009	No diagnostic data.
Mangione 2001	Abnormal screening results only.
Markov 2008	Unable to obtain paper.
Maymon 2001a	No Down's syndrome pregnancies in study population.
Maymon 2001b	No normal test results included therefore unable to extract meaningful data

(Continued)

Maymon 2002	No Down's syndrome pregnancies in study population.
Maymon 2004	No Down's syndrome pregnancies in study population.
Maymon 2005	Modelled data.
McDuffie 1996	USS dating on screen positive women only.
Meier 2002	Observed versus expected cases of Down's syndrome in a population
Merkatz 1984	Gestational age not confirmed by ultrasound scan.
Merz 2005	Editorial.
Merz 2008	Data available for only combined ultrasound marker (nuchal translucency) and serum tests
Metzenbauer 2001	Normal pregnancies only.
Metzenbauer 2002	Unable to extract useful data.
Mikic 1999	No Down's syndrome pregnancies in study population.
Miller 1991	Unable to extract useful data.
Milunsky 1989	Fewer than 80% gestational age estimated by USS.
Milunsky 1996	Fewer than 80% gestational age estimated by USS.
Minobe 2002	Gestational age greater than specified limits.
Miron 2008	No diagnostic data.
Miron 2009	No diagnostic data.
Miron 2010	No diagnostic data.
Miyamura 1999	Unable to extract useful data.
Moghadam 1998	Unable to extract useful data.
Monni 2000	Less than 5 Down's syndrome pregnancies.
Monni 2002	Review article.
Mooney 1994	Greater than 24 weeks' gestation.
Muhcu 2008	No diagnostic data.

(Continued)

Muller 1994	No Down's syndrome pregnancies in study population.
Muller 1996	Unable to extract useful data.
Muller 1999	Unable to extract useful data.
Muller 2002a	Gestational age greater than 24 weeks.
Muller 2002b	Unable to extract meaningful data - unable to separate double- and triple-test data
Muller 2003b	No Down's syndrome pregnancies in study population.
Murta 2002	Unable to extract useful data.
Musone 2000	Unable to extract useful data.
Musto 1986	Fewer than 80% USS dated.
Myrick 1990	Unable to extract useful data.
Naidoo 2008	Not specific Down's syndrome results.
Nau 2009a	No diagnostic data.
Nau 2009b	No diagnostic data.
Neveux 1996a	No Down's syndrome pregnancies in population.
Neveux 1996b	Unable to extract useful data.
Ng 2004	Unable to extract useful data.
Nicolaides 1992	Study of outcomes of abnormal NT results.
Nicolaides 2000	Review article.
Nicolaides 2004	Review article.
Nicolaides 2005a	Unable to obtain translation - appears to be a review article
Nicolaides 2005b	Unable to obtain translation - appears to be a review article
Nicolaides 2005c	Unable to obtain translation - appears to be a review article
Nicolaides 2005d	Unable to obtain translation - appears to be a review article
Nicolaides 2005e	Unable to obtain translation - appears to be a review article



(Continued)

Nicolaides 2005f	Review article.
Niemimaa 2001b	No Down's pregnancies in study population.
Niemimaa 2002	No Down's syndrome pregnancies in population.
Niemimaa 2003	No Down's syndrome pregnancies in population.
Noble 1997a	Unable to extract useful data.
Norgaard 1990	Less than 80% of gestational ages confirmed by USS.
Norton 1992	Unable to extract useful data.
Novakov-Mikic 2007	Out of first trimester screening time frame.
O'Brien 1997a	No Down's syndrome pregnancies in population.
O'Brien 1997b	No Down's syndrome pregnancies in population.
Odibo 2004	Gestational age of greater than 14 weeks in USS population.
Odibo 2007	Second trimester ultrasound.
Odibo 2008	Second trimester ultrasound.
Odibo 2009	No results presented.
Offerdal 2008	Second trimester ultrasound.
Ognibene 1999	Unable to extract useful data.
Oh 2007	No diagnostic data.
Olajide 1989	Unable to extract useful data.
Onda 1996	Unable to extract useful data.
Onda 1998	Unable to extract useful data.
Onda 2000	Less than 80% follow-up.
Orlandi 2002	No Down's syndrome pregnancies in study population.
Ozkaya 2010	Only healthy pregnancies.
Paladini 2007	No diagnostic data.

(Continued)

Palka 1998	Twin data used in calculation of the median.
Palomaki 1989	Fewer than 80% USS dated.
Palomaki 1993	No Down's syndrome pregnancies in population.
Palomaki 1994	No Down's syndrome pregnancies in population.
Palomaki 1996	Meta-analysis.
Palomaki 2005	Unable to extract meaningful data.
Panburana 2001	Less than 5 Down's syndrome pregnancies in population.
Pandya 1994	Study of outcomes of abnormal nuchal translucency results.
Pandya 1995	Review article.
Papadopoulou 2008	No diagnostic data.
Parra-Cordero 2007	Second trimester ultrasound.
Paterlini-Brechot 2007	Editorial, no new data.
Paul 2001	Unable to extract useful data.
Peralta 2005	Unable to extract useful data.
Perenc 1998	No Down's syndrome pregnancies in study population.
Perheentupa 2002	No Down's syndrome pregnancies in population.
Perona 1998	Smokers versus non smokers.
Persico 2008	Second trimester ultrasound.
Petervari 2000	Unable to extract useful data.
Petrocik 1989	Likely fewer than 80% USS dated.
Phillips 1992	Gestational age confirmed by USS in less than 80% of population
Phillips 1993	Gestational age confirmed by USS in less than 80% of population
Pihl 2008	Only 2 cases of Down's syndrome.
Pinette 2003	Women screened prior to recruitment.

(Continued)

Platt 2004	Unable to extract useful data.
Podobnik 1995	Abnormal results only.
Poon 2009	No diagnostic data.
Prefumo 2002	Comparison of prevalence and prediction.
Prefumo 2004	Comparison of a marker in women of different ethnic origins.
Price 1998	Unable to extract useful data.
Páez 2004	Unable to obtain translation.
Raty 2000	No Down's syndrome pregnancies in population.
Rembouskos 2004	Unable to extract useful data.
Ren 1992	Review article.
Renier 1998	Method of ascertainment of gestational age unclear. Twin gestations included in general population
Resta 1990	Second trimester USS.
Reynders 1997	Fewer than 5 Down's cases.
Reynolds 1989	Explanation of mathematical techniques.
Reynolds 1999	Unable to extract useful data.
Reynolds 2008	Not full diagnostic data.
Ribbert 1996	No Down's syndrome pregnancies in study population.
Rice 2005	Down's syndrome pregnancies excluded from study.
Rich 1991	Unable to extract useful data.
Roberts 1995	No Down's syndrome pregnancies in study population.
Robertson 1991	Editorial.
Rode 2003	No Down's pregnancies.
Ronge 2006	Editorial - summary of FASTER results.
Rose 1995	Review article.

(Continued)

Ross 1997	Review article.
Rotmensch 1996	Unable to extract useful data.
Rotmensch 1999	No Down's syndrome pregnancies in study population.
Rozenberg 2006	USS greater than 14 weeks' gestation.
Rudnicka 2002	No Down's syndrome pregnancies in population.
Ryall 1992	Unable to determine method of confirmation of gestational age
Ryall 2001	High-risk results only included (i.e. no screen-negative group for comparison)
Räty 2002	No Down's pregnancies in population.
Sabriá 2002	Unable to ascertain how numbers calculated and from which populations
Sacchini 2003	Unable to extract useful data.
Sahota 2009	No diagnostic data.
Sahota 2010a	Included in <a href="#">Sahota 2010</a> .
Salazar 2007	Unable to obtain paper.
Salazar 2008	Only one case of Down's syndrome.
Saller 1997	Down's syndrome secondary to Robertsonian translocation only. No controls
Salomon 2001	No Down's syndrome pregnancies in population.
Salonen 1997	Fewer than 80% had gestational age estimated by USS.
Saltvedt 2005	Gestation greater than 14 weeks for nuchal scanning.
Saridogan 1996	Down's syndrome and Edward's syndrome affected pregnancies only
Savoldelli 1993	Unable to extract useful data.
Schielen 2009	Full study information not given.
Schiott 2006	Unable to extract useful data.
Schmidt 2007a	Not specific to Down's syndrome.
Schmidt 2007b	No separate Down's syndrome data.

(Continued)

Schmidt 2007c	No diagnostic data.
Schmidt 2008a	Not specific to Down's syndrome.
Schmidt 2008b	Not specific to Down's syndrome.
Schmidt 2008c	Not specific to Down's syndrome.
Schmidt 2010	No follow-up data for test negatives.
Schuchter 1998	No Down's pregnancies in study population.
Scott 1995	Less than 5 Down's syndrome pregnancies in study population.
Seeds 1990	Review article.
Seki 1995	No Down's syndrome pregnancies in study population.
Shenhav 2003	No Down's syndrome pregnancies.
Shintaku 1989	Unable to extract useful data.
Shulman 2003	No Down's syndrome pregnancies in population.
Sieroszewski 2008	No Down's syndrome specific information for specificity.
Simon-Bouy 1999	Review article.
Simpson 1986	Gestational age confirmed by USS in less than 80% of population
Smith 1990	Analysis of screen-positive results.
Smith 1996	Review/meta-analysis.
Smith 1999	Unable to extract useful data.
Smith-Bindman 2001	Meta-analysis of second trimester ultrasound markers.
Smith-Bindman 2003	Population study, not examining DTA.
Snijders 1995	Study of prevalence, not screening.
Snijders 1999	Study of prevalence, not screening.
Soergel 2006	Less than 80% follow-up.
Sokol 1998	Observation of Down's prevalence stratified by age.

(Continued)

Sonek 2003	Editorial.
Sonek 2007	Second trimester ultrasound.
Sood 2010	No diagnostic data.
Sooklim 2010	Second trimester ultrasound.
Spencer 1985	Fewer than 80% USS dated.
Spencer 1991a	Likely fewer than 80% USS dated.
Spencer 1991b	Unable to extract useful data.
Spencer 1992	Unable to extract useful data.
Spencer 1993a	Fewer than 80% USS dated.
Spencer 1993b	No Down's pregnancies in study population.
Spencer 1993c	Unable to extract useful data.
Spencer 1993d	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1993e	Unable to extract useful data.
Spencer 1995	No Down's pregnancies in population.
Spencer 1996	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1997	Statistical modelling, aneuploid pregnancies only in study population
Spencer 1998a	No Down's pregnancies in population.
Spencer 1998b	Unable to extract useful data.
Spencer 1999b	Review.
Spencer 1999c	Statistical methods paper.
Spencer 2000a	Examination of median shifts rather than an evaluation of screening
Spencer 2000b	No Down's syndrome pregnancies in population.
Spencer 2000c	No Down's syndrome pregnancies in population.
Spencer 2000d	No Down's cases.

(Continued)

Spencer 2000e	Male versus female fetuses.
Spencer 2000f	No Down's cases in population.
Spencer 2000g	No Down's pregnancies in population.
Spencer 2000h	No Down's pregnancies in population.
Spencer 2000i	Comparison of fetal sex.
Spencer 2001a	No Down's syndrome pregnancies in population.
Spencer 2001b	Unable to extract useful data.
Spencer 2001c	Unable to extract useful data.
Spencer 2001d	Unable to extract useful data.
Spencer 2001e	No Down's syndrome pregnancies in population.
Spencer 2002b	No Down's pregnancies.
Spencer 2002c	Risk validation study.
Spencer 2002d	No Down's syndrome pregnancies in population.
Spencer 2002e	Demonstration of median changes with time, rather than evaluation of screening
Spencer 2003a	No Down's pregnancies in population.
Spencer 2003b	No Down's pregnancies in population.
Spencer 2003c	Calculation of weight correction factor.
Spencer 2003d	Fewer than 5 Down's syndrome pregnancies.
Spencer 2004	Calculation of smoking correction factor.
Spencer 2005a	No Down's pregnancies.
Spencer 2005b	No Down's pregnancies.
Spencer 2005c	Comparison of 2 different assays - not actual screening evaluation
Spencer 2008	Unable to extract appropriate data for unaffected pregnancies

(Continued)

Spong 1999	Comparison of male and female fetuses.
Staboulidou 2009	No diagnostic data.
Stevens 1998	Literature review.
Stoll 1992	Review article.
Stressig 2011	Second trimester ultrasound.
Su 2002	Unable to extract useful data.
Suchet 1995	Review article.
Suchy 1990	Unable to ascertain method of confirmation of gestational age
Summers 2003a	Only 55% gestational ages estimated by USS.
Summers 2003b	No Down's syndrome pregnancies in study population.
Suntharasaj 2005	Examination of inter-observer variation in NT scanning.
Susman 2010	No diagnostic data.
Sutton 2004	Unable to extract useful data.
Suzuki 1998	Unable to extract useful data.
Tabor 1987	Gestational age not confirmed by USS.
Tanski 1999	Information on screen positive pregnancies only.
Thilaganathan 1998	No Down's syndrome pregnancies in study population.
Thilaganathan 1999	Editorial.
Tislaric 2002	No Down's syndrome pregnancies in population.
Torok 1997	Unable to extract useful data.
Torrington 2009	Not possible to obtain full diagnostic data.
Trninic-Pjevic 2007	Unable to obtain translation.
Tsai 2001	Less than 5 Down's syndrome pregnancies in study population.
Valerio 1996	Fewer than 80% pregnancies had gestational age estimated by USS



(Continued)

Van Blerk 1992	Unable to extract useful data.
Van Dyke 2007	Not possible to obtain full diagnostic data.
Van Heesch, 2006	No Down's syndrome pregnancies in study population. Software comparison study
Van Lith 1991	Unable to extract useful data.
Van Lith 1993	Unable to extract useful data.
Van Lith 1994	Unable to extract useful data.
Veress 1986	Unable to extract useful data.
Veress 1988	Unable to extract useful data.
Vergani 2008	Second trimester ultrasound.
Vintzileos 2003	Second trimester USS.
Wald 1988a	Less than 80% had gestational age confirmed by ultrasound.
Wald 1988b	Gestational age not confirmed by USS.
Wald 1991	No Down's pregnancies in study.
Wald 1992a	Less than 80% had gestational age confirmed by ultrasound.
Wald 1992b	No Down's pregnancies in study.
Wald 1992c	No Down's pregnancies in study.
Wald 1993	No USS dating.
Wald 1994	No Down's syndrome pregnancies in population.
Wald 1994a	Review article.
Wald 1996a	No Down's pregnancies.
Wald 1996b	Dated by LMP.
Wald 1996c	No Down's syndrome pregnancies in population.
Wald 1996d	Gestational age greater than 24 weeks.
Wald 1997	Data modelled on 3 separate populations of women.

(Continued)

Wald 1998	Unable to extract useful data.
Wald 1999a	Unable to extract useful data.
Wald 1999b	Gestational age not confirmed by USS.
Wald 1999c	No Down's syndrome pregnancies.
Wald 1999d	Modelled on several studies, some of which have no USS dating
Wald 2003b	No cases.
Wald 2003c	Less than 80% had gestational age confirmed by USS.
Wald 2006	Modelled on SURRUS data.
Wallace 1994	Unable to extract useful data.
Wallace 1997	No Down's syndrome pregnancies in study population.
Wang 2010	Second trimester ultrasound.
Ward 2005	Review article.
Watt 1996a	No Down's syndrome pregnancies in study population.
Watt 1996b	No Down's syndrome pregnancies in study population.
Wax 2007	No diagnostic data.
Weinans 2001	Unable to extract useful data.
Weinans 2004	Study of women's views on screening.
Weisz 2007	Cohort split into people having different tests and non-representative samples of women assessed for each test
Welborn 1994	Abnormal results only (cystic hygroma).
Wenstrom 1993	Less than 80% of pregnancies had gestational age confirmed by USS
Wenstrom 1995a	Adjustment factors.
Wenstrom 1995b	Less than 80% of pregnancies had gestational age confirmed by USS
Wetta 2011	No diagnostic data.
Whitlow 1998a	Unable to extract useful data.

(Continued)

Whitlow 1998b	Unable to extract useful data.
Whitlow 1999	Unable to extract useful data.
Williamson 1994	Likely fewer than 80% USS dated.
Wilson 2000	Review.
Wojdemann 2001	No Down's syndrome pregnancies in study population.
Wong 2003	Less than 5 Down's syndrome pregnancies in population.
Wright 2006	Mathematical model.
Wright 2007	Simulation study, no new data.
Xie 2010	Only cases of false negatives and true negatives included.
Yagel 1998	Second trimester USS.
Yamamoto 2001a	Unable to extract useful data.
Yamamoto 2001b	Method of determination of gestational age unclear.
Yamamoto 2001c	Unable to extract useful data.
Yaron 2001	Male versus female fetuses.
Ye 1995	Unable to obtain translation.
Yoshida 2000	Fewer than 80% pregnancies had gestational age estimated by USS
Zalel 2008	No diagnostic data.
Zeitune 1991	Only aneuploid pregnancies included in study.
Zelop 2005	No Down's cases in population.
Zhang 2011	No diagnostic data.
Zhao 1998	Unable to obtain translation.
Zhong 2011	Second trimester ultrasound.
Zoppi 2003	Inappropriate study design.

CME: continuing medical education

CVS: chorionic villus sampling  
DTA: diagnostic test accuracy  
FISH technique: fluorescence in situ hybridization  
LMP: last menstrual period  
NT: nuchal translucency  
USS: ultrasound scan

## DATA

Presented below are all the data for all of the tests entered into the review.

### Tests. Data tables by test

Test	No. of studies	No. of participants
1 1T PAPP-A, 5% FPR	4	2837
2 1T PAPP-A, $\leq 5^{\text{th}}$ percentile	1	22280
3 1T PAPP-A, mixed cut-points	6	25510
4 1T free $\beta$ hCG, 5% FPR	4	4280
5 1T total hCG, 5FPR	2	2482
6 1T AFP, 5% FPR	2	2248
10 1T AFP, mixed cut-points	3	2724
11 1T Inhibin, 5FPR	3	2098
12 1T ADAM 12, 5FPR	1	579
13 1T SPI, 5% FPR	3	1080
17 ba'hcg' ratio, 0.25MoM	1	476
18 1T uE3, 5% FPR	1	1110
19 1T PIGF, 5FPR	1	699
20 1T PAPP-A and 1T free $\beta$ hCG, 5% FPR	2	795
21 1T PAPP-A and 1T free $\beta$ hCG, mixed cut-points	2	795
22 1T PAPP-A and 1T AFP, 5% FPR	1	96
23 1T PAPP-A and 1T ITA, 3% FPR	1	344
24 1T PAPP-A and 1T ITA, 5% FPR	1	344
25 1T free $\beta$ hCG and 1T Inhibin, 5% FPR	1	876
26 1T free $\beta$ hCG and 1T AFP, 5% FPR	1	1138
27 1T PAPP-A and 1T ITA, 10% FPR	1	344
28 1T PAPP-A, 1T free $\beta$ hCG and 1T ITA, 5% FPR	1	344
29 1T PAPP-A, 1T free $\beta$ hCG and 1T ITA, 3% FPR	1	344
30 1T PAPP-A, 1T free $\beta$ hCG and 1T ITA, 10% FPR	1	344
31 1T total hCG, 1T free $\alpha$ hCG and 1T progesterone, 0.34 MoM	1	129
32 Age, 1T Inhibin, risk 1:100	1	40
33 Age, 1T Inhibin, risk 1:250	1	40
34 Age, 1T Inhibin, risk 1:400	1	40

35 Age, 1T Inhibin, 5FPR	1	1110
36 Age, 1T Inhibin, mixed cut-points	2	1150
37 Age, 1T PAPP-A, 5FPR	5	3491
38 Age, 1T PAPP-A, mixed cut-points	6	13742
39 Age, 1T free $\beta$ hCG, 5FPR	7	5893
40 Age, 1T free $\beta$ hCG, risk 1:384	1	512
41 Age, 1T free $\beta$ hCG, mixed cut-points	9	16656
42 Age, 1T total hCG, risk 1:384	1	512
43 Age, 1T total hCG, mixed cut-points	2	1622
44 Age, 1T AFP, 5FPR	2	1397
45 Age, 1T AFP, risk 1:384	1	512
46 Age, 1T AFP, mixed cut-points	3	1909
47 Age, 1T uE3, risk 1:384	1	512
48 Age, 1T uE3, mixed cut-points	2	799
49 Age, 1T free $\alpha$ hCG, risk 1:384	1	512
50 Age, 1T SP1, 5FPR	2	804
51 Age, 1T ProMBP, risk 1:250	1	181
52 Age, 1T ITA, 5FPR	1	278
53 Age, 1T ADAM 12, risk 1:400	2	703
54 Age, 1T PAPP-A and 1T free $\beta$ hCG, risk 1:250	11	60484
55 Age, 1T PAPP-A and 1T free $\beta$ hCG, 5FPR	17	49827
56 Age, 1T PAPP-A and 1T free $\beta$ hCG, mixed cut-points	31	158878
57 Age, 1T PAPP-A and 1T free $\beta$ hCG, mixed cut-points without 5FPR	20	138731
58 Age, 1T total hCG and 1T PAPP-A, 5FPR	2	4327
59 Age, 1T PAPP-A and 1T Inhibin, risk 1:100	1	41
60 Age, 1T PAPP-A and 1T Inhibin, risk 1:250	1	40
61 Age, 1T PAPP-A and 1T Inhibin, risk 1:400	1	40
62 Age, 1T PAPP-A and 1T Inhibin, 5FPR	1	1110
63 Age, 1T PAPP-A and 1T Inhibin, mixed cut-points	2	1150
64 Age, 1T PAPP-A and 1T ITA, 5FPR	2	622
65 Age, 1T PAPP-A and 1T AFP, 5FPR	2	2705
66 Age, 1T free $\beta$ hCG and 1T Inhibin, risk 1:100	1	40

67	Age, 1T free $\beta$ hCG and 1T Inhibin, risk 1:250	1	40
68	Age, 1T free $\beta$ hCG and 1T Inhibin, risk 1:400	1	40
69	Age, 1T free $\beta$ hCG and 1T Inhibin, 5FPR	1	1110
70	Age, 1T free $\beta$ hCG and 1T Inhibin, mixed cut-points	2	1150
71	Age, 1T free $\beta$ hCG and 1T AFP, 5FPR	3	2992
72	Age, 1T free $\beta$ hCG and 1T AFP, risk 1:250	1	1656
73	Age, 1T free $\beta$ hCG and 1T AFP, risk 1:384	1	512
74	Age, 1T free $\beta$ hCG and 1T AFP, mixed cut-points	5	5160
75	Age, 1T AFP and 1T uE3, risk 1:384	1	512
76	Age, 1T AFP and 1T free $\alpha$ hCG, risk 1:384	1	512
77	Age, 1T free $\beta$ hCG and 1T total hCG, risk 1:384	1	512
78	Age, 1T free $\beta$ hCG and 1T uE3, risk 1:384	1	512
79	Age, 1T free $\beta$ hCG and 1T uE3, 5FPR	1	287
80	Age, 1T free $\beta$ hCG and 1T uE3, mixed cut-points	2	799
81	Age, 1T free $\beta$ hCG and 1T SP1, 5FPR	1	60
82	Age, 1T free $\beta$ hCG and 1T SP1 risk 1:250	1	60
83	Age, 1T AFP and 1T total hCG, 1:384	1	512
84	Age, 1T free $\beta$ hCG and 1T free $\alpha$ hCG, risk 1:384	1	512
85	Age, 1T total hCG and 1T uE3, risk 1:384	1	512
86	Age, 1T total hCG and 1T Inhibin, 5FPR	1	1110
87	Age, 1T total hCG and 1T free $\alpha$ hCG, risk 1:384	1	512
88	Age, 1T uE3 and 1T free $\alpha$ hCG, risk 1:384	1	512
89	Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T AFP, 5FPR	2	2705
90	Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T AFP, mixed cut-points	3	8188
91	Age, 1T free $\beta$ hCG, 1T AFP and 1T uE3, 5FPR	1	287

92 Age, 1T free $\beta$ hCG, 1T AFP and 1T uE3, risk 1:384	1	512
93 Age, 1T free $\beta$ hCG, 1T AFP and 1T uE3, mixed cut-points	2	799
94 Age, 1T total hCG, 1T AFP and 1T uE3, risk 1:384	1	512
95 Age, 1T total hCG, 1T AFP and 1T uE3, mixed cut-points	2	1505
96 Age, 1T AFP, free $\alpha$ hCG and 1T uE3, risk 1:384	1	512
97 Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T Inhibin, 5FPR	1	1110
98 Age, 1T PAPP-A, 1T total hCG and 1T Inhibin, 5FPR	1	1110
99 Age, 1T PAPP-A, sp1 and 1T ProMBP, 5FPR	1	192
100 Age, 1T PAPP-A, sp1 and 1T ProMBP, risk 1:250	1	192
101 Age, 1T free $\beta$ hCG, 1T total hCG, 1T AFP and 1T uE3, risk 1:384	1	512
102 Age, 1T total hCG, 1T AFP, 1T uE3 and 1T free $\alpha$ hCG, risk 1:384	1	512
103 Age, 1T PAPP-A, 1T free $\beta$ hCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR	1	1110
104 Age, 1T PAPP-A, 1T total hCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR	1	1110
105 Age, 1T free $\beta$ hCG, 1T total hCG, 1T AFP, 1T uE3 and 1T free $\alpha$ hCG, risk 1:384	1	512
106 Age, 1T hPL, risk 1:250	1	183
107 Age, 1T hPL, 1T PAPP-A, risk 1:250	1	183
108 Age, 1T hPL, 1T free $\beta$ hCG, risk 1:250	1	183
109 Age, 1T hPL, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250	1	183
110 Age, 1T PGH, risk 1:250	1	335
111 Age, 1T PGH, 1T PAPP-A, risk 1:250	1	335
112 Age, 1T PGH, 1T free $\beta$ hCG, risk 1:250	1	335
113 Age, 1T PGH, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250	1	335
114 Age, 1T GHBP, risk 1:250	1	335
115 Age, 1T GHBP, 1T PAPP-A, risk 1:250	1	335

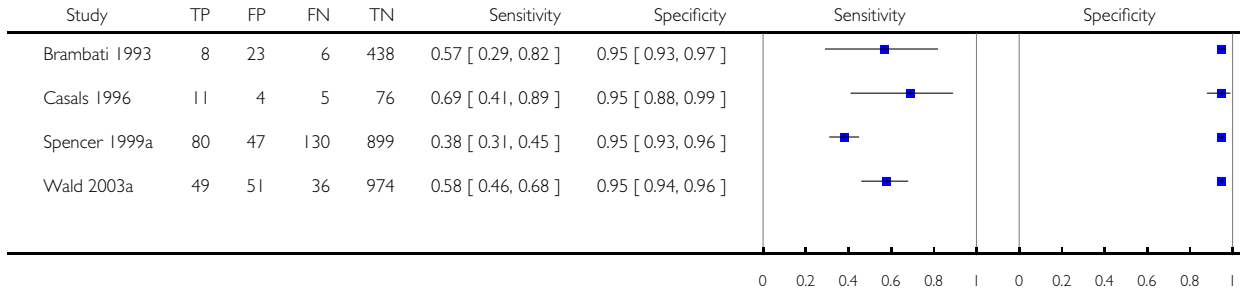


116 Age, 1T GHBP, 1T free $\beta$ hCG, risk 1:250	1	335
117 Age, 1T GHBP, 1T PGH, risk 1:250	1	335
118 Age, 1T GHBP, 1T PAPP-A, 1T free $\beta$ hCG , risk 1:250	1	335
119 Age, 1T GHBP, 1T PGH, 1T PAPP-A, 1T free $\beta$ hCG , risk 1:250	1	335
120 Age, 1T ADAM 12, risk 1:250	1	531
121 Age, 1T ADAM 12, 1T PAPP-A, 1T free $\beta$ hCG, risk 1:250	3	1501
122 Age, PIGF, 1T PAPP-A, 1T free $\beta$ hCG, 5FPR	2	1144
123 Age, 1T PAPP-A and 1T free $\beta$ hCG, risk 1:300	4	41172
124 Age, 1T PAPP-A, 1T Hyperglycosylated hCG, 5FPR	1	10775
128 Age, ADAM 12, 1T PAPP-A, 5FPR	1	691
129 Age, ADAM 12, 1T PAPP-A, 1T free $\beta$ hCG, 5FPR	2	1222
130 Age, 1T PIGF, 5FPR	1	699
131 1T PIGF, 1T PAPP-A, 1T free $\beta$ hCG, 5FPR	1	699
132 Age, 1T ADAM 12, 1T PAPP-A, 1T free $\beta$ hCG, mixed cut-points	3	1501
133 Age, 1T PAPP-A, 1T free $\beta$ hCG and 1T Inhibin, risk 1:250	1	40
134 Age, 1T PAPP-A, 1T free $\beta$ hCG, and 1T Inhibin, mixed cut-points	2	1150

### Test 1. IT PAPP-A, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

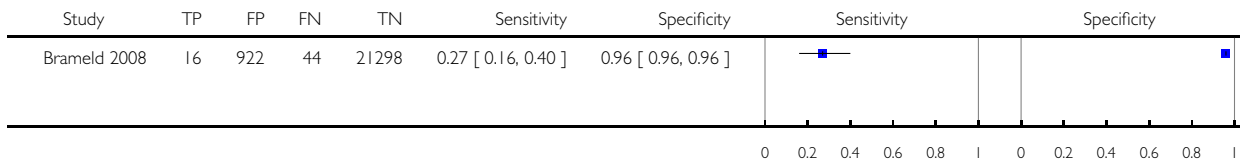
Test: 1 IT PAPP-A, 5% FPR



### Test 2. IT PAPP-A, ≤5<sup>th</sup> percentile.

Review: First trimester serum tests for Down's syndrome screening

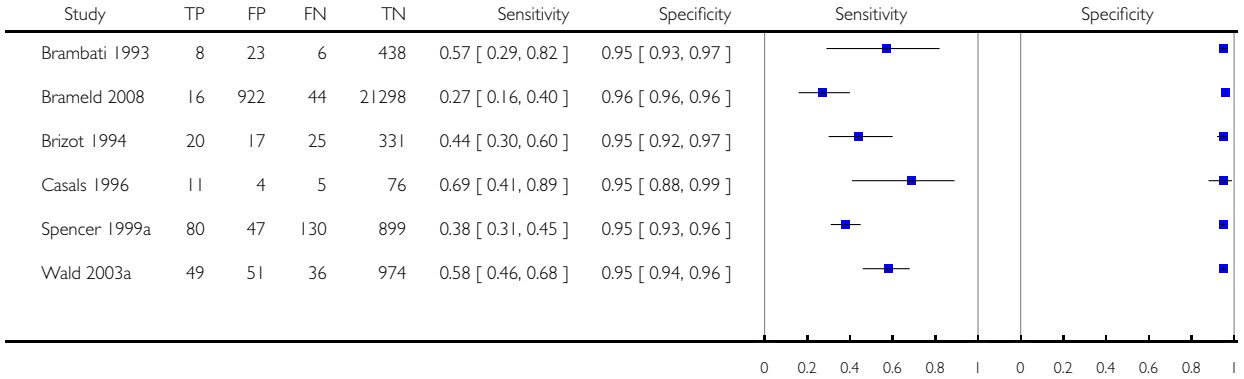
Test: 2 IT PAPP-A, ≤5<sup>th</sup> percentile



### Test 3. IT PAPP-A, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

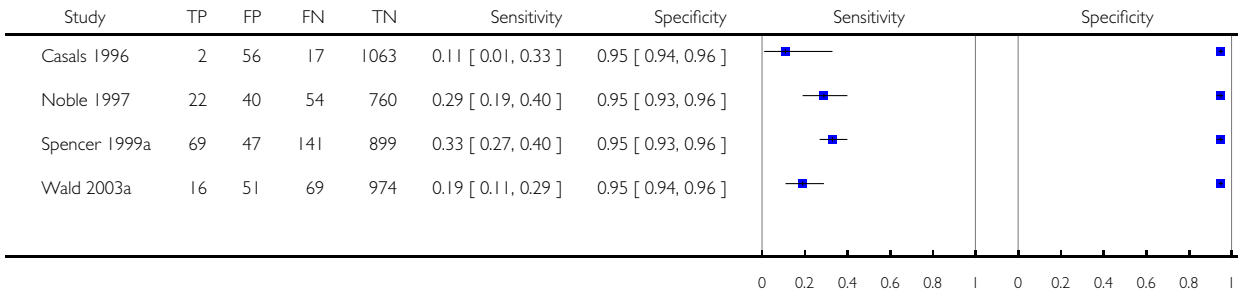
Test: 3 IT PAPP-A, mixed cut-points



### Test 4. IT free $\beta$ hCG, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

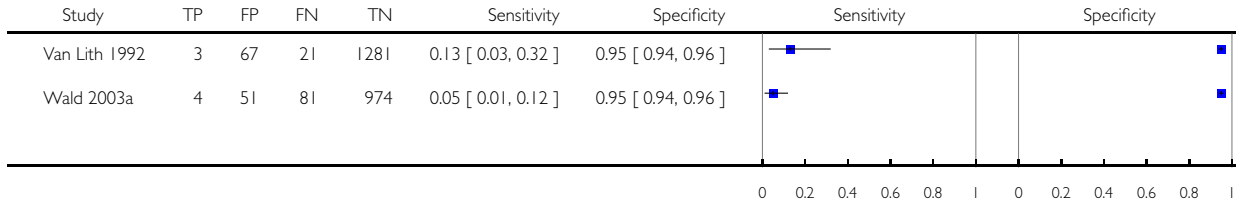
Test: 4 IT free hCG, 5% FPR



### Test 5. IT total hCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

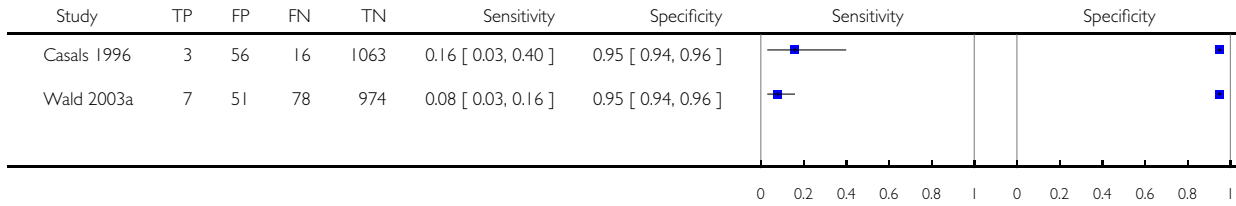
Test: 5 IT total hCG, 5FPR



### Test 6. IT AFP, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

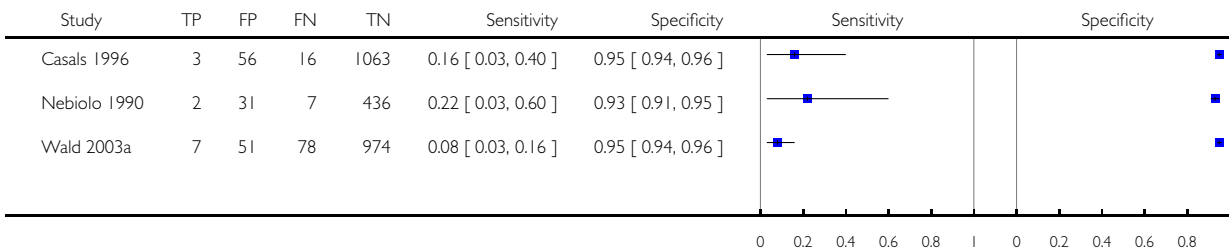
Test: 6 IT AFP, 5% FPR



### Test 10. IT AFP, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

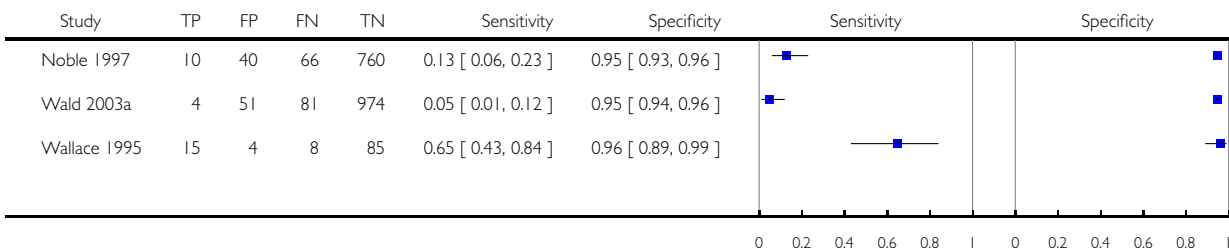
Test: 10 IT AFP, mixed cut-points



### Test 11. IT Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

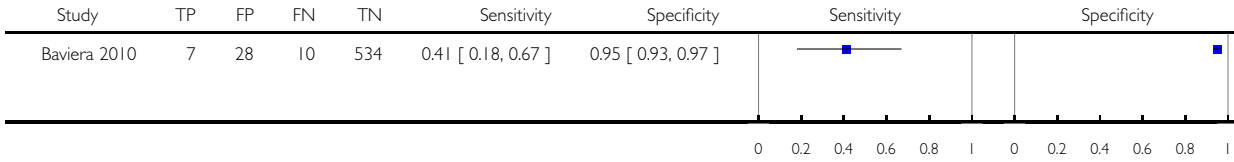
Test: 11 IT Inhibin, 5FPR



### Test 12. IT ADAM I2, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

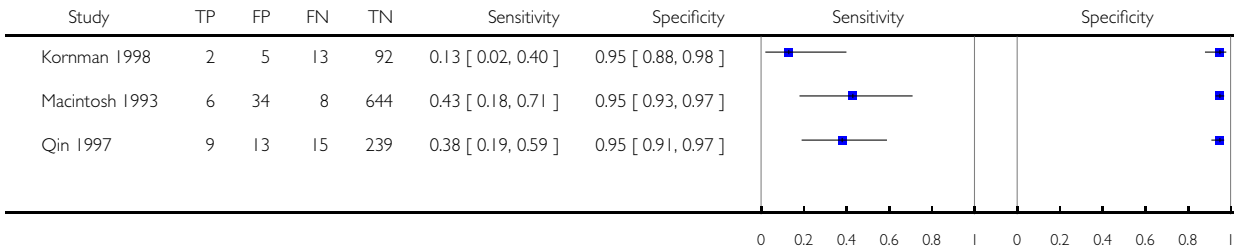
Test: I2 IT ADAM I2, 5FPR



### Test 13. IT SPI, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

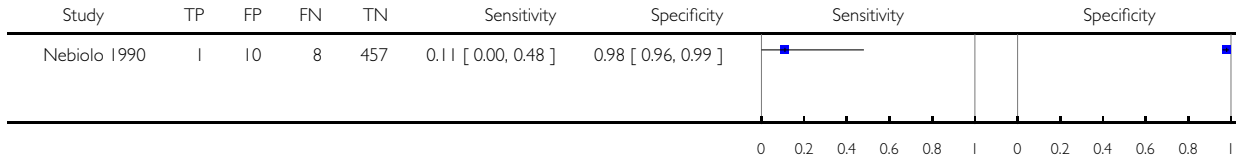
Test: I3 IT SPI, 5% FPR



### Test 17. ba'hcg'ratio, 0.25MoM.

Review: First trimester serum tests for Down's syndrome screening

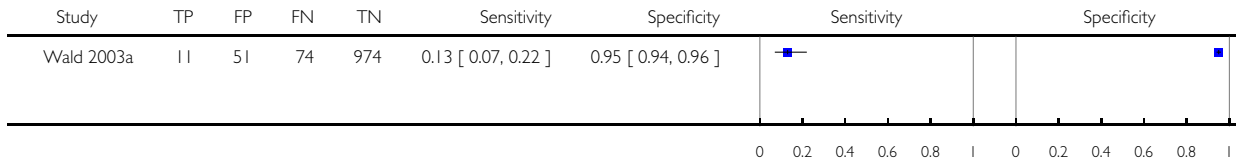
Test: 17 ba'hcg'ratio, 0.25MoM



### Test 18. 1T uE3, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

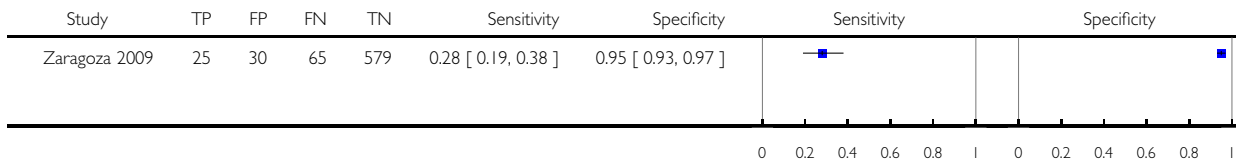
Test: 18 1T uE3, 5% FPR



### Test 19. 1T PIGF, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

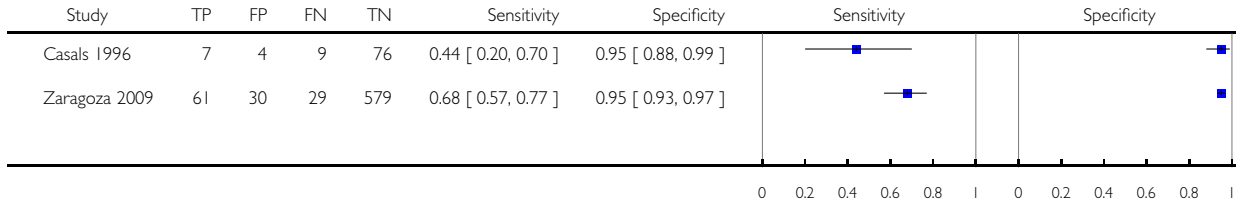
Test: 19 1T PIGF, 5FPR



### Test 20. IT PAPP-A and IT free $\beta$ hCG, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

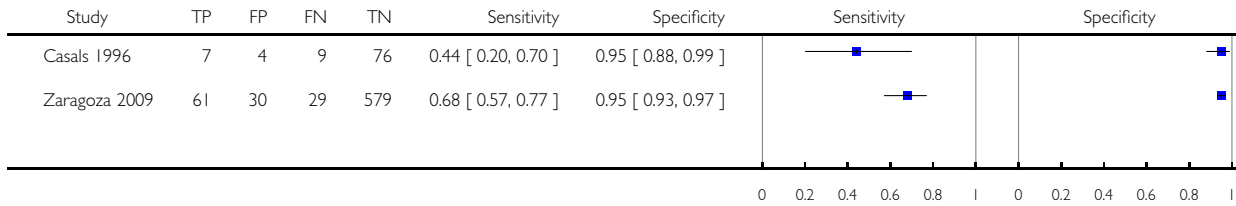
Test: 20 IT PAPP-A and IT free hCG, 5% FPR



### Test 21. IT PAPP-A and IT free $\beta$ hCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 21 IT PAPP-A and IT free hCG, mixed cut-points

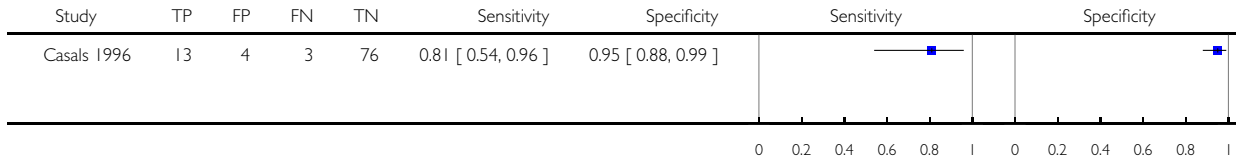




### Test 22. IT PAPP-A and IT AFP, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

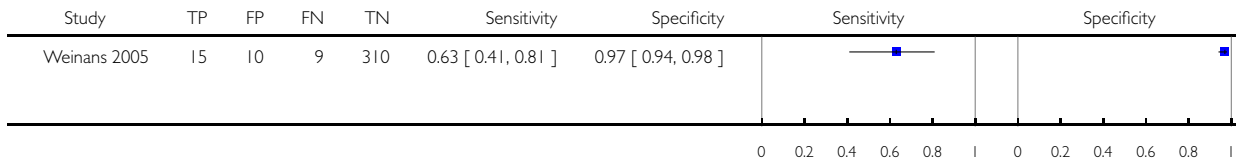
Test: 22 IT PAPP-A and IT AFP, 5% FPR



### Test 23. IT PAPP-A and IT ITA, 3% FPR.

Review: First trimester serum tests for Down's syndrome screening

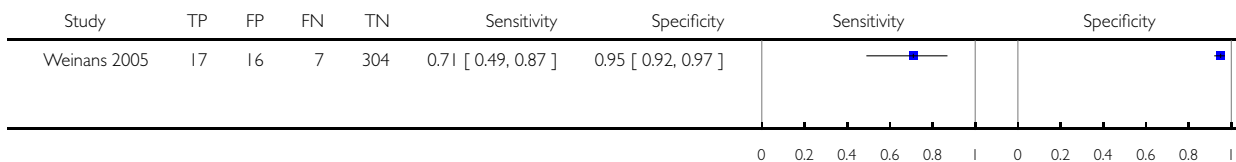
Test: 23 IT PAPP-A and IT ITA, 3% FPR



### Test 24. IT PAPP-A and IT ITA, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

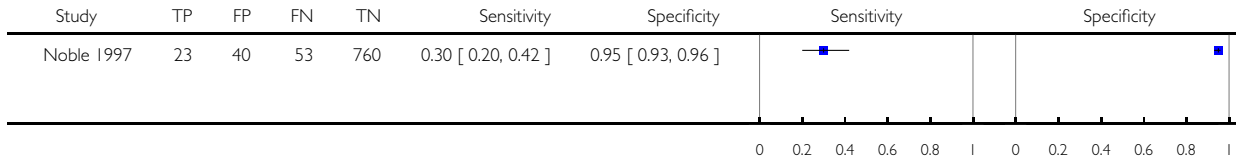
Test: 24 IT PAPP-A and IT ITA, 5% FPR



### Test 25. IT free $\beta$ hCG and IT Inhibin, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

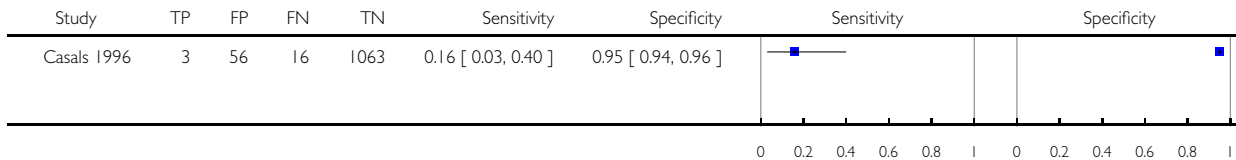
Test: 25 IT free hCG and IT Inhibin, 5% FPR



### Test 26. IT free $\beta$ hCG and IT AFP, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

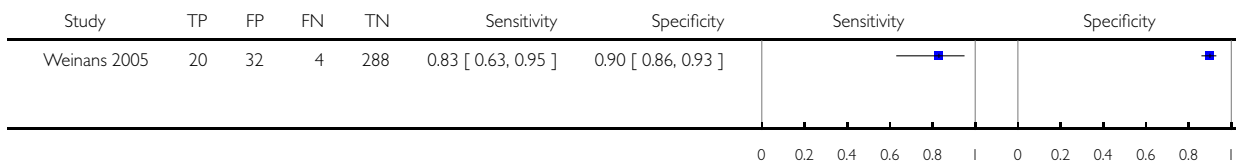
Test: 26 IT free hCG and IT AFP, 5% FPR



### Test 27. IT PAPP-A and IT ITA, 10% FPR.

Review: First trimester serum tests for Down's syndrome screening

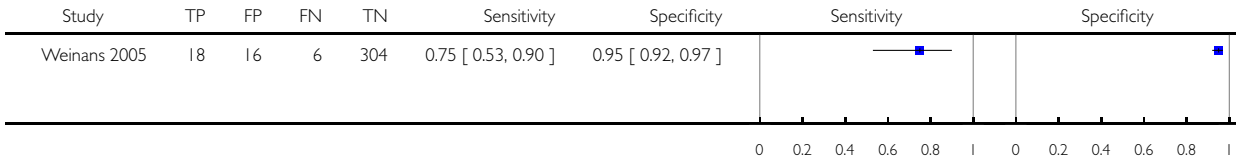
Test: 27 IT PAPP-A and IT ITA, 10% FPR



**Test 28. IT PAPP-A, IT free  $\beta$ hCG and IT ITA, 5% FPR.**

Review: First trimester serum tests for Down's syndrome screening

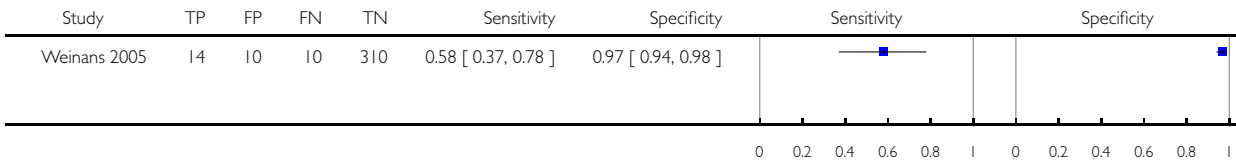
Test: 28 IT PAPP-A, IT free hCG and IT ITA, 5% FPR



**Test 29. IT PAPP-A, IT free  $\beta$ hCG and IT ITA, 3% FPR.**

Review: First trimester serum tests for Down's syndrome screening

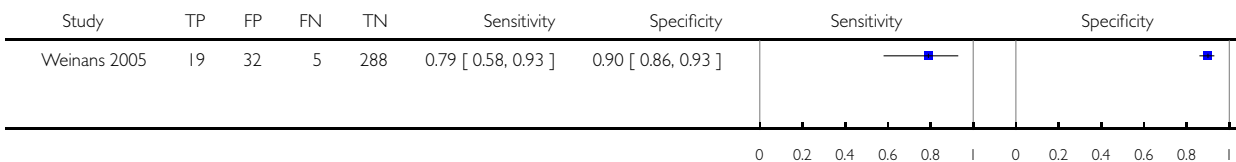
Test: 29 IT PAPP-A, IT free hCG and IT ITA, 3% FPR



**Test 30. IT PAPP-A, IT free  $\beta$ hCG and IT ITA, 10% FPR.**

Review: First trimester serum tests for Down's syndrome screening

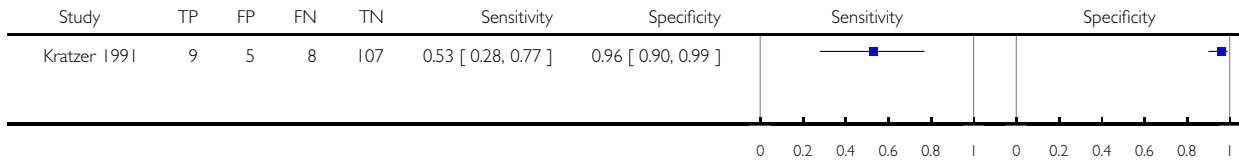
Test: 30 IT PAPP-A, IT free hCG and IT ITA, 10% FPR



### Test 31. IT total hCG, IT free $\alpha$ hCG and IT progesterone, 0.34 MoM.

Review: First trimester serum tests for Down's syndrome screening

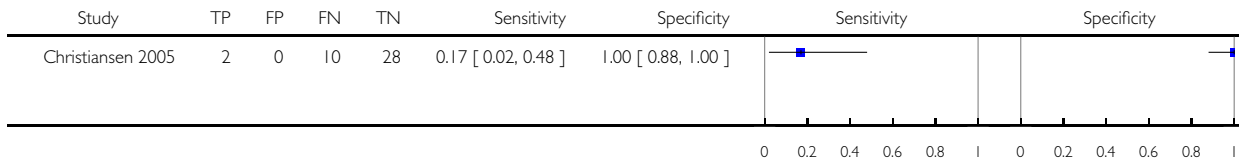
Test: 31 IT total hCG, IT free  $\alpha$  hCG and IT progesterone, 0.34 MoM



### Test 32. Age, IT Inhibin, risk 1:100.

Review: First trimester serum tests for Down's syndrome screening

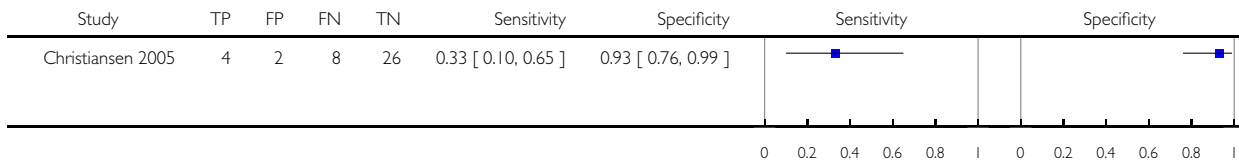
Test: 32 Age, IT Inhibin, risk 1:100



### Test 33. Age, IT Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

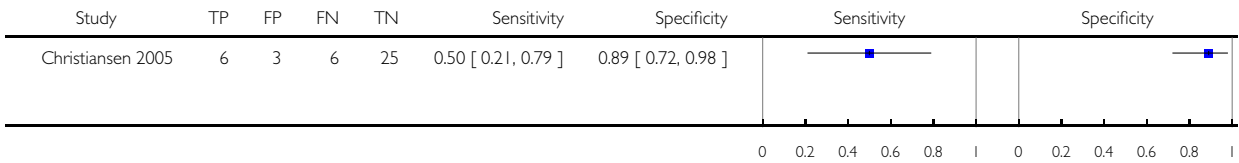
Test: 33 Age, IT Inhibin, risk 1:250



### Test 34. Age, IT Inhibin, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

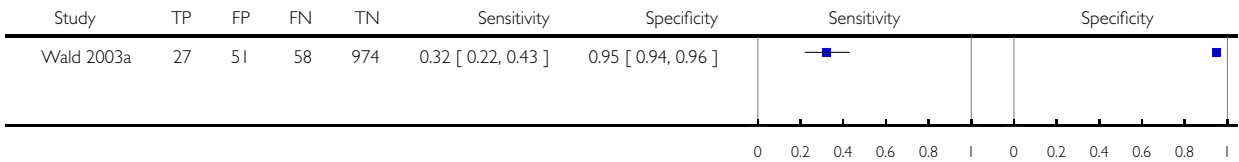
Test: 34 Age, IT Inhibin, risk 1:400



### Test 35. Age, IT Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

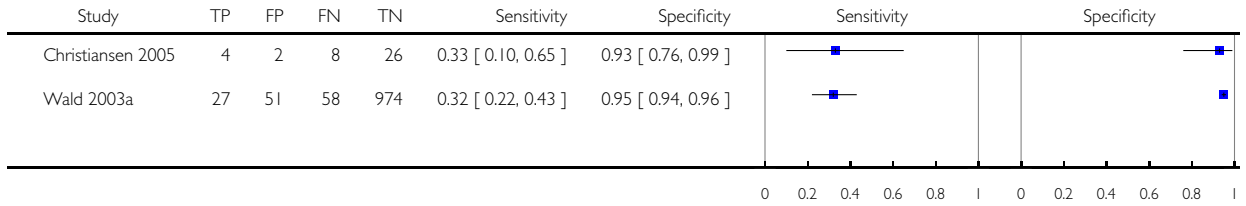
Test: 35 Age, IT Inhibin, 5FPR



### Test 36. Age, IT Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

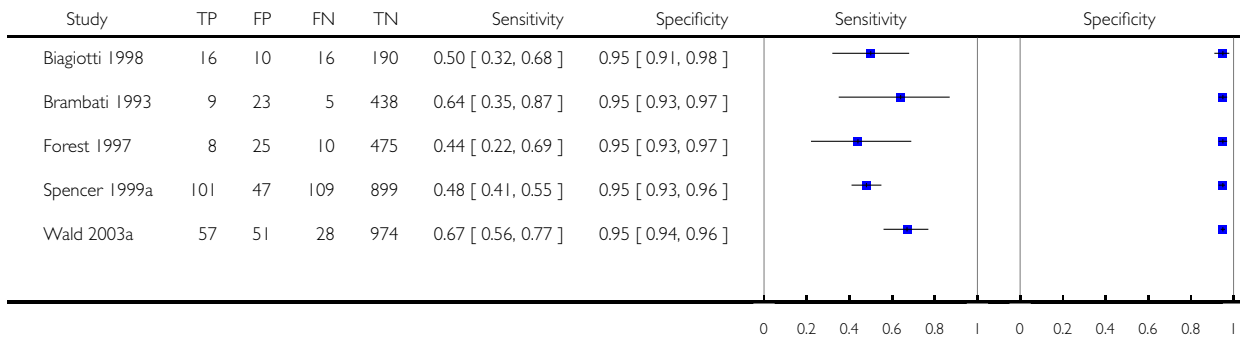
Test: 36 Age, IT Inhibin, mixed cut-points



### Test 37. Age, IT PAPP-A, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

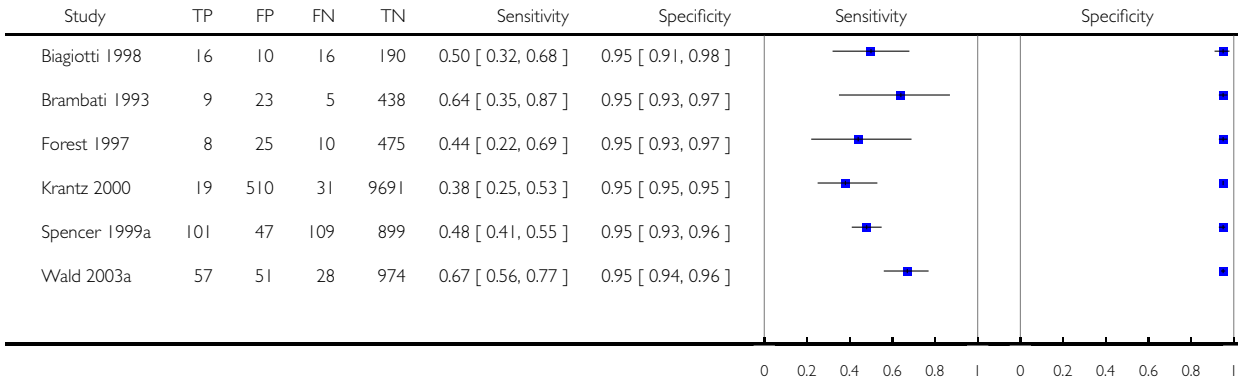
Test: 37 Age, IT PAPP-A, 5FPR



### Test 38. Age, IT PAPP-A, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

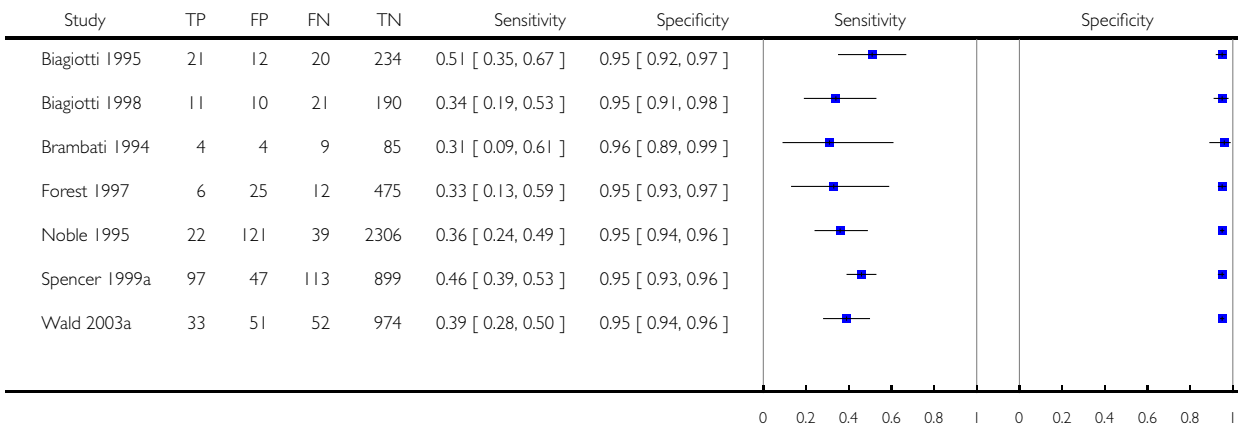
Test: 38 Age, IT PAPP-A, mixed cut-points



### Test 39. Age, IT free $\beta$ hCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

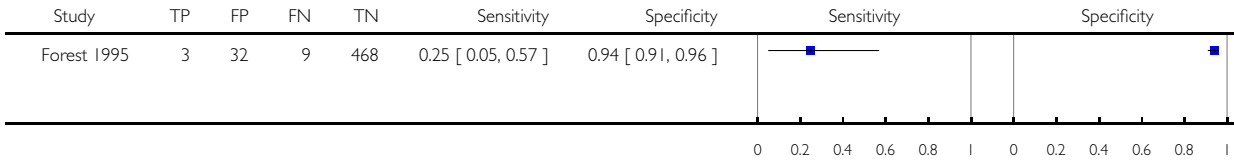
Test: 39 Age, IT free hCG, 5FPR



### Test 40. Age, IT free $\beta$ hCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

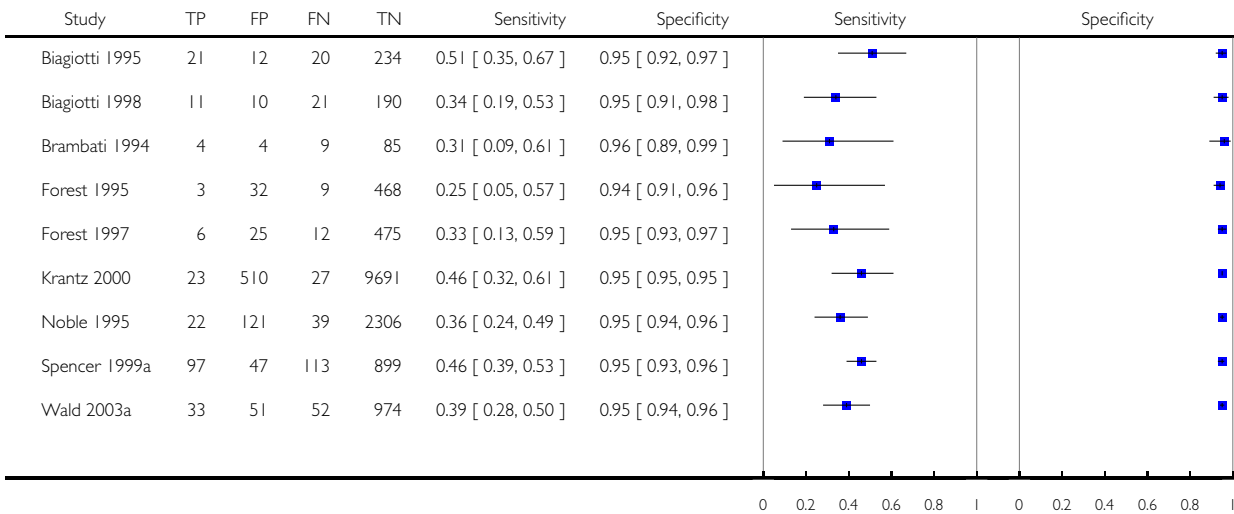
Test: 40 Age, IT free hCG, risk 1:384



### Test 41. Age, IT free $\beta$ hCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 41 Age, IT free hCG, mixed cut-points

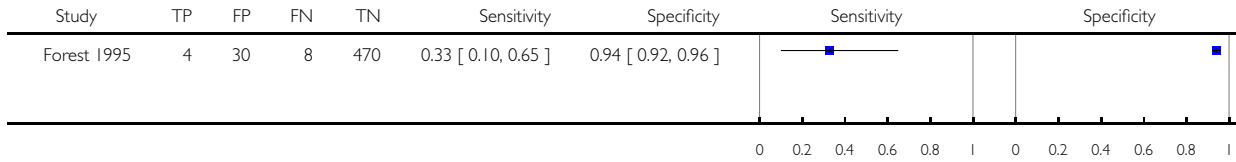




### Test 42. Age, IT total hCG,risk I:384.

Review: First trimester serum tests for Down's syndrome screening

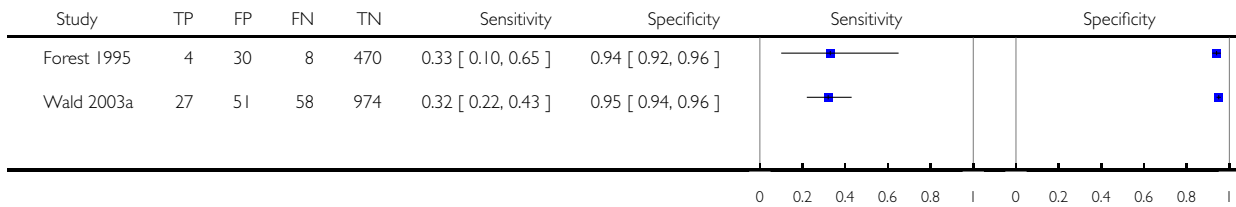
Test: 42 Age, IT total hCG,risk I:384



### Test 43. Age, IT total hCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

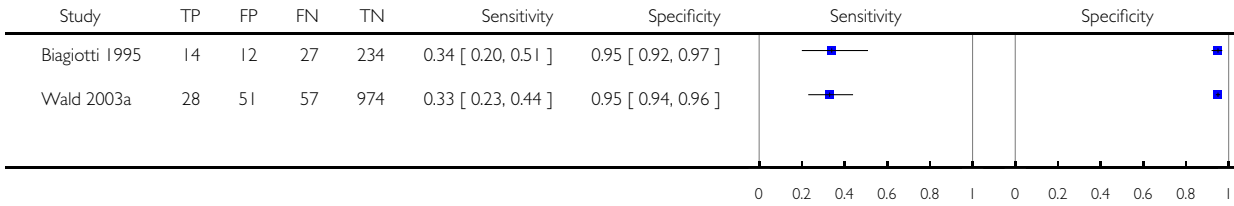
Test: 43 Age, IT total hCG, mixed cut-points



### Test 44. Age, IT AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

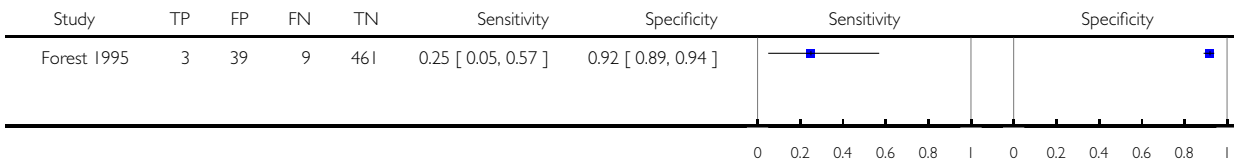
Test: 44 Age, IT AFP, 5FPR



### Test 45. Age, IT AFP, risk1:384.

Review: First trimester serum tests for Down's syndrome screening

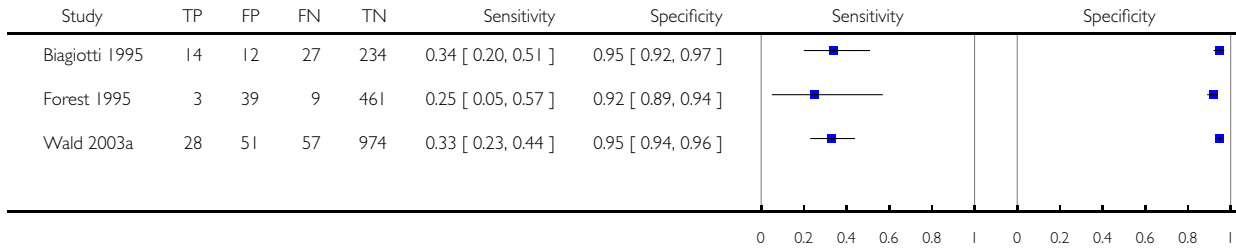
Test: 45 Age, IT AFP, risk1:384



### Test 46. Age, IT AFP,mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

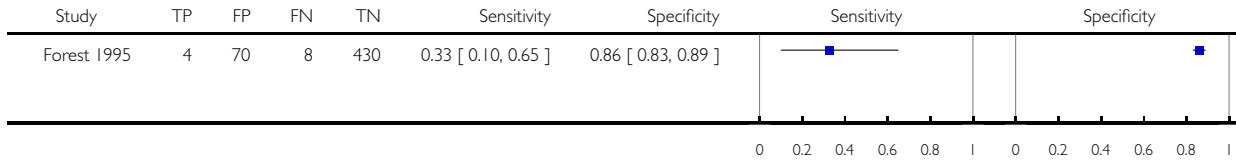
Test: 46 Age, IT AFP,mixed cut-points



### Test 47. Age, IT uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

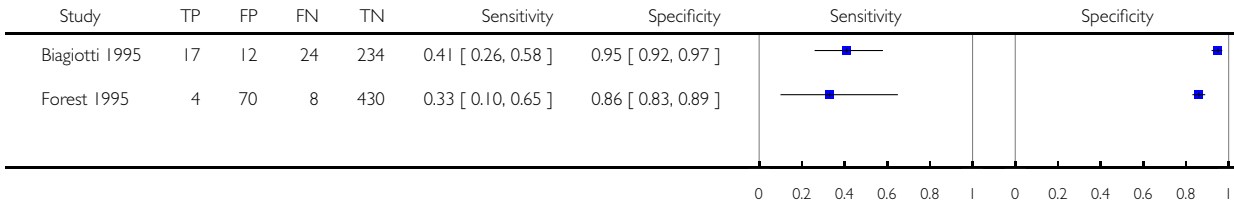
Test: 47 Age, IT uE3, risk 1:384



### Test 48. Age, IT uE3, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

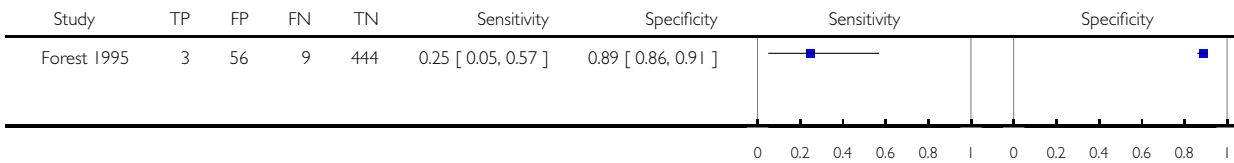
Test: 48 Age, IT uE3, mixed cut-points



### Test 49. Age, IT free $\alpha$ hCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

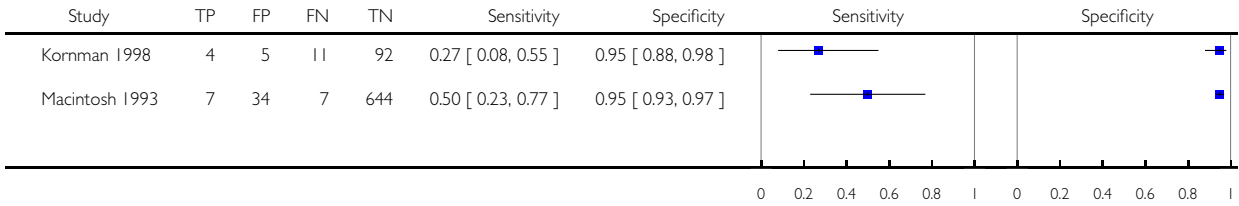
Test: 49 Age, IT free  $\alpha$  hCG, risk 1:384



### Test 50. Age, IT SPI, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

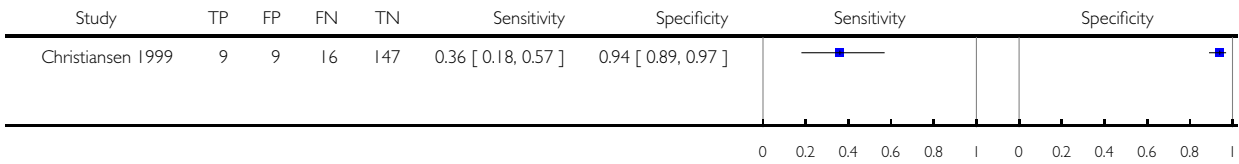
Test: 50 Age, IT SPI, 5FPR



### Test 51. Age, IT ProMBP, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

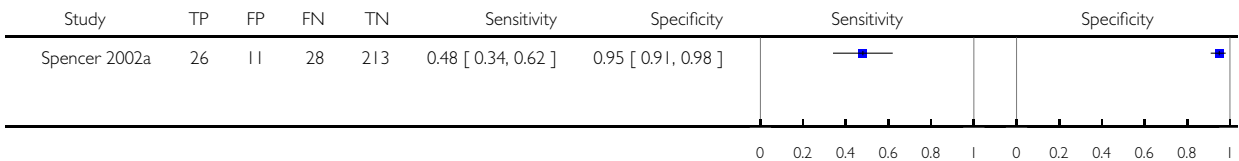
Test: 51 Age, IT ProMBP, risk 1:250



### Test 52. Age, IT ITA, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

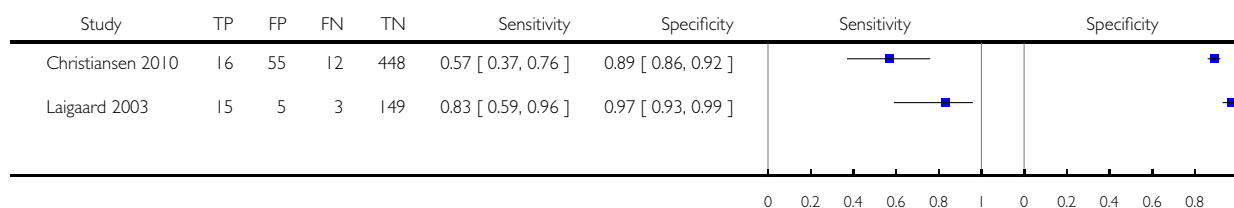
Test: 52 Age, IT ITA, 5FPR



### Test 53. Age, IT ADAM 12, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

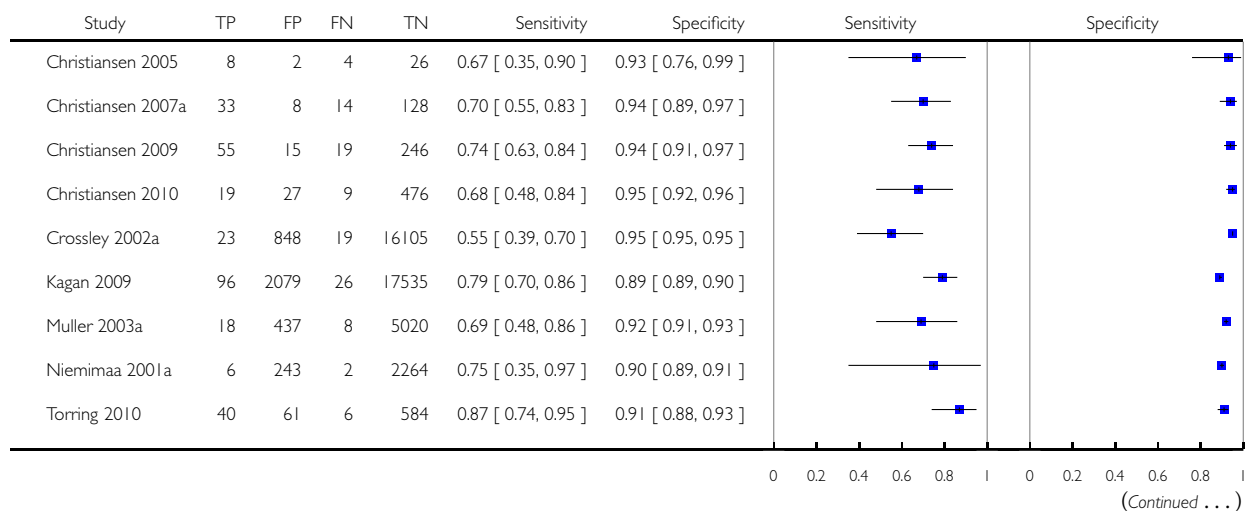
Test: 53 Age, IT ADAM 12, risk 1:400



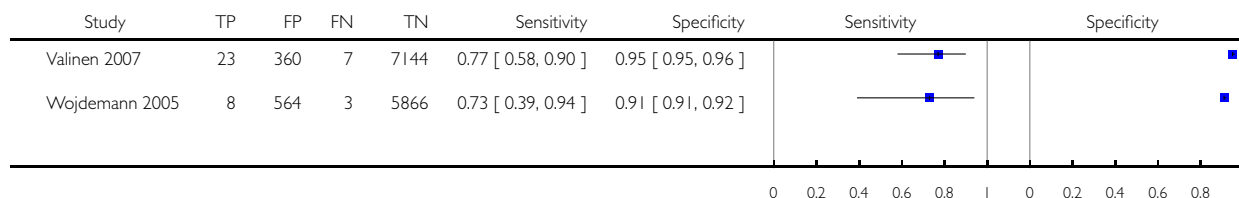
### Test 54. Age, IT PAPP-A and IT free $\beta$ hCG, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 54 Age, IT PAPP-A and IT free hCG, risk 1:250



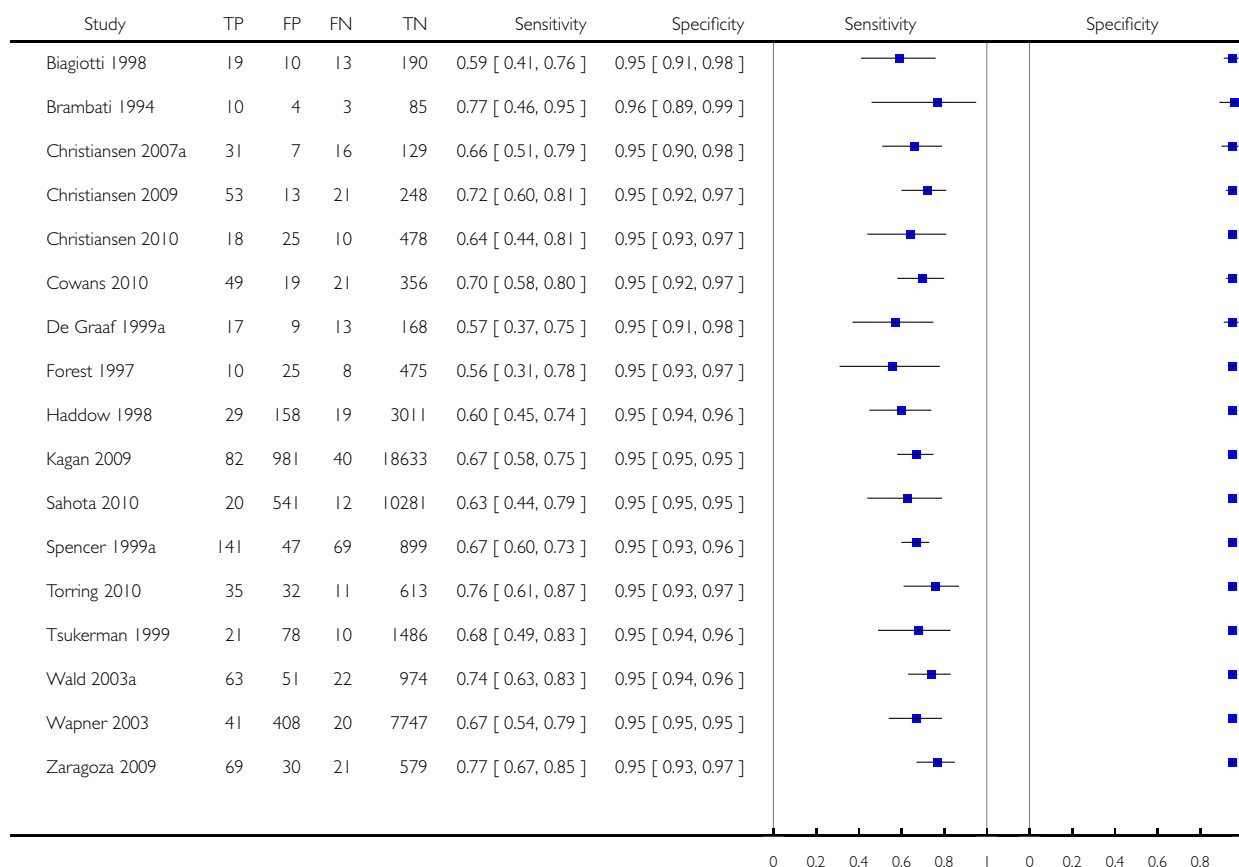
(... Continued)



### Test 55. Age, IT PAPP-A and IT free $\beta$ hCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

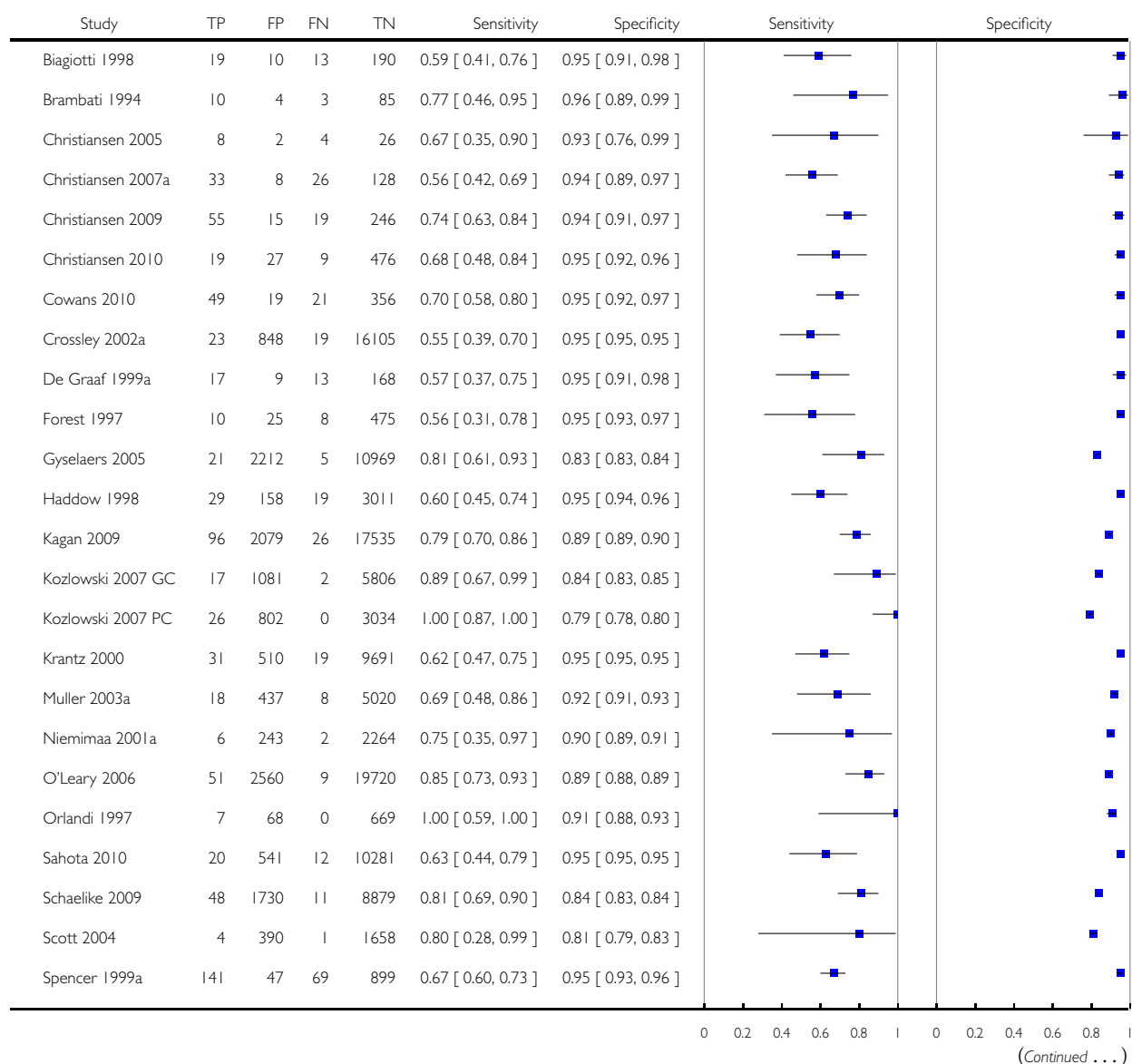
Test: 55 Age, IT PAPP-A and IT free hCG, 5FPR



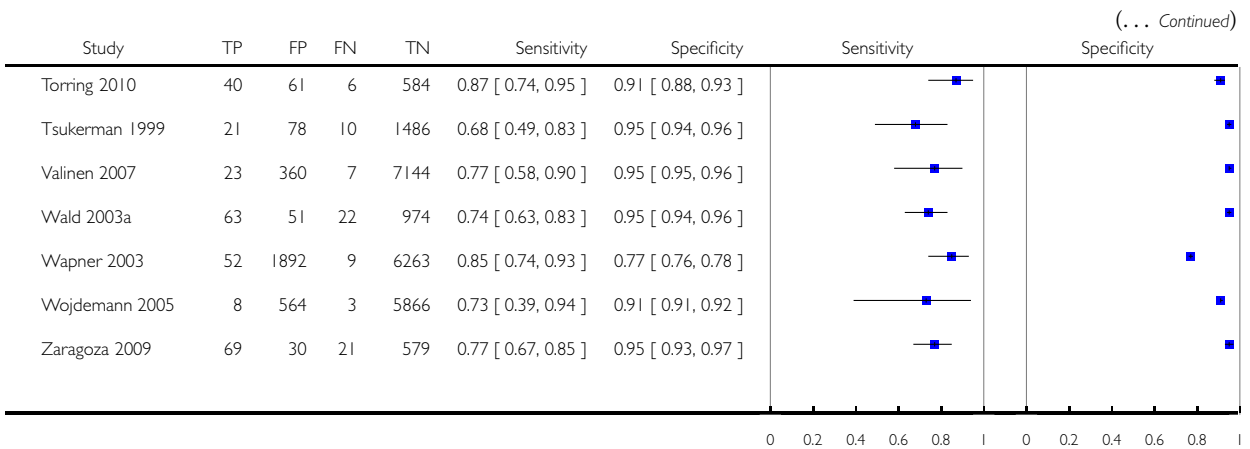
### Test 56. Age, IT PAPP-A and IT free $\beta$ hCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 56 Age, IT PAPP-A and IT free hCG, mixed cut-points



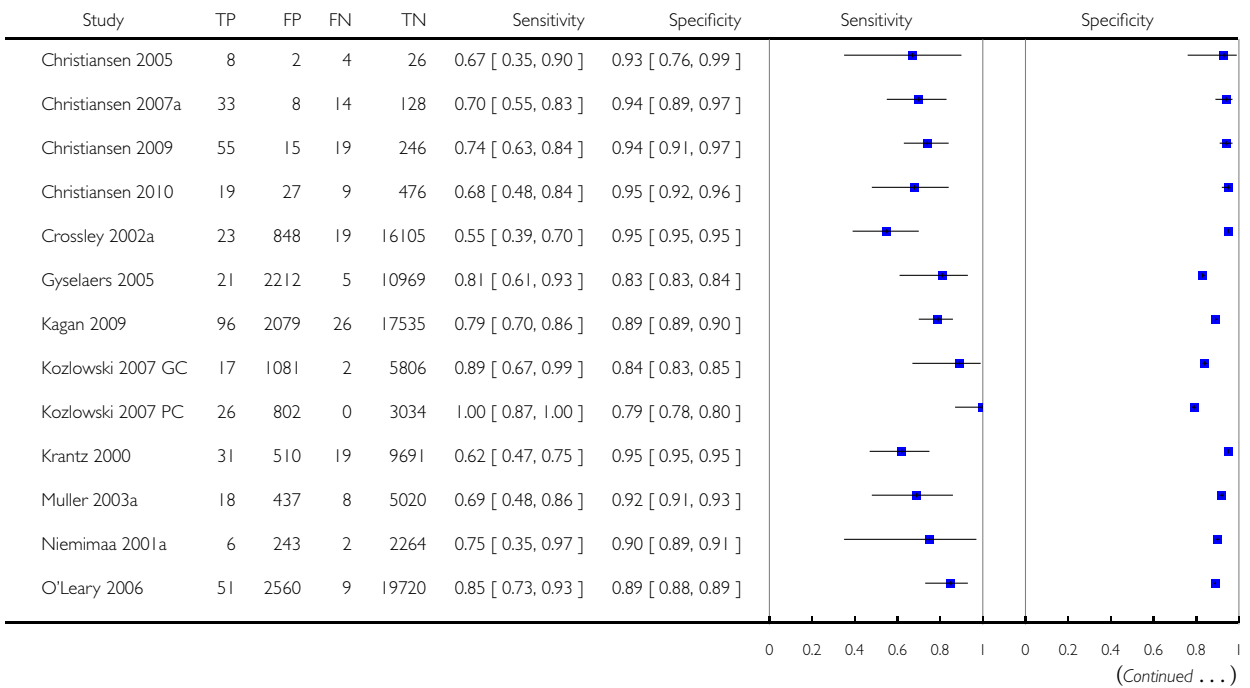




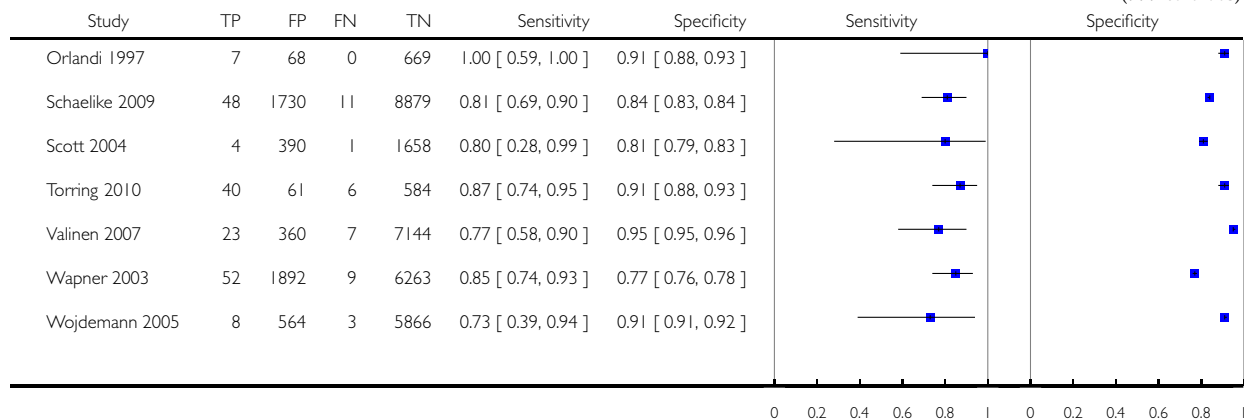
### Test 57. Age, IT PAPP-A and IT free $\beta$ hCG, mixed cut-points without 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 57 Age, IT PAPP-A and IT free hCG, mixed cut-points without 5FPR



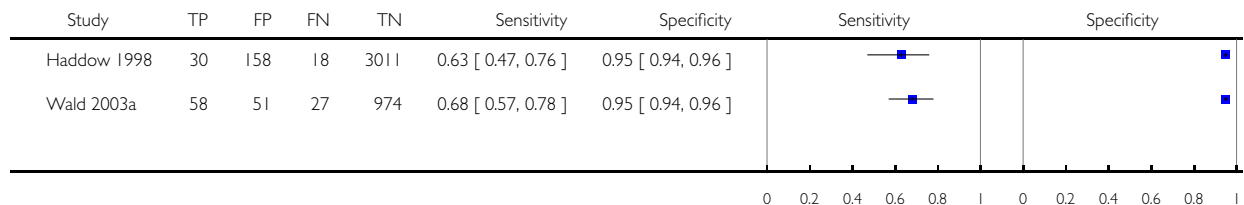
(... Continued)



### Test 58. Age, IT total hCG and IT PAPP-A, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

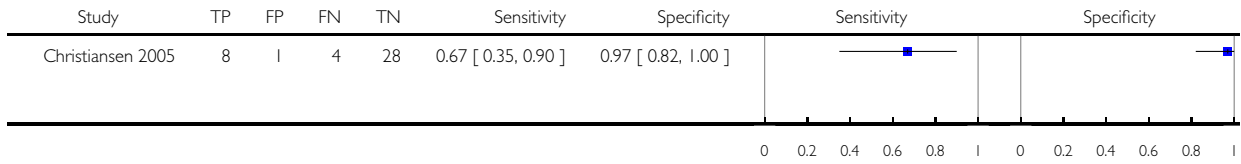
Test: 58 Age, IT total hCG and IT PAPP-A, 5FPR



### Test 59. Age, IT PAPP-A and IT Inhibin, risk 1:100.

Review: First trimester serum tests for Down's syndrome screening

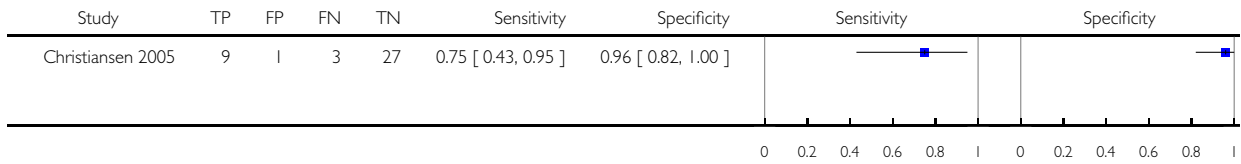
Test: 59 Age, IT PAPP-A and IT Inhibin, risk 1:100



### Test 60. Age, IT PAPP-A and IT Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

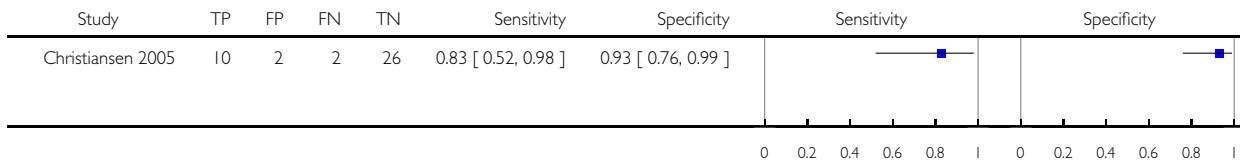
Test: 60 Age, IT PAPP-A and IT Inhibin, risk 1:250



### Test 61. Age, IT PAPP-A and IT Inhibin, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

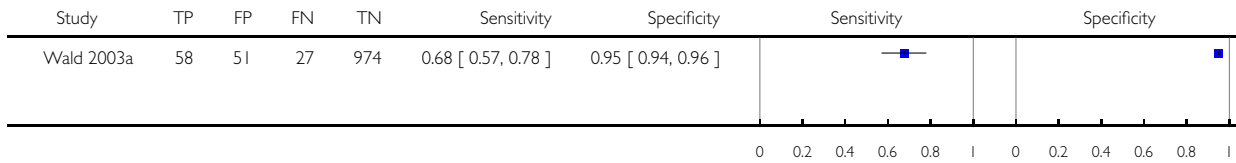
Test: 61 Age, IT PAPP-A and IT Inhibin, risk 1:400



### Test 62. Age, IT PAPP-A and IT Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

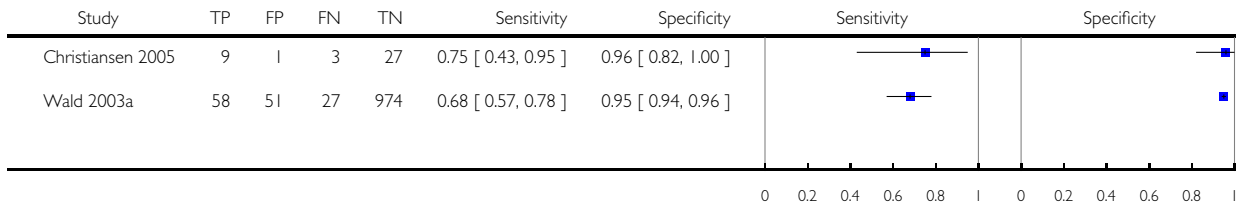
Test: 62 Age, IT PAPP-A and IT Inhibin, 5FPR



### Test 63. Age, IT PAPP-A and IT Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

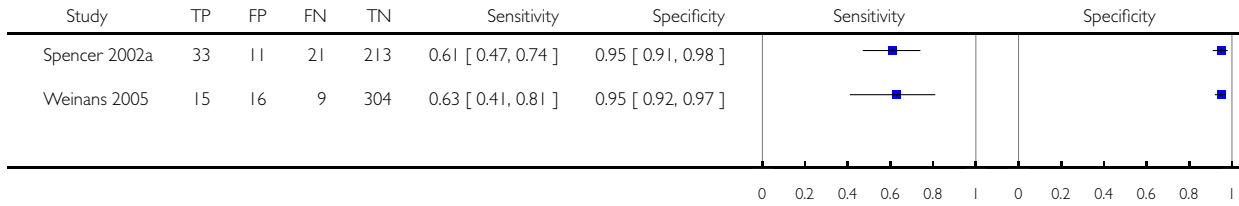
Test: 63 Age, IT PAPP-A and IT Inhibin, mixed cut-points



### Test 64. Age, IT PAPP-A and IT ITA, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

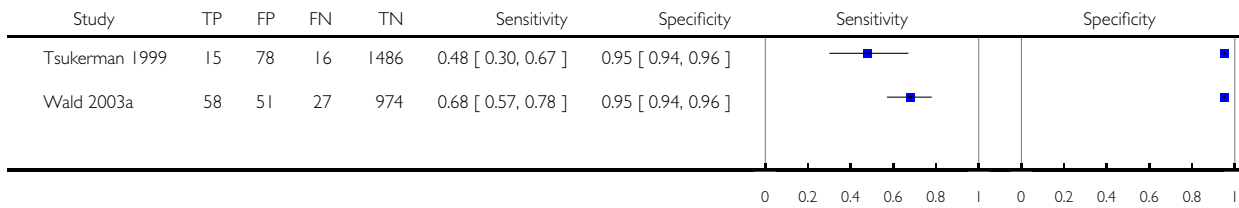
Test: 64 Age, IT PAPP-A and IT ITA, 5FPR



### Test 65. Age, IT PAPP-A and IT AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

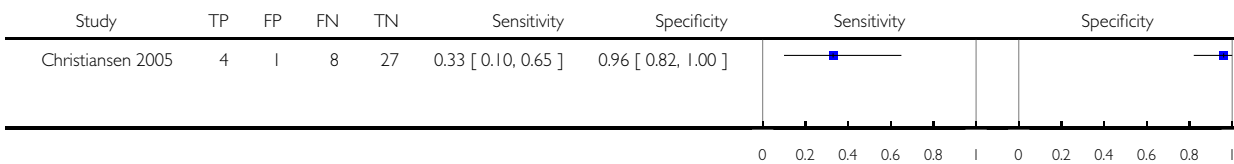
Test: 65 Age, IT PAPP-A and IT AFP, 5FPR



### Test 66. Age, IT free $\beta$ hCG and IT Inhibin, risk 1:100.

Review: First trimester serum tests for Down's syndrome screening

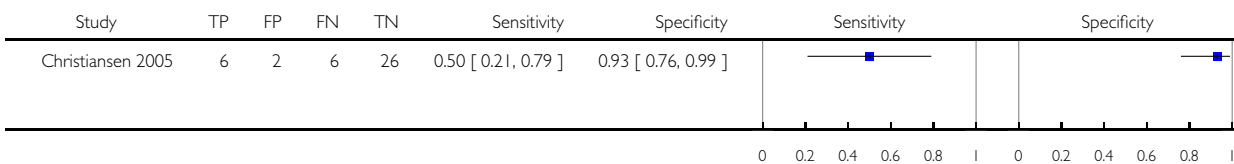
Test: 66 Age, IT free hCG and IT Inhibin, risk 1:100



### Test 67. Age, IT free $\beta$ hCG and IT Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

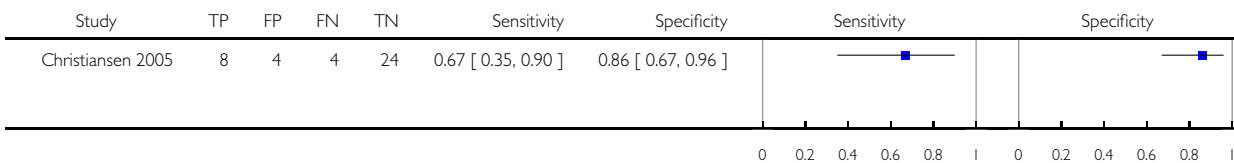
Test: 67 Age, IT free hCG and IT Inhibin, risk 1:250



### Test 68. Age, IT free $\beta$ hCG and IT Inhibin, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

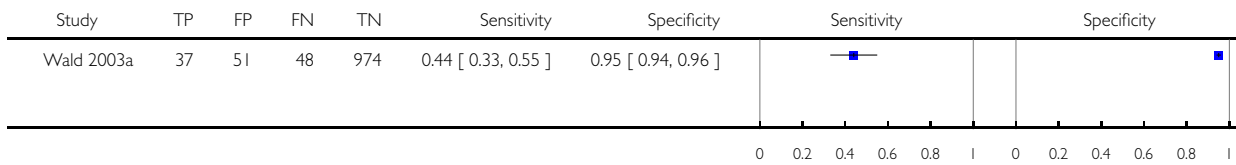
Test: 68 Age, IT free hCG and IT Inhibin, risk 1:400



### Test 69. Age, IT free $\beta$ hCG and IT Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

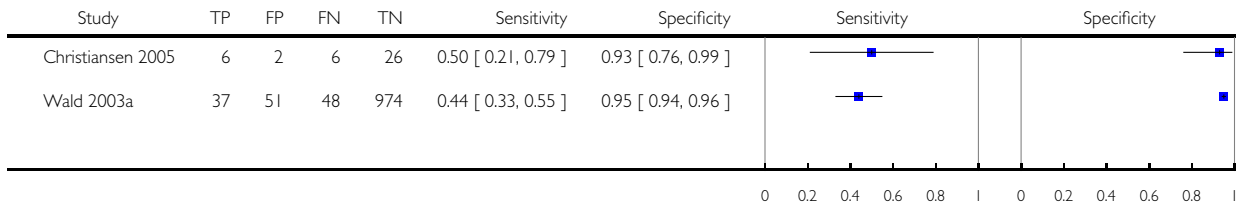
Test: 69 Age, IT free hCG and IT Inhibin, 5FPR



### Test 70. Age, IT free $\beta$ hCG and IT Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

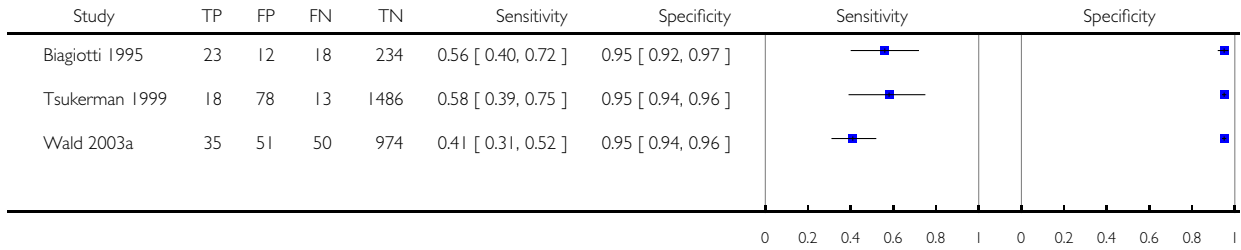
Test: 70 Age, IT free hCG and IT Inhibin, mixed cut-points



### Test 71. Age, IT free $\beta$ hCG and IT AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

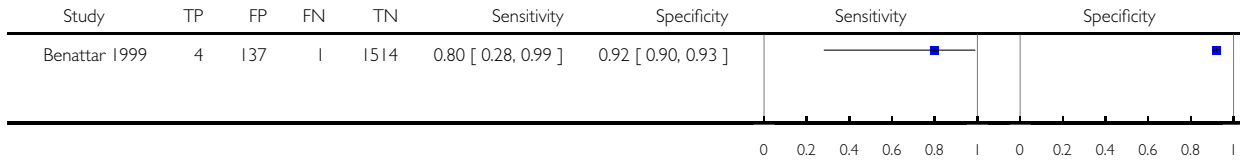
Test: 71 Age, IT free hCG and IT AFP, 5FPR



### Test 72. Age, IT free $\beta$ hCG and IT AFP, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 72 Age, IT free hCG and IT AFP, risk 1:250

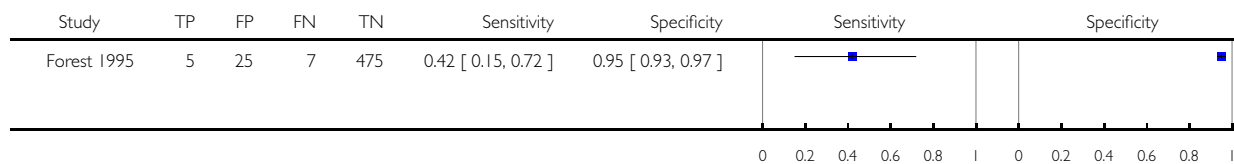




### Test 73. Age, IT free $\beta$ hCG and IT AFP, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

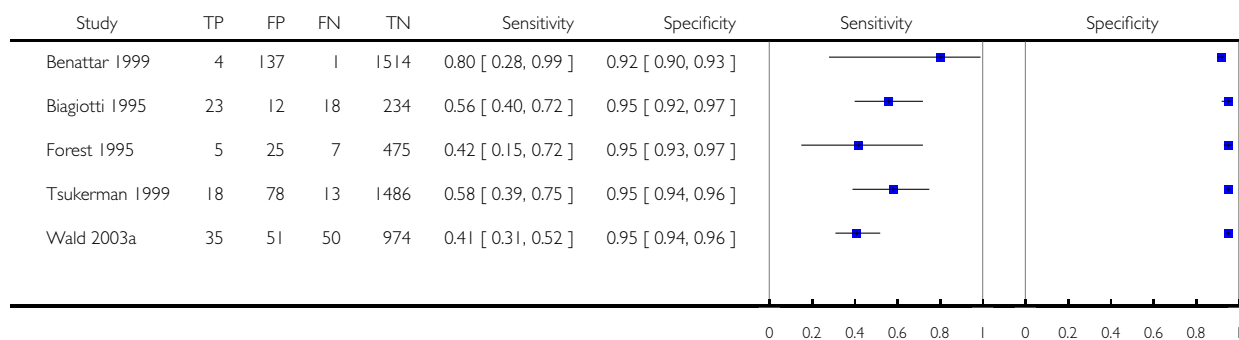
Test: 73 Age, IT free hCG and IT AFP, risk 1:384



### Test 74. Age, IT free $\beta$ hCG and IT AFP, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

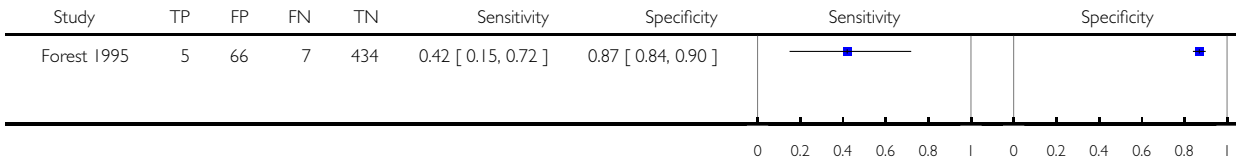
Test: 74 Age, IT free hCG and IT AFP, mixed cut-points



**Test 75. Age, IT AFP and IT uE3, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

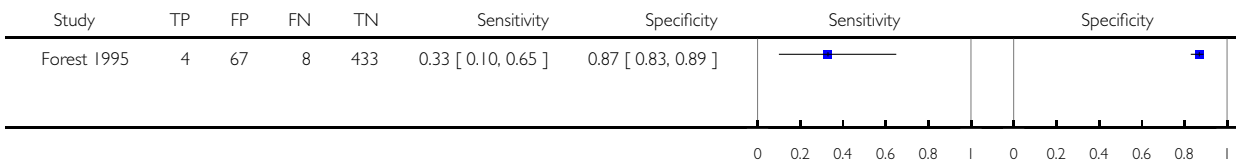
Test: 75 Age, IT AFP and IT uE3, risk 1:384



**Test 76. Age, IT AFP and IT free  $\alpha$ hCG, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

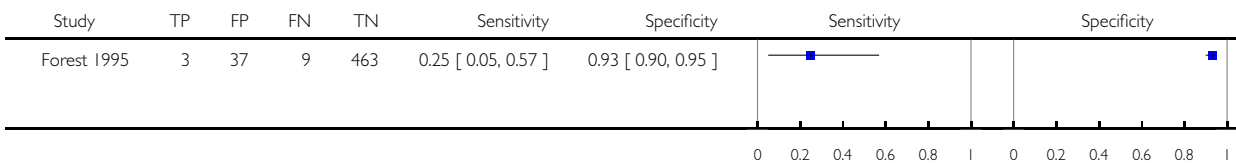
Test: 76 Age, IT AFP and IT free  $\alpha$ hCG, risk 1:384



**Test 77. Age, IT free  $\beta$ hCG and IT total hCG, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

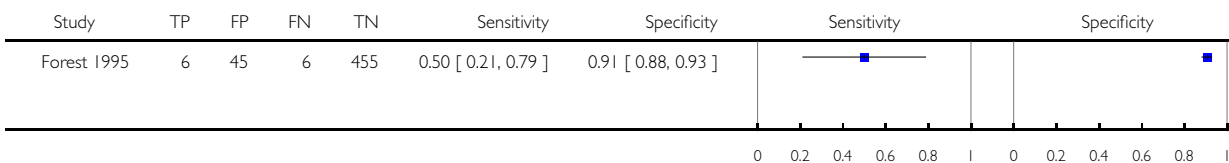
Test: 77 Age, IT free  $\beta$ hCG and IT total hCG, risk 1:384



### Test 78. Age, IT free $\beta$ hCG and IT uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

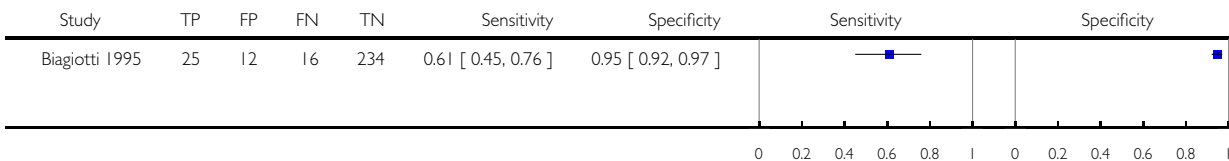
Test: 78 Age, IT free hCG and IT uE3, risk 1:384



### Test 79. Age, IT free $\beta$ hCG and IT uE3, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

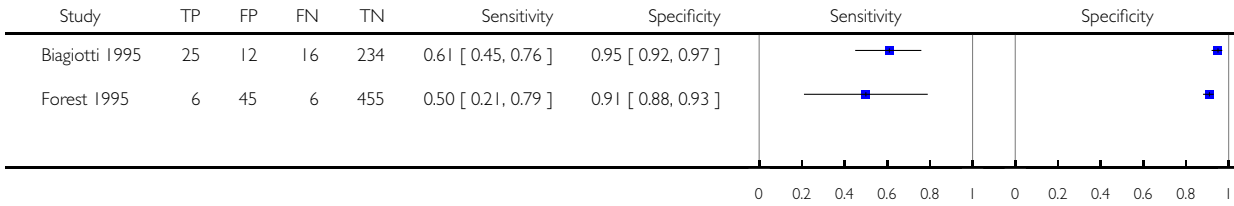
Test: 79 Age, IT free hCG and IT uE3, 5FPR



### Test 80. Age, IT free $\beta$ hCG and IT uE3, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

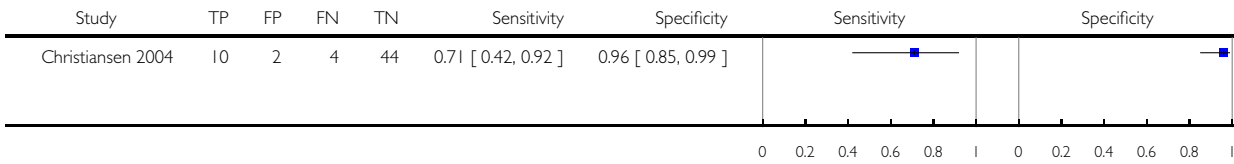
Test: 80 Age, IT free hCG and IT uE3, mixed cut-points



### Test 81. Age, IT free $\beta$ hCG and IT SPI, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

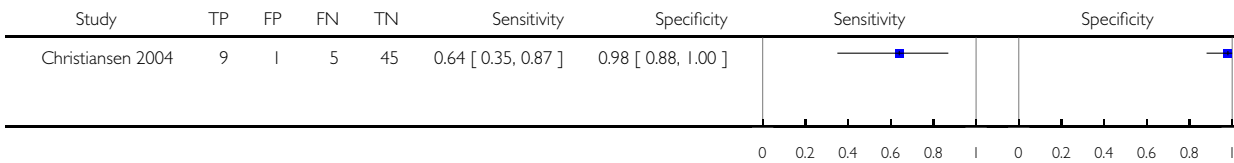
Test: 81 Age, IT free hCG and IT SPI, 5FPR



### Test 82. Age, IT free $\beta$ hCG and IT SPI risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

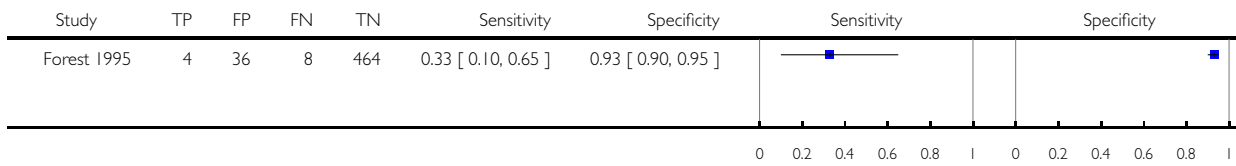
Test: 82 Age, IT free hCG and IT SPI risk 1:250



### Test 83. Age, IT AFP and IT total hCG, 1:384.

Review: First trimester serum tests for Down's syndrome screening

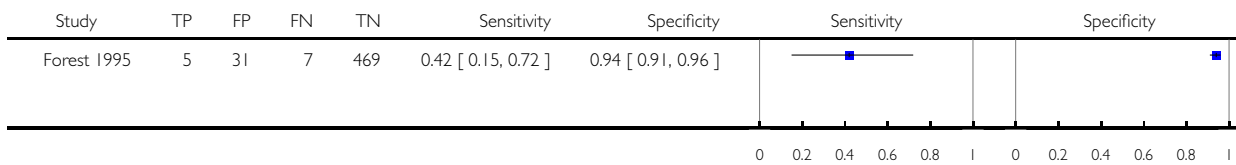
Test: 83 Age, IT AFP and IT total hCG, 1:384



### Test 84. Age, IT free $\beta$ hCG and IT free $\alpha$ hCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

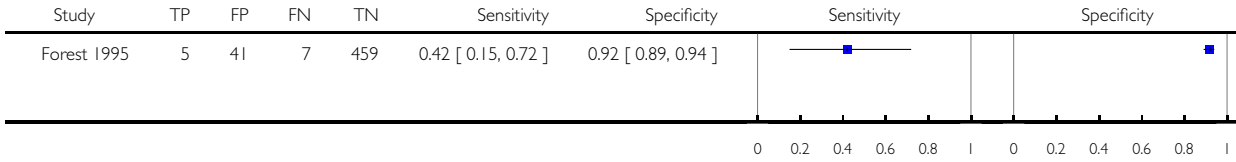
Test: 84 Age, IT free  $\beta$ hCG and IT free  $\alpha$ hCG, risk 1:384



**Test 85. Age, IT total hCG and IT uE3, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

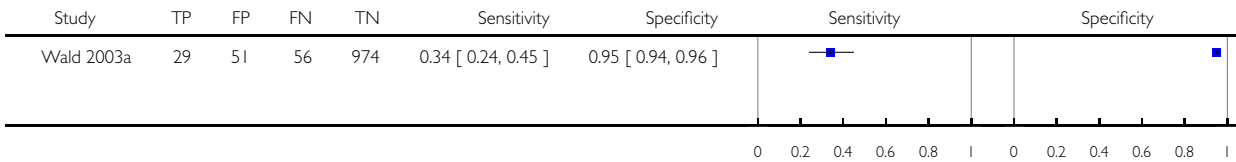
Test: 85 Age, IT total hCG and IT uE3, risk 1:384



**Test 86. Age, IT total hCG and IT Inhibin, 5FPR.**

Review: First trimester serum tests for Down's syndrome screening

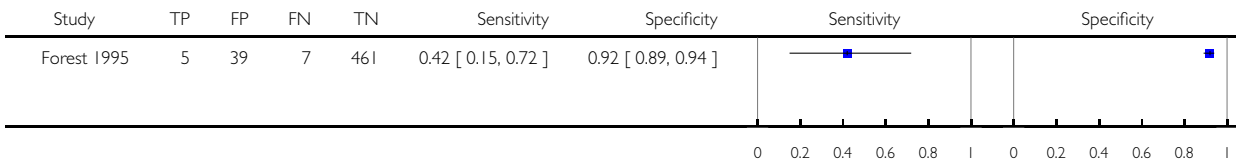
Test: 86 Age, IT total hCG and IT Inhibin, 5FPR



**Test 87. Age, IT total hCG and IT free  $\alpha$ hCG, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

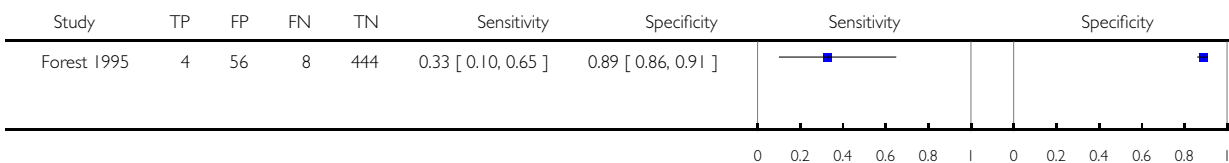
Test: 87 Age, IT total hCG and IT free  $\alpha$  hCG, risk 1:384



### Test 88. Age, IT uE3 and IT free $\alpha$ hCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

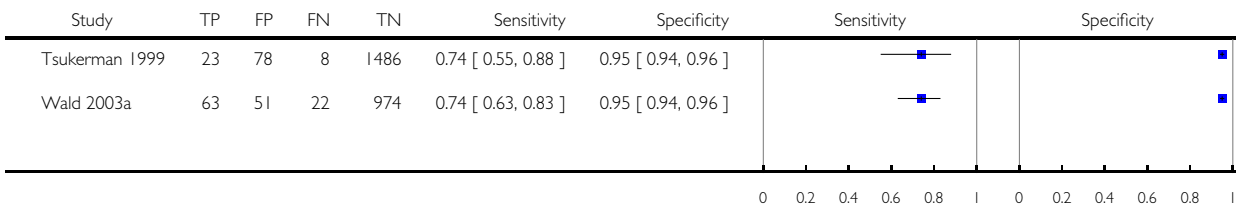
Test: 88 Age, IT uE3 and IT free  $\alpha$  hCG, risk 1:384



### Test 89. Age, IT PAPP-A, IT free $\beta$ hCG and IT AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

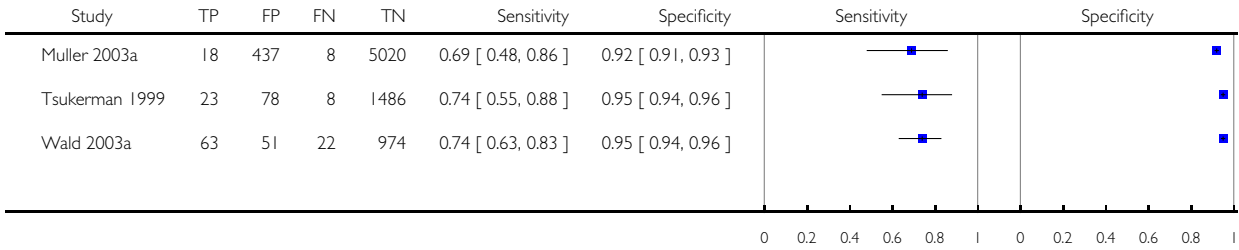
Test: 89 Age, IT PAPP-A, IT free  $\beta$  hCG and IT AFP, 5FPR



### Test 90. Age, IT PAPP-A, IT free $\beta$ hCG and IT AFP, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

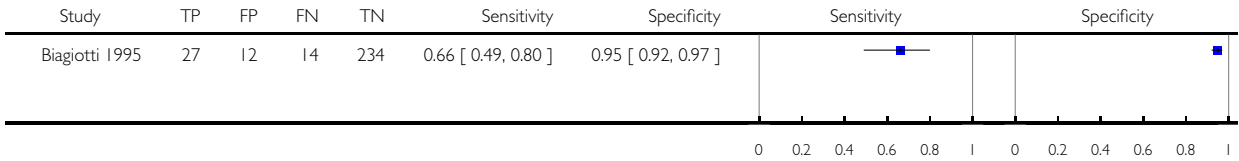
Test: 90 Age, IT PAPP-A, IT free hCG and IT AFP, mixed cut-points



### Test 91. Age, IT free $\beta$ hCG, IT AFP and IT uE3, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 91 Age, IT free hCG, IT AFP and IT uE3, 5FPR

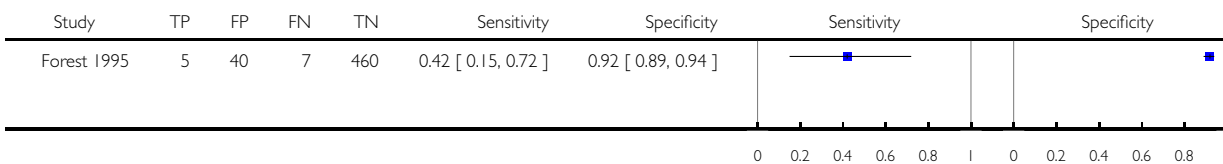




### Test 92. Age, IT free $\beta$ hCG, IT AFP and IT uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

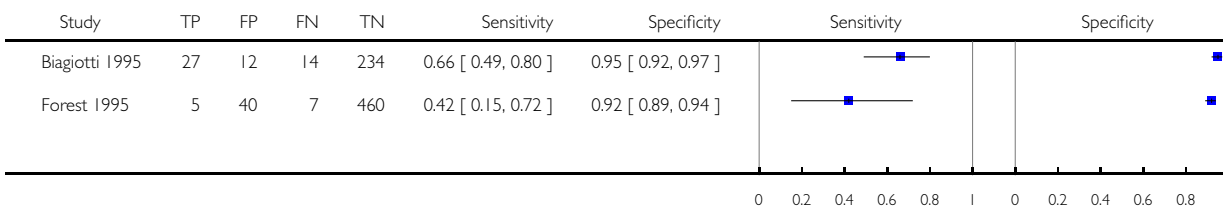
Test: 92 Age, IT free hCG, IT AFP and IT uE3, risk 1:384



### Test 93. Age, IT free $\beta$ hCG, IT AFP and IT uE3, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

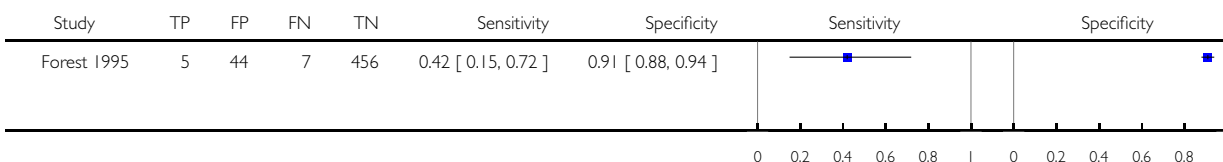
Test: 93 Age, IT free hCG, IT AFP and IT uE3, mixed cut-points



### Test 94. Age, IT total hCG, IT AFP and IT uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

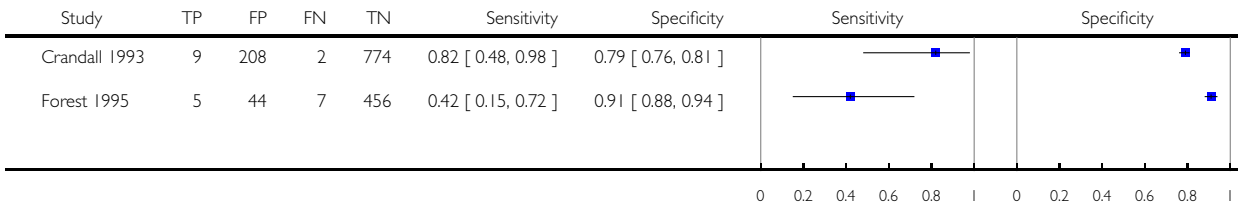
Test: 94 Age, IT total hCG, IT AFP and IT uE3, risk 1:384



**Test 95. Age, IT total hCG, IT AFP and IT uE3, mixed cut-points.**

Review: First trimester serum tests for Down's syndrome screening

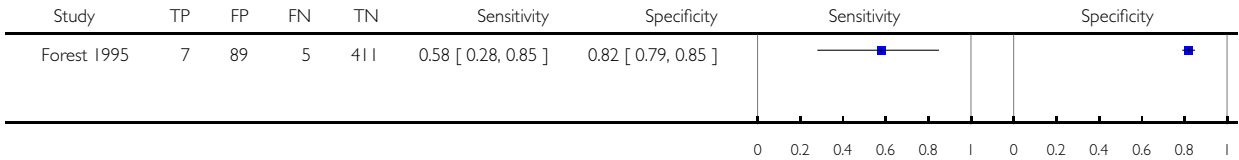
Test: 95 Age, IT total hCG, IT AFP and IT uE3, mixed cut-points



**Test 96. Age, IT AFP, free  $\alpha$ hCG and IT uE3, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

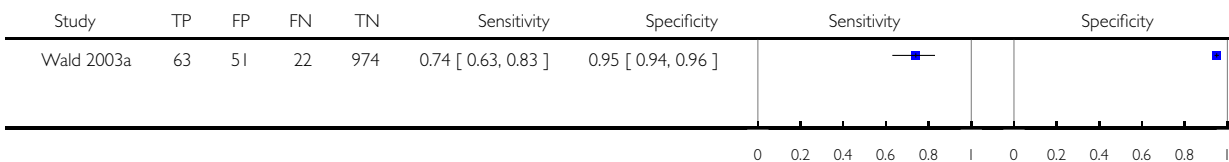
Test: 96 Age, IT AFP, free  $\alpha$  hCG and IT uE3, risk 1:384



### Test 97. Age, IT PAPP-A, IT free $\beta$ hCG and IT Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

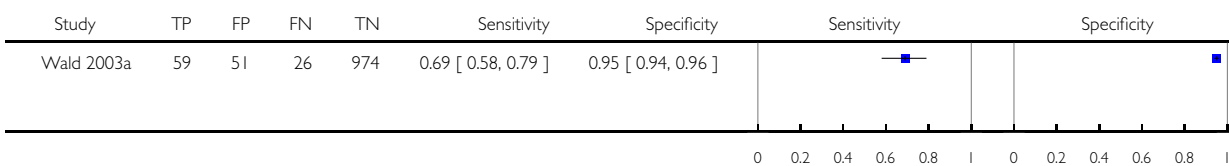
Test: 97 Age, IT PAPP-A, IT free hCG and IT Inhibin, 5FPR



### Test 98. Age, IT PAPP-A, IT total hCG and IT Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

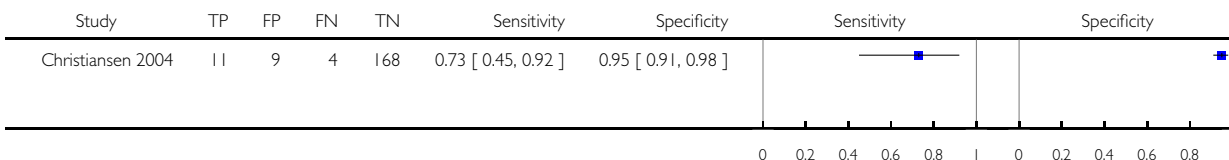
Test: 98 Age, IT PAPP-A, IT total hCG and IT Inhibin, 5FPR



### Test 99. Age, IT PAPP-A, spI and IT ProMBP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

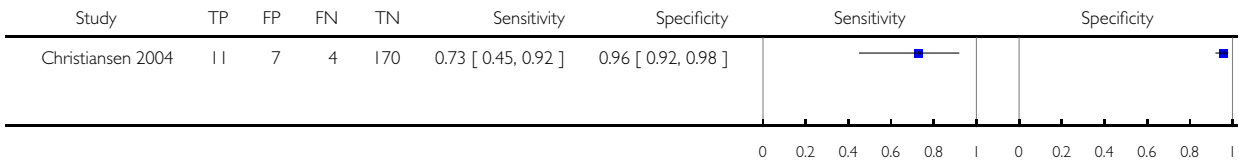
Test: 99 Age, IT PAPP-A, spI and IT ProMBP, 5FPR



**Test 100. Age, IT PAPP-A, spI and IT ProMBP, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

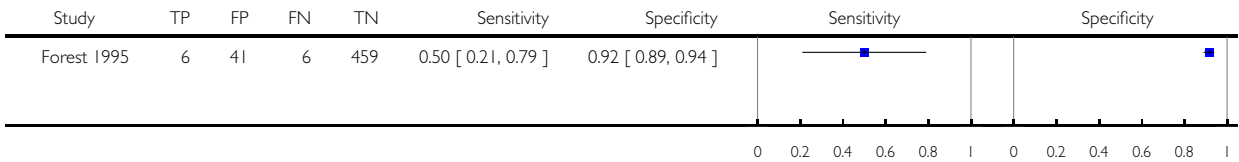
Test: 100 Age, IT PAPP-A, spI and IT ProMBP, risk 1:250



**Test 101. Age, IT free  $\beta$ hCG, IT total hCG, IT AFP and IT uE3, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

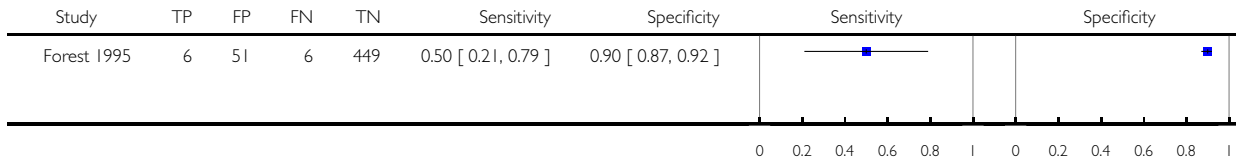
Test: 101 Age, IT free  $\beta$ hCG, IT total hCG, IT AFP and IT uE3, risk 1:384



**Test 102. Age, IT total hCG, IT AFP, IT uE3 and IT free  $\alpha$ hCG, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

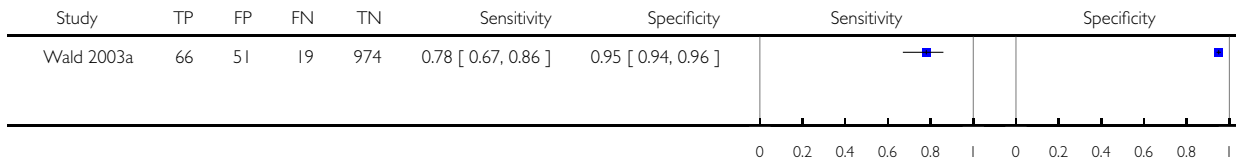
Test: 102 Age, IT total hCG, IT AFP, IT uE3 and IT free  $\alpha$  hCG, risk 1:384



**Test 103. Age, IT PAPP-A, IT free  $\beta$ hCG, IT AFP, IT uE3 and IT Inhibin, 5FPR.**

Review: First trimester serum tests for Down's syndrome screening

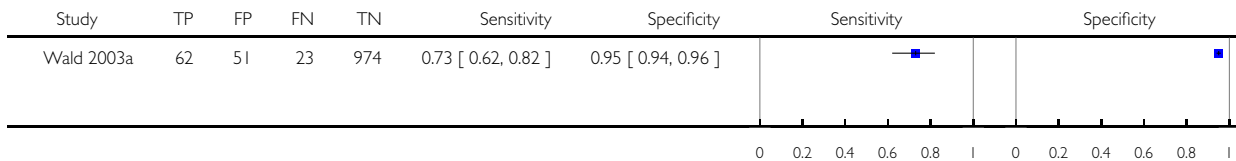
Test: 103 Age, IT PAPP-A, IT free hCG, IT AFP, IT uE3 and IT Inhibin, 5FPR



**Test 104. Age, IT PAPP-A, IT total hCG, IT AFP, IT uE3 and IT Inhibin, 5FPR.**

Review: First trimester serum tests for Down's syndrome screening

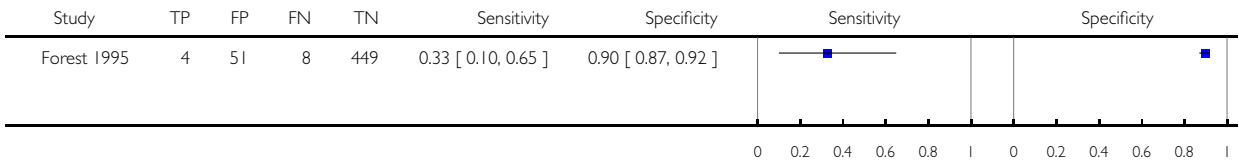
Test: 104 Age, IT PAPP-A, IT total hCG, IT AFP, IT uE3 and IT Inhibin, 5FPR



**Test 105. Age, IT free  $\beta$ hCG, IT total hCG, IT AFP, IT uE3 and IT free  $\alpha$ hCG, risk 1:384.**

Review: First trimester serum tests for Down's syndrome screening

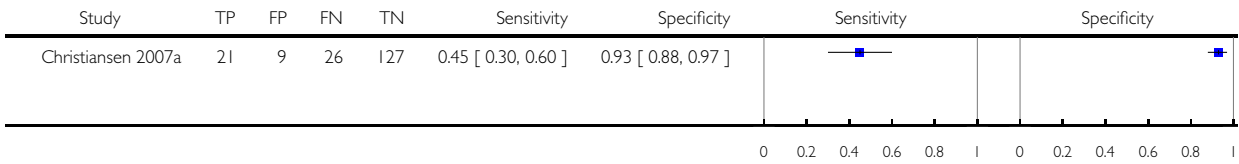
Test: 105 Age, IT free hCG, IT total hCG, IT AFP, IT uE3 and IT free  $\alpha$  hCG, risk 1:384



**Test 106. Age, IT hPL, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

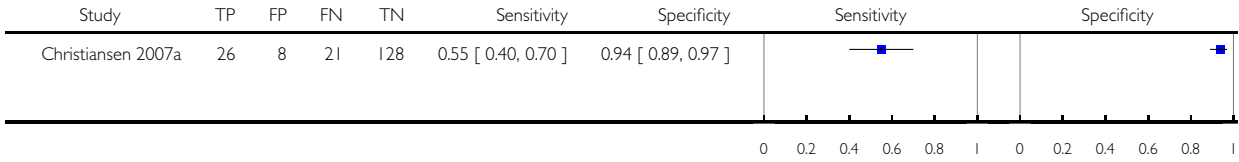
Test: 106 Age, IT hPL, risk 1:250



**Test 107. Age, IT hPL, IT PAPP-A, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

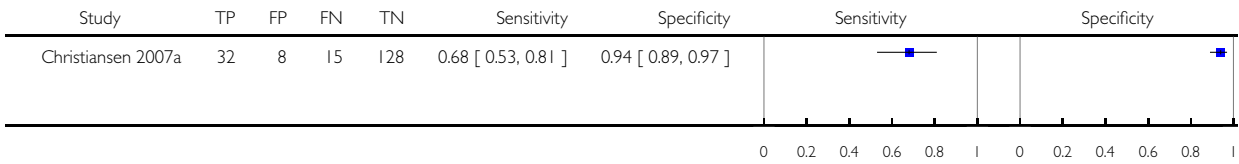
Test: 107 Age, IT hPL, IT PAPP-A, risk 1:250



**Test 108. Age, IT hPL, IT free hCG, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

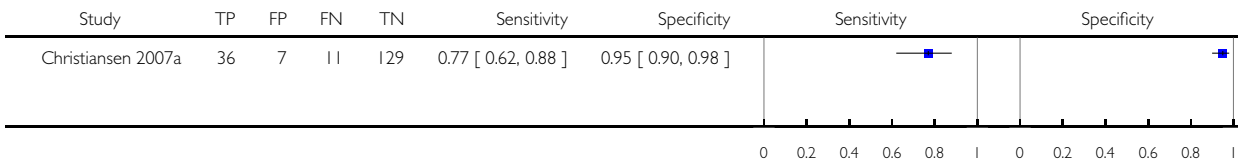
Test: 108 Age, IT hPL, IT free hCG, risk 1:250



**Test 109. Age, IT hPL, IT PAPP-A, IT free hCG, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

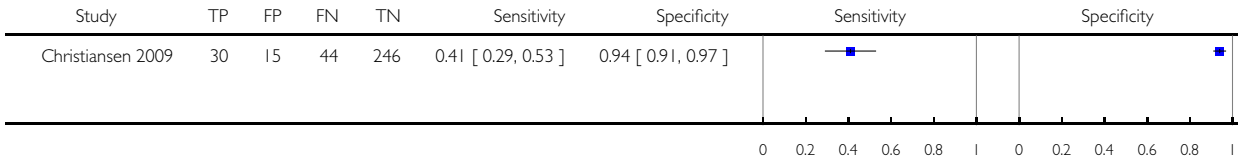
Test: 109 Age, IT hPL, IT PAPP-A, IT free hCG, risk 1:250



**Test I 10. Age, IT PGH, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

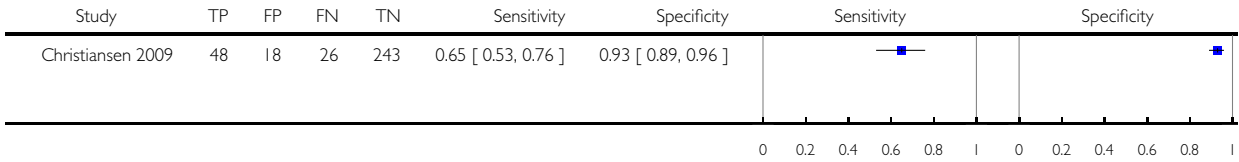
Test: I 10 Age, IT PGH, risk 1:250



**Test I 11. Age, IT PGH, IT PAPP-A , risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

Test: I 11 Age, IT PGH, IT PAPP-A , risk 1:250

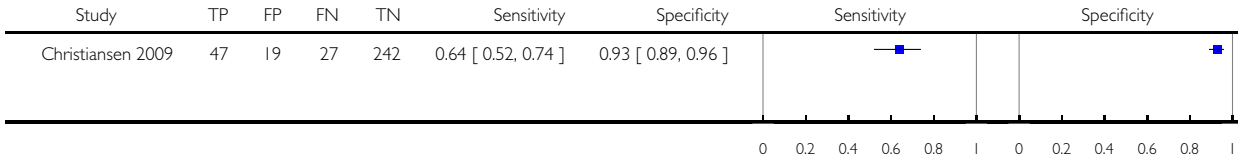




### Test 112. Age, IT PGH, IT free $\beta$ hCG , risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

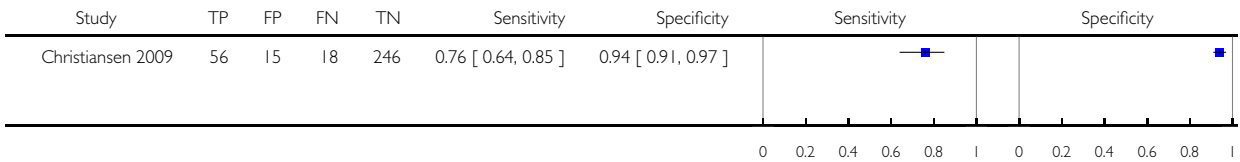
Test: 112 Age, IT PGH, IT free hCG , risk 1:250



### Test 113. Age, IT PGH, IT PAPP-A, IT free $\beta$ hCG , risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

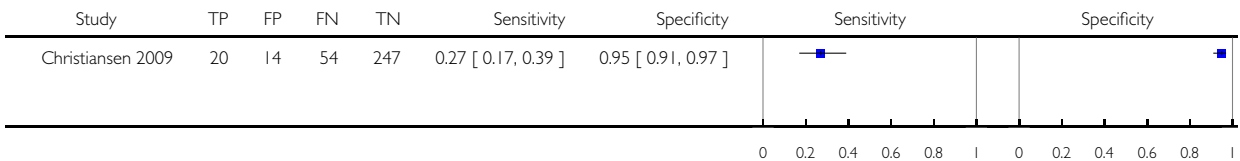
Test: 113 Age, IT PGH, IT PAPP-A, IT free hCG , risk 1:250



### Test 114. Age, IT GHBP, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

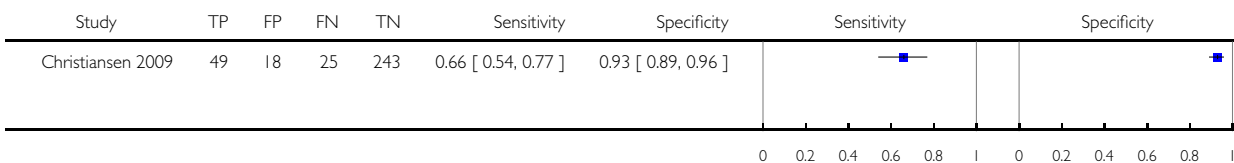
Test: 114 Age, IT GHBP, risk 1:250



### Test 115. Age, IT GHBP, IT PAPP-A, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

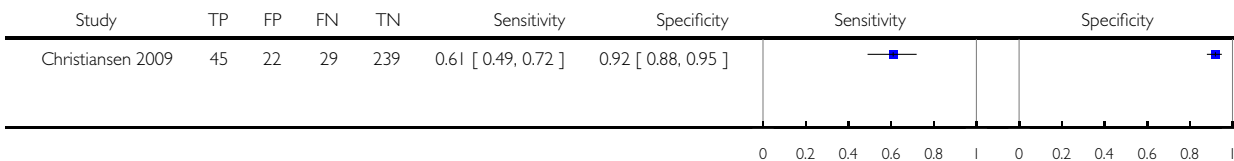
Test: 115 Age, IT GHBP, IT PAPP-A, risk 1:250



### Test 116. Age, IT GHBP, IT free $\beta$ hCG, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

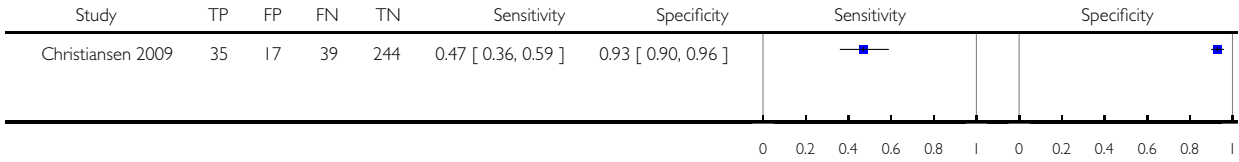
Test: 116 Age, IT GHBP, IT free hCG, risk 1:250



**Test I17. Age, IT GHBP, IT PGH, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

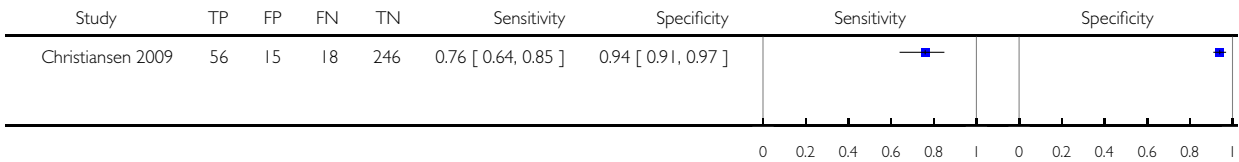
Test: I17 Age, IT GHBR, IT PGH, risk 1:250



**Test I18. Age, IT GHBP, IT PAPP-A, IT free βhCG , risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

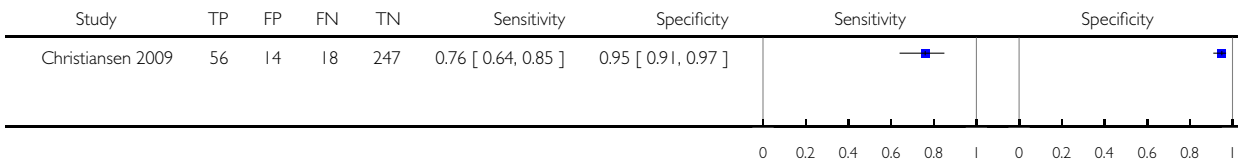
Test: I18 Age, IT GHBR, IT PAPP-A, IT free hCG , risk 1:250



**Test I19. Age, IT GHBP, IT PGH, IT PAPP-A, IT free βhCG , risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

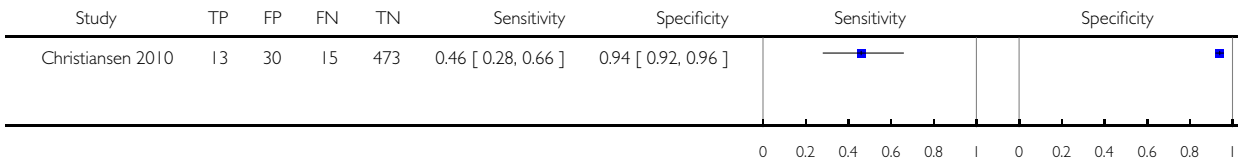
Test: I19 Age, IT GHBR, IT PGH, IT PAPP-A, IT free hCG , risk 1:250



**Test 120. Age, IT ADAM 12, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

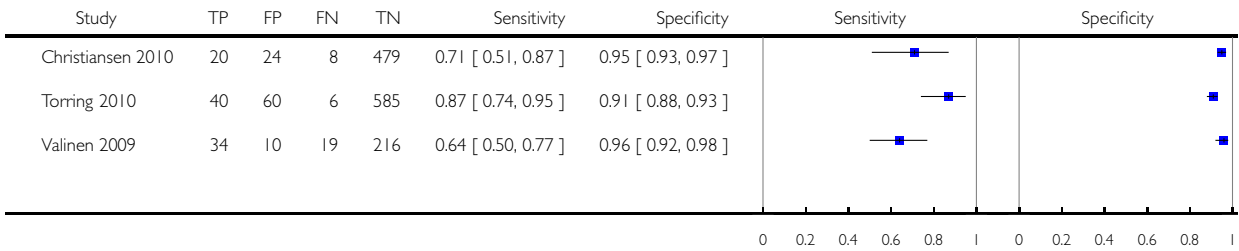
Test: 120 Age, IT ADAM 12, risk 1:250



**Test 121. Age, IT ADAM 12, IT PAPP-A, IT free βhCG, risk 1:250.**

Review: First trimester serum tests for Down's syndrome screening

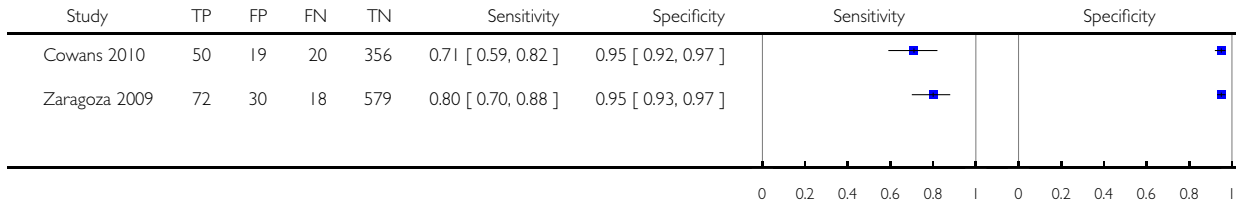
Test: 121 Age, IT ADAM 12, IT PAPP-A, IT free hCG, risk 1:250



### Test 122. Age, PIGF, IT PAPP-A, IT free $\beta$ hCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

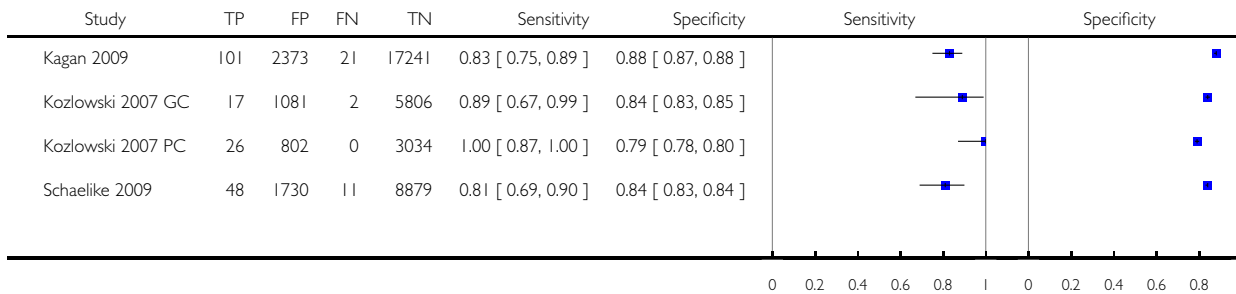
Test: 122 Age, PIGF, IT PAPP-A, IT free hCG, 5FPR



### Test 123. Age, IT PAPP-A and IT free $\beta$ hCG, risk 1:300.

Review: First trimester serum tests for Down's syndrome screening

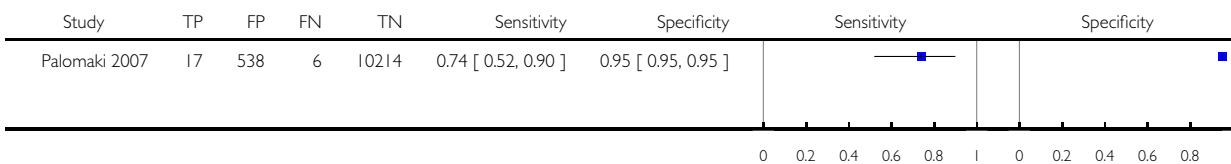
Test: 123 Age, IT PAPP-A and IT free hCG, risk 1:300



### Test 124. Age, IT PAPP-A, IT Hyperglycosylated hCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

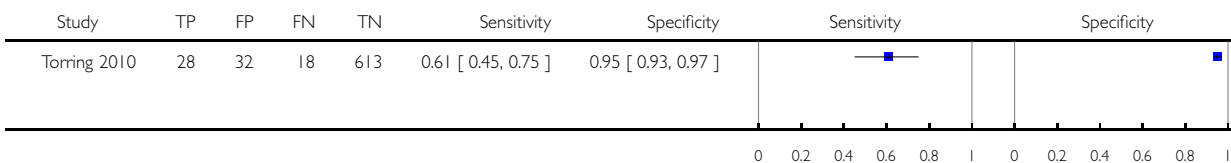
Test: 124 Age, IT PAPP-A, IT Hyperglycosylated hCG, 5FPR



### Test 128. Age, ADAM 12, IT PAPP-A, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

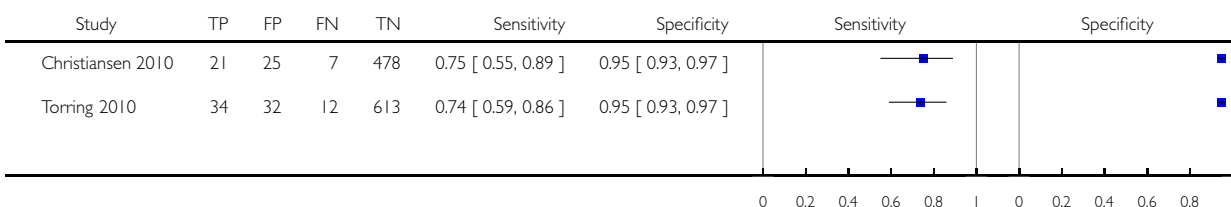
Test: 128 Age, ADAM 12, IT PAPP-A, 5FPR



### Test 129. Age, ADAM 12, IT PAPP-A, IT free βhCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

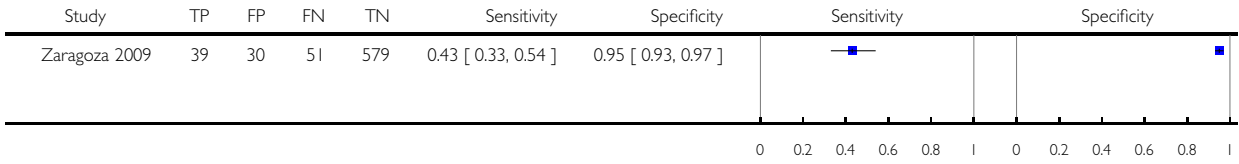
Test: 129 Age, ADAM 12, IT PAPP-A, IT free hCG, 5FPR



### Test 130. Age, IT PIGF, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

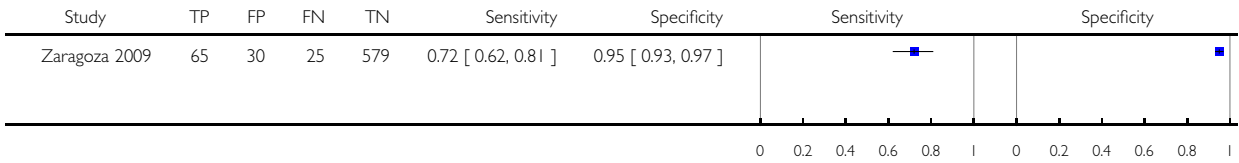
Test: 130 Age, IT PIGF, 5FPR



### Test 131. IT PIGF, IT PAPP-A, IT free βhCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

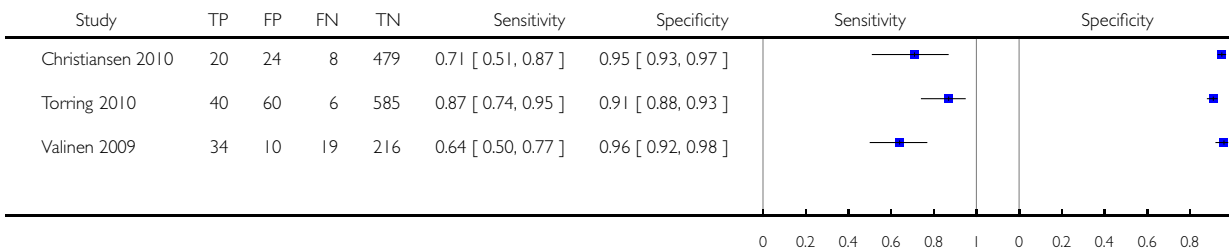
Test: 131 IT PIGF, IT PAPP-A, IT free hCG, 5FPR



### Test 132. Age, IT ADAM 12, IT PAPP-A, IT free $\beta$ hCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

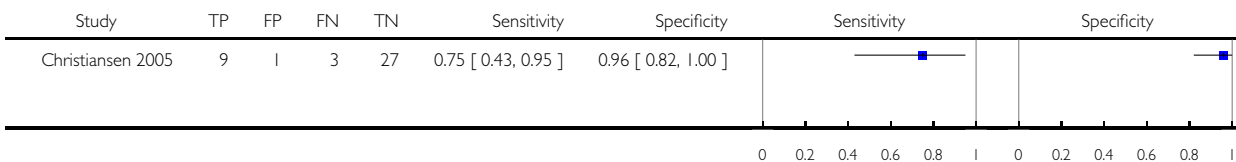
Test: 132 Age, IT ADAM 12, IT PAPP-A, IT free hCG, mixed cut-points



### Test 133. Age, IT PAPP-A, IT free $\beta$ hCG and IT Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 133 Age, IT PAPP-A, IT free hCG and IT Inhibin, risk 1:250

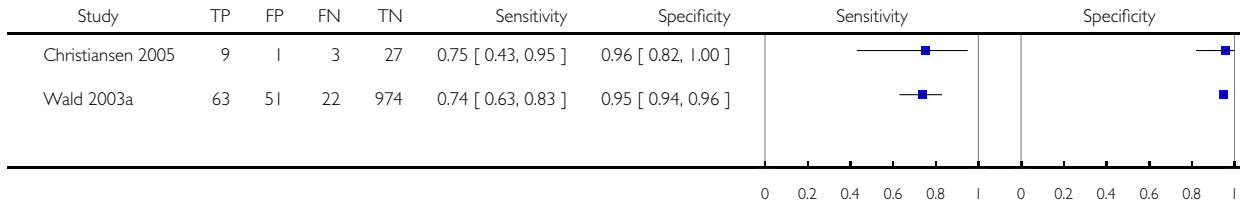




### Test 134. Age, 1T PAPP-A, 1T free $\beta$ hCG, and 1T Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 134 Age, 1T PAPP-A, 1T free hCG, and 1T Inhibin, mixed cut-points



## ADDITIONAL TABLES

Table 1. Direct comparisons of the sensitivity of nine test strategies at the 5% false positive rate

Ratio of sensitivity (95% CI), P value for comparison (studies)	Free $\beta$ hCG	PAPP-A	Age, free $\beta$ hCG	Age, PAPP-A	Age, PAPP-A, free $\beta$ hCG	Age, free $\beta$ hCG, AFP	Age, ADAM 12, PAPP-A, free $\beta$ hCG	Age, PAPP-A, free $\beta$ hCG, AFP
PAPP-A	1.78 (1.10 to 2.88), P = 0.02 (2)							
Age, free $\beta$ hCG	1.67 (1.11 to 2.50), P = 0.013 (2)	0.94 (0.68 to 1.29), P = 0.70 (2)						
Age, PAPP-A	2.15 (1.37 to 3.38), P = 0.001 (2)	1.20 (0.86 to 1.67), P = 0.29 (3)	1.26 (1.02 to 1.57), P = 0.034 (4)					
Age, PAPP-A, free $\beta$ hCG	2.62 (1.77 to 3.87), P < 0.001 (2)	1.47 (1.09 to 2.00), P = 0.012 (2)	1.61 (1.31 to 1.98), P < 0.001 (5)	1.26 (1.04 to 1.52), P = 0.02 (4)				

**Table 1. Direct comparisons of the sensitivity of nine test strategies at the 5% false positive rate** (Continued)

<b>Age, free βhCG, AFP</b>	2.19 (1.31 to 3.64), P = 0.002 (1)	0.71 (0.52 to 0.98), P = 0.03 (1)	1.08 (0.80 to 1.46), P = 0.62 (2)	0.61 (0.46 to 0.82), P < 0.001 (1)	0.63 (0.47 to 0.86), P = 0.004 (2)					
<b>Age, ADAM 12, PAPP-A, free βhCG</b>	-	-	-	-	1.04 (0.85 to 1.26), P = 0.71 (2)					
<b>Age, PAPP- A, free βhCG, AFP</b>	3.94 (2.49 to 6.23), P < 0.001 (1)	1.29 (1.03 to 1.60), P = 0.024 (1)	1.91 (1.42 to 2.56), P < 0.001 (1)	1.11 (0.91 to 1.34), P = 0.31 (1)	1.02 (0.88 to 1.20), P = 0.77 (2)	1.62 (1.19 to 2.19), P = 0.002 (2)				
<b>Age, PIGF, PAPP-A, free βhCG</b>	-	-	-	-	1.03 (0.91 to 1.17), P = 0.61 (2)					

- indicates that no comparative study was available for the pair of tests.

Direct comparisons were made only using data from studies which compared each pair of tests on the same women. Where there were at least two studies, meta-analysis was performed to summarise and compare the sensitivities. The ratio of sensitivities was computed by division of the sensitivity for the column by the sensitivity for the row. If the ratio of sensitivity is greater than one then the sensitivity of the test for the column is higher than that for the row, if less than one the sensitivity of the test in the row is higher than in the column. All test comparisons that were evaluated by only one study were from Wald 2003. The ratio of the sensitivities for test comparisons from a single study were calculated as a ratio of two proportions.

**ADAM12:** a disintegrin and metalloprotease; **AFP:** alpha-fetoprotein; **βhCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **PAPP-A:** pregnancy-associated plasma protein A; **PIGF:** placental growth factor; **PROMBP:** proform of eosinophil major basic protein

**Table 2. Indirect comparisons of the sensitivity of nine test strategies at the 5% false positive rate**

Ratio of sensi- tivity (95% CI) , P value for com- parison			Free βhCG	PAPP-A	Age, free βhCG	Age, PAPP-A	Age, PAPP-A, free βhCG	Age, free βhCG, AFP	Age, ADAM 12, PAPP-A, free βhCG	Age, PAPP-A, free βhCG, AFP
		<b>Studies (cases/ women)</b>	4 (390/ 4280)	4 (325/ 2837)	7 (460/ 5893)	5 (359/ 3491)	17 (1037/ 49827)	3 (157/ 2992)	2 (74/ 1222)	2 (116/ 2705)
	<b>Studies (cases/ women)</b>	<b>Sensi- tivity %</b>	25 (18 to 34)	52 (39 to 65)	42 (36 to 48)	55 (46 to 63)	68 (65 to 71)	49 (39 to 60)	74 (63 to 83)	74 (65 to 83)

**Table 2. Indirect comparisons of the sensitivity of nine test strategies at the 5% false positive rate** (Continued)

	women)	(95% CI)								81)
<b>PAPP-A</b>	4 (325/ 2837)	52 (39 to 65)	2.05 (1.37 to 3.09), P = 0.001							
<b>Age, free βhCG</b>	7 (460/ 5893)	42 (36 to 48)	1.66 (1.17 to 2.36), P = 0.004	0.81 (0.61 to 1.08), P = 0.15						
<b>Age, PAPP-A</b>	5 (359/ 3491)	55 (46 to 63)	2.16 (1.51 to 3.10), P < 0.001	1.05 (0.78 to 1.42), P = 0.73	1.30 (1.05 to 1.61), P = 0.015					
<b>Age, PAPP-A, free βhCG</b>	17 (1037/ 49827)	68 (65 to 71)	2.70 (1.95 to 3.73), P < 0.001	1.31 (1.02 to 1.70), P = 0.037	1.62 (1.40 to 1.88), P < 0.001	1.25 (1.05 to 1.47), P = 0.01				
<b>Age, free βhCG, AFP</b>	3 (157/ 2992)	49 (39 to 60)	1.95 (1.33 to 2.86), P = 0.001	0.95 (0.69 to 1.32), P = 0.76	1.18 (0.92 to 1.51), P = 0.20	0.90 (0.69 to 1.17), P = 0.45	0.72 (0.59 to 0.89), P = 0.003			
<b>Age, ADAM 12, PAPP-A, free βhCG</b>	2 (74/ 1222)	74 (63 to 83)	2.94 (2.07 to 4.16), P < 0.001	1.43 (1.07 to 1.90), P = 0.014	1.77 (1.46 to 2.14), P < 0.001	1.36 (1.10 to 1.67), P = 0.004	1.09 (0.95 to 1.25), P = 0.24	1.50 (1.17 to 1.92), P = 0.001		
<b>Age, PAPP-A, free βhCG, AFP</b>	2 (116/ 2705)	74 (65 to 81)	2.93 (2.09 to 4.11), P < 0.001	1.43 (1.08 to 1.88), P = 0.011	1.76 (1.48 to 2.10), P < 0.001	1.35 (1.11 to 1.64), P = 0.002	1.09 (0.97 to 1.22), P = 0.16	1.50 (1.19, to 1.89). P = 0.001	1.00 (0.84 to 1.18), P = 0.98	
<b>Age, PIGE, PAPP-A, free βhCG</b>	2 (160/ 1144)	76 (69 to 82)	3.01 (2.16 to 4.20), P < 0.001	1.47 (1.12 to 1.91), P = 0.005	1.81 (1.54 to 2.14), P < 0.001	1.39 (1.16 to 1.67), P < 0.001	1.12 (1.01 to 1.23), P = 0.024	1.54 (1.23 to 1.93), P < 0.001	1.03 (0.87 to 1.20), P = 0.75	1.03 (0.90 to 1.18), P = 0.7

Ratio of sensitivities were computed by division of the sensitivity for the column by the sensitivity for the row. If the ratio of sensitivity is greater than one then the sensitivity of the test for the column is higher than that for the row, if less than one the sensitivity of the test in the row is higher than in the column.

**AFP:** alpha-fetoprotein; **αhCG:** alpha human chorionic gonadotrophin; **βhCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **PAPP-A:** Pregnancy-associated plasma protein A

**Table 3. Summary of study characteristics**

Study	PAPP-A, free βhCG and age*	Maternal age (years)	Reference standard	Population	Study design	Study location
<a href="#">Baviera 2010</a>		Mean 35.3 for Down's cases, 30.4 for control	Amniocentesis or follow-up to birth	Routine screening	Case-control	Italy
<a href="#">Benattar 1999</a>		Mean 32 (16-46), 8.3% > 35	Amniocentesis due to maternal age > 38 years (6.1% or women). Karyotyping encouraged for women with positive result on one or more index test. No details of reference standard for index test negative women	Routine screening	Prospective cohort	France
<a href="#">Biagiotti 1995</a>		Not reported	Amniocentesis or CVS	High-risk referral for invasive testing	Case-control	Italy
<a href="#">Biagiotti 1998</a>	X	Unclear (maybe all ≥ 38)	Amniocentesis or CVS	High-risk referral for invasive testing	Retrospective case-control	Italy
<a href="#">Brambati 1993</a>		Median 38 (20-47)	CVS	High-risk referral for invasive testing	Retrospective cohort	Italy
<a href="#">Brambati 1994</a>	X	Not reported	CVS	High-risk referral for invasive testing	Case-control	Italy
<a href="#">Brameld 2008</a>		Median 31 (14-47), 20% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Australia
<a href="#">Brizot 1994</a>		Median 38 (22-45)	Fetal karyotyping	High-risk referral for invasive testing	Retrospective case-control	UK

**Table 3. Summary of study characteristics** (Continued)

Casals 1996		94.4% > 35	CVS	High-risk referral for invasive testing	Retrospective case-control	Spain
Christiansen 1999		Not reported	Karyotyping	High-risk referral for invasive testing	Case-control	Denmark
Christiansen 2004		Not reported	CVS (for 120 of cases of Down's) or follow-up to birth (for 36 of cases of Down's)	Routine screening	Case-control	Denmark
Christiansen 2005		Not reported	Karyotyping	Screening programmes for syphilis and Down's syndrome	Case-control	Denmark
Christiansen 2007a	X	Median 37.7 (24-48) for Down's cases, 36.4 (22-44) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Christiansen 2009	X	Median 37.5 for Down's cases, 36.4 for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Christiansen 2010	X	Median 36 (25-44) for Down's cases, 29 (17-45) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Cowans 2010	X	Mean 37.0 (IQR 32.9-40.5) for Down's cases, 32.4 (IQR 29.0-35.9) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	UK
Crandall 1993		90% > 35	Amniocentesis	High-risk referral for invasive testing	Retrospective cohort	USA
Crossley 2002a		Median 29.9, 15.4% $\geq$ 35	CVS offered where women had high NT measurements.	Routine screening	Prospective cohort	UK

**Table 3. Summary of study characteristics** (Continued)

			Also amniocentesis or follow-up to birth			
De Graaf 1999a	X	Not reported	Amniocentesis or CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Forest 1995		Mean 29.1 (SD 4.7), 10.7% $\geq$ 35	Follow-up to birth	Routine screening	Case-control	Canada
Forest 1997	X	Mean 27.9, 10.7% $\geq$ 35	Follow-up to birth	Routine screening	Case-control	Canada
Gyselaers 2005		Not reported	Amniocentesis, CVS and postnatal karyotyping	Routine screening	Prospective cohort	Belgium
Haddow 1998	X	Median 37 (15-51)	Amniocentesis or CVS	High-risk referral for invasive testing	Prospective cohort	USA
Kagan 2009	X	Mean 35.4 (14.1-52.2)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	UK
Kornman 1998		Not reported	CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Kozlowski 2007 GC		Median 32 (15-48), 26.4% $\geq$ 35	Karyotyping or follow-up to birth	Routine screening	Cohort	Germany
Kozlowski 2007 PC		Median 34 (14-46), 43.2% $\geq$ 35	Karyotyping or follow-up to birth	Routine screening	Cohort	Germany
Krantz 2000		34.7% $\geq$ 35	Not reported	Routine screening	Prospective cohort	USA
Kratzer 1991		Missing	CVS	High-risk referral for invasive testing	Case-control	USA
Laigaard 2003		Not reported	Karyotyping, unclear reference standard for con-	Routine screening	Case-control	Denmark

**Table 3. Summary of study characteristics** (Continued)

			controls			
Macintosh 1993		Median 38 (27-40)	CVS	High-risk referral for invasive testing	Retrospective cohort	UK and Italy
Muller 2003a		Not reported	Invasive testing (offered to women with high NT measurement) or follow-up to birth	Routine screening	Retrospective cohort	France
Nebiolo 1990		Approximately 75% $\geq$ 35	CVS	High-risk referral for invasive testing	Retrospective cohort	Italy
Niemimaa 2001a		17.5% $\geq$ 35	Invasive testing (patients considered high-risk based on NT screening) or follow-up to birth	Routine screening	Prospective cohort	Finland
Noble 1995		Median 34 (15-47), 47% $\geq$ 35	Karyotyping performed (27%), ultrasound examination at 20 weeks (65%), or follow-up to birth (9%)	Routine screening in a high-risk population	Prospective cohort	UK
Noble 1997		Median 34 (15-47)	CVS, follow-up to birth not reported	Routine screening	Case-control	UK
O'Leary 2006		Median 31 (14-47), 20% $\geq$ 35 years	CVS or amniocentesis (women assessed to be high risk on screening) or follow-up to birth	Routine screening	Prospective cohort	Australia
Orlandi 1997		Range 15-46, 35% $\geq$ 35	Not reported	Routine screening	Prospective cohort	Italy

**Table 3. Summary of study characteristics** (Continued)

Palomaki 2007		Mean maternal age 32.3 years (SD 4.6 years)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Canada
Qin 1997		Not reported	CVS, amniocentesis, karyotyping at birth, unclear reference standard for control	Routine screening	Case-control	Denmark
Sahota 2010	X	Median 33.1, 30.1% $\geq$ 35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	China
Schaelike 2009		31.0% $\geq$ 35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Germany
Scott 2004		Median 32 (15-44), 29% $\geq$ 35	Invasive testing or follow-up to birth	Routine screening	Prospective cohort	Australia
Spencer 1999a	X	Median cases 38 (19-46), controls 36 (15-47)	Invasive testing (high-risk women) or follow-up to birth	Referred for invasive testing or self-referred for screening	Case-control	UK
Spencer 2002a		Median cases 36 (20-44), controls 30 (16-41)	Not reported	Routine screening	Case-control	UK
Torring 2010	X	Mean 35 for Down's, 31 for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Tsukerman 1999	X	Not reported	Karyotyping, karyotyping at birth, follow-up to birth not reported	Routine screening	Case-control	Belarus
Valinen 2007		Mean 29.6, 18.6% $\geq$ 35	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Finland
Valinen 2009		Not reported	Karyotyping or follow-up to birth	Routine screening	Case-control	Finland



**Table 3. Summary of study characteristics** (Continued)

Van Lith 1992		Not reported	CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Wald 2003a	X	Missing	Invasive testing (following second trimester screening) or follow-up to birth	Routine screening	Case-control	UK and Austria
Wallace 1995		Mean 32 (22-44) for Down's cases, 28 (19-38) for controls	Not reported	Routine screening	Case-control	UK
Wapner 2003	X	Mean 35 (SD 4.6), 50% $\geq$ 35	Invasive testing, miscarriage with cytogenetic testing, follow-up to birth	Routine screening	Prospective cohort	USA
Weinans 2005		Mean 38 (SD 2.7) for Down's cases, 37 (SD 3.0) for controls	CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Wojdemann 2005		Mean 29, 10.8% $\geq$ 35	Invasive testing (in cases of increased risk) or follow-up to birth	Routine screening	Prospective cohort	Denmark
Zaragoza 2009	X	Median 37.9 (19.1-46.5) for Down's cases, 32.7 (16.1-45.2) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	UK

\*The PAPP-A, free  $\beta$ hCG and age test combination was the only test evaluated by at least 10 studies. X indicates that the test was evaluated in the study.

CVS: chorionic villus sampling; IQR: interquartile range; SD: standard deviation.

## APPENDICES

### Appendix I. Search Strategy

Database: Ovid MEDLINE

---

- 1 exp Prenatal Diagnosis/
- 2 nuchal translucency.mp.
- 3 exp Pregnancy-Associated Plasma Protein-A/
- 4 pregnancy associated plasma protein a.mp.
- 5 papp-a.mp.
- 6 exp Chorionic Gonadotropin, beta Subunit, Human/
- 7 (b-hcg or bhcg).mp.
- 8 human chorionic gonadotropin.mp.
- 9 exp alpha-Fetoproteins/
- 10 alphafetoprotein\$.mp.
- 11 alpha-fetoprotein\$.mp.
- 12 afp.mp.
- 13 (unconjugated estriol or unconjugated oestriol).mp.
- 14 ue3.mp.
- 15 exp INHIBINS/
- 16 inhibin a.mp.
- 17 ultrasound.mp.
- 18 amniocentesis/
- 19 chorion\$ vill\$ sampling.mp.
- 20 Chorionic Villi-Sampling/
- 21 nasal bone.mp.
- 22 tricuspid regurgitation.mp.
- 23 ductus venosus.mp
- 24 marker\$.mp.
- 25 screen\$.mp.
- 26 detect\$.mp.
- 27 accura\$.mp.
- 28 predict\$.mp.
- 29 ROC.mp.
- 30 ROC curve/
- 31 AUC.mp.
- 32 Area under curve/
- 33 exp false negative reactions/ or exp false positive reactions/
- 34 (false positive\$ or false negative\$).mp.
- 35 likelihood ratio\$.mp.
- 36 sensitiv\$.mp.
- 37 specific\$.mp.
- 38 diagnos\$.ti,ab.
- 39 "reproducibility of results".mp.
- 40 reference value\$.mp.
- 41 reference standard\$.mp.
- 42 exp Down Syndrome/
- 43 downs syndrome.mp.
- 44 down syndrome.mp.
- 45 trisomy 21.mp.
- 46 Aneuploidy/
- 47 aneuploidy.mp.

48 Mosaicism/  
 49 mosaicism.mp.  
 50 or/1-41  
 51 or/42-49  
 52 50 and 51  
 53 (antenatal\$ or prenatal\$ or trimester\$ or pregnan\$ or fetus or foetus or fetal or foetal).mp.  
 54 52 and 53  
 55 animal/ not (humans/ and animal/)  
 56 54 not 55

\*\*\*\*\*

Embase via Dialog Datastar

1. PRENATAL-DIAGNOSIS#.DE.  
 2. FETUS-ECHOGRAPHY#.DE.  
 3. PREGNANCY-ASSOCIATED-PLASMA-PROTEIN-A#.DE.  
 4. CHORIONIC-GONADOTROPIN-BETA-SUBUNIT#.DE.  
 5. HCG.AB.  
 6. PAPP.AB.  
 7. ALPHA-FETOPROTEIN#.DE.  
 8. AFP.AB.  
 9. ALPHA ADJ FETOPROTEIN\$  
 10. ALPHAFETOPROTEIN\$  
 11. BETA ADJ HUMAN ADJ CHORIONIC ADJ GONADOTROPIN  
 12. PREGNANCY ADJ ASSOCIATED ADJ PLASMA ADJ PROTEIN  
 13. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).TI.  
 14. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).AB.  
 15. UE3  
 16. INHIBIN-A#.DE.  
 17. INHIBIN ADJ A  
 18. ULTRASOUND  
 19. AMNIOCENTESIS  
 20. CHORION-VILLUS-SAMPLING.DE.  
 21. NASAL ADJ BONE  
 22. TRICUSPID ADJ REGURGITATION  
 23. DUCTUS ADJ VENOSUS  
 24. MARKER OR MARKERS  
 25. SCREEN OR SCREENING  
 26. DETECT OR DETECTING OR DETECTION  
 27. FALSE ADJ POSITIVE\$  
 28. FALSE ADJ NEGATIVE\$  
 29. SENSITIVITY OR SENSITIVE OR SENSITIVITIES  
 30. SPECIFICITY OR SPECIFICITIES  
 31. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).TI.  
 32. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).AB.  
 33. ROC.AB.  
 34. AUC.AB.  
 35. AREA-UNDER-THE-CURVE.DE.  
 36. ROC-CURVE.DE.  
 37. ACCURA\$  
 38. PREDICT\$  
 39. REPRODUCIBILITY.DE.

40. REFERENCE ADJ VALUE\$
41. REFERENCE-VALUE.DE.
42. REFERENCE ADJ STANDARD\$
43. DOWN-SYNDROME#.DE.
44. DOWN ADJ SYNDROME OR DOWNS ADJ SYNDROME
45. TRISOMY ADJ '21'
46. MOSAICISM
47. ANEUPLOIDY
48. ANTENATAL\$ OR PRENATAL\$ OR PREGNANCY OR PREGNANT OR TRIMESTER\$ OR MATERNAL OR FETUS OR FOETUS OR FOETAL OR FETAL
49. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39 OR 40 OR 41 OR 42
50. 43 OR 44 OR 45 OR 46 OR 47
51. 48 AND 49 AND 50
52. HUMAN=YES
53. 51 AND 52

ADJ = adjacent      AB = abstract  
 TI = title      \$ = truncation symbol      DE = descriptor (similar to MeSH)

\*\*\*\*\*

CINAHL via OVID

- 
- 1 exp Prenatal Diagnosis/
  - 2 nuchal translucency.mp.
  - 3 pregnancy associated plasma protein.mp.
  - 4 papp\$.ti,ab.
  - 5 exp Gonadotropins, chorionic/
  - 6 (b-hcg or bhcg).mp.
  - 7 human chorionic gonadotropin.mp.
  - 8 exp alpha-Fetoproteins/
  - 9 alphafetoprotein\$.mp.
  - 10 alpha-fetoprotein\$.mp.
  - 11 afp.mp.
  - 12 (unconjugated estriol or unconjugated oestriol).mp.
  - 13 ue3.mp.
  - 14 inhibin\$.mp.
  - 15 ultrasound.mp.
  - 16 amniocentesis/
  - 17 chorion\$ vill\$ sampling.mp.
  - 18 Chorionic Villi-Sampling/
  - 19 nasal bone.mp.
  - 20 tricuspid regurgitation.mp.
  - 21 ductus venosus.mp.
  - 22 marker\$.mp.
  - 23 screen\$.mp.
  - 24 detect\$.mp.
  - 25 accura\$.mp.
  - 26 predict\$.mp.
  - 27 ROC.mp.
  - 28 ROC curve/
  - 29 AUC.mp.
  - 30 "area under curve".mp.

31 exp false negative reactions/ or exp false positive reactions/  
 32 (false positive\$ or false negative\$).mp.  
 33 likelihood ratio\$.mp.  
 34 sensitiv\$.mp.  
 35 specific\$.mp.  
 36 diagnos\$.ti,ab.  
 37 "reproducibility of results".mp.  
 38 reference value\$.mp.  
 39 reference standard\$.mp.  
 40 exp Down Syndrome/  
 41 downs syndrome.mp.  
 42 down syndrome.mp.  
 43 trisomy 21.mp.  
 44 aneuploidy.mp.  
 45 mosaicism.mp.  
 46 (antenatal\$ or prenatal\$ or trimester\$ or pregnan\$ or fetus or foetus or fetal or foetal).mp.  
 47 or/1-39  
 48 or/40-45  
 49 47 and 48 and 46

\*\*\*\*\*

#### Search terms and instructions for Biosis

The following search terms were entered separately in standard search box (select 'Titles/subject/abstract' from the drop-down box on the right of the search box).

1. "reference standard\*"
2. "reference value\*"
3. "reproducibility of results"
4. diagnos\*
5. sensitiv\*
6. specific\*
7. "likelihood ratio\*"
8. "false negative\*"
9. "false positive"
10. "area under curve"
11. ROC
12. AUC
13. predict\*
14. detect\*
15. marker\*
16. screen\*
17. accura\*
18. "ductus venosus"
19. "nasal bone"
20. "tricuspid regurgitation"
21. "chorion\* vill\* sampling"
22. amniocentesis
23. ultrasound
24. inhibin\*
25. "unconjugated oestriol"
26. "unconjugated estriol"
27. afp
28. "alpha fetoprotein\*"



Down  
 Trisomy  
 Aneuploidy  
 Pregnant  
 Pregnancy  
 Pregnancies  
 Mosaicism

\*\*\*\*\*

## Appendix 2. Glossary of terms (adapted in part from the UK National Screening Committee Glossary)

Abnormal ductus venosus flow velocity	The ductus venosus is a vessel in the fetus which allows oxygenated blood from the placenta to bypass the fetal liver and flow straight to the heart. In conditions such as Down's syndrome the pressure in this vessel can be abnormally high
Absent nasal bone	Absence of the bone that forms the bridge of the nose, which may be detected at ultrasound scan during early pregnancy
Affected individuals	Those individuals who are affected by the disorder for which they are being screened
Amniocentesis	Amniocentesis is an invasive procedure which involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks' gestation
Chorionic villus sampling (CVS)	Chorionic villus sampling involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks' gestation
Combined test	First trimester test (up to 13 + 6 weeks of pregnancy) based on combining nuchal translucency (NT) measurement with free beta-hCG, pregnancy-associated plasma protein A (PAPP-A) and the woman's age
Diagnostic accuracy	The amount of agreement between the information from the index test and the reference standard (see below)
Diagnostic test	A definitive test, performed after a positive screening test result that gives a diagnosis (i.e. yes or no)?
Double test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG $\beta$ either free beta-hCG or total hCG), together with the woman's age
First trimester	Pregnancy from conception up to 13 weeks and 6 days.
Iatrogenic	A disease or condition in a patient occurring as a result of treatment
Index test	A test or group of tests being evaluated in a systematic review

(Continued)

Integrated test	Measurements performed at different times of pregnancy combined into a single test result. Unless otherwise specified, 'integrated test' refers to the combination of nuchal translucency measurement and PAPP-A in the first trimester, with the quadruple test (see below) in the second
Mosaicism	This is a condition in which person has some cells containing a normal number of chromosomes, and some containing an abnormal number. The more abnormal cells there are, the greater the effect
Multiple of the median (MOM)	The serum test concentration for a pregnant woman divided by the average (median) for unaffected pregnancies in a defined population at the same stage of pregnancy
Quadruple test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of AFP, uE3, free beta-hCG (or total hCG), and inhibin-A together with the woman's age
Reference Standard	The best available method for establishing the presence or absence of the target disease or condition
Second trimester	Pregnancy from 14 weeks to 28 weeks' gestation. Note that for the purposes of this Cochrane review, second trimester testing refers to the period of 14 to 24 weeks' gestation
Tricuspid regurgitation	Leakiness of or backflow of blood through the tricuspid valve of the heart. The tricuspid valve separates the upper and lower chambers of the right side of the heart
Triple test	Second trimester test (from 14 up to 24 weeks of pregnancy) based on the measurement of AFP, unconjugated oestriol (uE3), and hCG (either total hCG or free beta-hCG) together with the woman's age
Trisomy	The presence of an extra chromosome resulting in three copies of a particular chromosome instead of the normal two
Translocation	Part of one chromosome is broken off and attached to another chromosome. This does not usually cause the individual any problems as they have a normal amount of chromosomes, but in an abnormal arrangement. It can be passed on as an extra chromosome to offspring, resulting in conditions such as Down's syndrome

### Appendix 3. QUADAS questionnaire

QUADAS criteria included the following 10 questions.

1. Was the spectrum of women representative of the women who will receive the test in practice? (criteria met if the sample was selected from a wide range of childbearing ages, or selected from a specified 'high risk' group such as over 35s, family history of Down's Syndrome, multiple pregnancy or diabetes mellitus, provided all affected and unaffected fetuses included that could be tested at the time point when the screening test would be applied; criteria not met if the sample taken from a select or unrepresentative group of women (i.e. private practice), was an atypical screening population or recruited at a later time point when selection could be affected by selective fetal loss)

2. Is the reference standard likely to correctly classify the target condition? (amniocentesis, chorionic villus sampling, postnatal karyotyping, miscarriage with cytogenetic testing of the fetus, a phenotypically normal baby or birth registers are all regarded as meeting this criteria)



3. Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?
4. Did women receive the same reference standard regardless of the index test result?
5. Was the reference standard independent of the index test result (i.e. the index test did not form part of the reference standard)?
6. Were the index test results interpreted without knowledge of the results of the reference standard?
7. Were the reference standard results interpreted without knowledge of the results of the index test?
8. Were the same clinical data (i.e. maternal age and weight, ethnic origin, gestational age) available when test results were interpreted as would be available when the test is used in practice?
9. Were uninterpretable/intermediate test results reported?
10. Were withdrawals from the study explained?

## **CONTRIBUTIONS OF AUTHORS**

KA undertook the searches, applied eligibility criteria, extracted and entered data and wrote the first and second draft of the review.

ZA applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

JD supervised and planned the review, checked data extraction, supervised statistical analyses and wrote the second draft of the review.

JN applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

BG checked data extraction and undertook statistical analyses.

MP applied eligibility criteria, extracted and entered data for the updated literature search, and entered characteristics of studies information.

YT checked data extraction, undertook statistical analyses, contributed to the first draft of the review, and approved the final draft of the review.

## **DECLARATIONS OF INTEREST**

KA: None known.

ZA: None known.

JD: None known.

JN: None known.

BG: None known.

MP: None known.

YT: None known.

## **SOURCES OF SUPPORT**

## Internal sources

- University of Birmingham, UK.

Funding of the research time of JD and BG

## External sources

- NIHR Health Technology Assessment Programme, UK.

Project grant - need to have reference number etc. Jim/Zarko can you add please?

- NIHR Health Technology Assessment Programme, UK.

Funding for the Cochrane Reviews of Diagnostic Test Accuracy Support Unit, based at the University of Birmingham (JD).

## DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The protocol intended to investigate several additional outcomes downstream from test accuracy, should they be reported in the test accuracy studies. When we attempted to extract this information however, it was found to be available in very few studies, and where such information was found it was difficult to extract meaningful data to allow for comparison between studies, as data were not reported in a universal manner. In several studies such outcomes were estimated rather than measured. Often they were not reported at all. The outcomes stated in the protocol which have not been included are: harms of testing; need for further testing; side effects of test; interventions and side effects; other abnormalities detected by testing; spontaneous miscarriage; miscarriage subsequent to invasive procedure, with or without normal karyotype; fetal karyotype; termination of pregnancy (prior to definitive testing or in a karyotypically normal pregnancy and following confirmation of Down's syndrome or following detection of other chromosomal abnormalities); stillbirth; livebirth of affected and unaffected fetus; uptake of definitive testing by women.

The following refinements to the eligibility criteria were imposed to ensure that the quality of the included literature remained high. We excluded studies that identified fewer than five Down's syndrome pregnancies in their study population. We excluded studies that had less than 80% follow-up of participants.

In addition, the analytical strategy was informed by the volume of tests and studies included, and developed so that we focused on key tests and test combinations by a) only meta-analysed tests that were included in four or more studies or b) showed more than 70% sensitivity for more than 95% specificity. In addition, a requirement that a minimum of 10 studies for a single test was required before subgroup analysis was undertaken. Consequently several possible sources of heterogeneity were not investigated due to lack of data.

## NOTES

This is one of a suite of planned systematic diagnostic test reviews planned for prenatal testing for fetal Down's syndrome. The plans for these reviews were described in a generic protocol (Allred 2010) published in the Cochrane Library in 2010. The five reviews were to be of: first trimester serum tests only; first trimester ultrasound tests alone, and in combination with first trimester serum tests; second trimester serum tests only; first and second trimester serum tests with and without first trimester ultrasound tests; and urine tests. One of these reviews has been published already (Allred 2012). Diagnostic test reviews are relatively new, and this project has proven much larger, more complex and difficult to complete than had been anticipated. Whilst not fulfilling the usual Cochrane up-to-date criteria (the electronic search was done in 2011), this review is published because it provides historical context in what is a rapidly-changing field, and because it is unlikely to ever be repeated.