Abstract

Aims

The aim of this review is to evaluate the available literature and to calculate a pooled diagnostic sensitivity and specificity for the different alpha-defensin test systems to diagnose peri-prosthetic infection.

Materials and Methods

Studies using alpha-defensin or Synovasure to diagnose periprosthetic joint infection were identified from systematic searches of electronic databases. Study quality was evaluated using the QUADAS tool. Meta-analysis was completed using a bivariate model.

Results

Eleven eligible studies were included. Median QUADAS score was 13 [I.Q.R. 13-13] out of 14. Significant conflicts of interest were identified in five studies.

Pooled sensitivity for the laboratory alpha-defensin test was 0.95 (95% CI 0.91-0.98) and specificity 0.97 (95% CI 0.95-0.98) for four studies with a threshold level of $5.2mgl^{-a}$. Pooled sensitivity for the lateral flow cassette test was 0.85 (95% CI 0.74-0.92) and specificity was 0.90 (95% CI 0.91-0.98). There was a statistically significant difference in sensitivity but not specificity.

Conclusion

Laboratory based alpha-defensin testing remains a promising tool for diagnosing periprosthetic joint infection. The lateral flow cassette has a significantly lower performance and pooled results are comparable to the leucocyte esterase test. Further studies are essential before the widespread adoption of the lateral flow cassette alpha-defensin test.

Take home message

Alpha defensin testing for periprosthetic joint infection has excellent sensitivity and specificity when performed in a laboratory.

The pooled sensitivity and specificity is much lower when results are combined for the lateral flow cassette (Synovasure devise)

Background

Infection was the cause for revision in 13,801 (11%) of the 130,195 revision hip or knee arthroplasties completed in the U.K. between 2003 and 2015[1]. Infection represents a devastating complication, with significant morbidity, mortality and cost. It has been estimated that the mean cost from each revision for infection is over £20,000[2]. As the incidence of joint arthroplasty increases, this burden from infection will also rise[3]. Reliable diagnosis of infection is vital for patients and clinicians to guide treatment decisions.

The diagnosis of periprosthetic joint infection is challenging. There is no individual gold standard test that has robust sensitivity and sensitivity, and hence a combination of investigations is required. In the initial Musculoskeletal Infection Society (MSIS) consensus guidelines, infection could be diagnosed with either one positive major criterion or four positive minor criteria[4]. These guidelines were adapted and updated in 2013, with one positive major criterion or three minor criteria identifying periprosthetic infection[5] (Table 1).

Table 1 MSIS and Updated MSIS Criteria for diagnosis of periprosthetic joint infection

MSIS (2011) Criteria[4] *1 major criterion or 4 or more minor criteria*		**Updated (2013) MSIS Criteria**[5] *1 major criterion or 3 or more minor criteria*	
Major	Minor	Major	Minor
A sinus tract communicating with the prosthesis	Elevated serum ESR and CRP	A sinus tract communicating with the joint	Elevated serum ESR and CRP
A single isolated pathogen from two or more samples from the prosthetic joint	Elevated synovial white cell count	A single isolated pathogen from two or more samples from the prosthetic joint	Elevated synovial white cell count or positive leucocyte esterase test strip
	Elevated synovial polymorphonuclear percentage		Elevated synovial polymorphonuclear percentage
	Presence of pus in the affected joint		Isolation of a microorganism in a periprosthetic sample
	Isolation of a microorganism in a periprosthetic sample		Positive histological analysis of periprosthetic tissue
	Greater than 5 neutrophils in 5 high power field in synovial histology at 400X magnification		

The search for novel biomarkers beyond conventional inflammatory markers (C-reactive protein, erythrocyte sedimentation rate (CRP, ESR)) has identified alpha-defensin as a potential diagnostic tool[6]. Alpha-defensin is a group of antimicrobial peptide that disrupts the synthesis of bacterial cell walls[7].

The alpha-defensin assay had been developed by CD Diagnostics. The peptide may be measured quantitatively with an enzyme linked immunosorbent assay (ELISA) in the laboratory or with a lateral flow cassette. The lateral flow cassette has been engineered to be used in hospital by theatre staff as

a point-of-care testing system. This has the advantage that a result may be obtained within 10 minutes of a sample being acquired.

Previous systematic reviews on the use of alpha-defensin have focused on early efficacy studies. Wyatt *et al.* undertook a review published in 2016 that identified six eligible studies and reported a pooled sensitivity of 100% (95% CI 82-100%) and specificity of 96% (95% CI 89-99%)[8]. However, four out of six studies were published by the same authors.[6,9–11]. One of these was a re-analysis of previously published samples[11]. Five out of the six studies had funding or financial links to CD Diagnostics[6,9–12] and none of the included studies evaluated the lateral flow cassette. There was significant heterogeneity in the studies and variation in test threshold values. This may have had a bearing on the results.

Xie *et al.* published a systematic review with a search date of January 2016 and included one additional study[13]. This review calculated a pooled sensitivity of 96% (95% CI 85-99%) and specificity of 95% (95% CI 89-98%). Three different threshold values were combined in this analysis and no studies were included using the lateral flow cassette.

Saleh *et al*. presented a systematic review of all synovial biomarkers for periprosthetic infection[14]. This review only identified three studies that studied alpha-defensin as the search was completed in mid-2015.

The aim of this systematic review was to evaluate the diagnostic utility of alpha-defensin for periprosthetic joint infections as previous reviews have not included more recent studies or studies using the lateral flow cassette. A sub-group analysis of tests performed with a lateral flow cassette (Synovasure device) was performed with studies pooled for sensitivity and specificity if three or more trials were identified that shared a threshold value.

Methods

The protocol for this review was registered with the PROSPERO database, registration number CRD42017069267[15]. The protocol had a minor alteration with the inclusion of a sub-group analysis of the lateral flow cassette when it became evident that this was a source of heterogeneity within the published studies.

Search strategy

Searches were designed with the input from an information specialist (DG). OVID Medline, EMBASE and PubMed were searched on 17th January 2018. The search strategies are provided in Appendix 1. Bibliographies of included studies and relevant review articles were also searched to identify potential additional titles.

Titles and abstracts were screened by one researcher (BM) to compile a list of potentially eligible studies. Final selection and data extraction by two researchers (BM & SD) and conflicts were resolved by consensus. Study quality was also evaluated by two researchers (BM & SD) using the QUADAS instrument[16]. Data collection was performed using the Joanna Briggs Institute Data Extraction Instrument[17]. Where required, authors were contacted to confirm details for the meta-analysis.

Inclusion and exclusion criteria

Studies were evaluated to identify all investigations using alpha-defensin to assess infection in periprosthetic joints using the original or revised MSIS criteria as gold standard. Conference abstracts were excluded, but authors and affiliations were used to identify subsequently published articles.

Data analysis

Heterogeneity was evaluated using graphical evaluation of forest plots and calculation of I^2 statistic using MetaDiSc 1.4 (Hospital Universitario Ramón y Cajal, Madrid)[18].

Where studies shared pre-specified test threshold values, meta-analysis was completed using a bivariate model to calculate pooled sensitivity and specificity. This was conducted using the MADA package for R, according to guidance from the Cochrane Diagnostic Accuracy Group[19,20]. Comparisons between groups were completed with a likelihood ratio test. Forest plots were generated in RevMan 5.3 (Cochrane Collaboration, Oxford) without performing meta-analysis for studies that did not share threshold values. Summary ROC curves were calculated in MetaDiSc 1.4.

Results

PRISMA flow diagram

Studies identified and excluded are shown in the flow diagram (Figure 4). A total of 179 studies were identified through database searches, and six from bibliographies. Following title screening, 30 studies remained, and 14 studies were subject to full text review. Eleven eligible studies were identified for inclusion in the qualitative and quantitative analysis.

Study characteristics

The study characteristics of included studies are summarised in Table 3. The total number of patients included within these studies was 1063. The total number of positive diagnoses of periprosthetic joint infection was 305.

Three studies that were included in previous systematic reviews were excluded from further analysis. Wyatt *et al.* [8] and Xie *et al.*[13] both included the 2014 paper by Deirmengian *et al*.[10]. This was excluded as the study re-analysed stored samples. The results from these samples had been published in a different paper, which was included in this review[9].

- Xie *et al.*[13] included a series from 1937 samples published by Deirmengian *et al.* in 2015[10]. This study was excluded as the reference standard was bacterial culture results rather than diagnosis of infection via either the MSIS or revised MSIS consensus guidelines[4,5].
- Frangiamore *et al's* paper on alpha defensin for diagnosis of periprosthetic shoulder infections was excluded as the reference test had not been validated as there is no consensus on diagnostic criteria for periprosthetic shoulder infection[21].

Participants

All the studies included patients with previous arthroplasty that were listed for revision. Hip and knee arthroplasties were the only joints studied in eight trials[6,9,12,22–28].One study included hip, knee shoulder and elbow arthroplasties[29].

Reference standard

MSIS criteria were used in five studies, though specific details regarding culture technique were not generally presented[6,9,12,24,26]. In Frangiamore *et al*.'s study the original MSIS criteria were adapted, as synovial leucocyte count and neutrophil percentage were not evaluated[24].

The revised MSIS criteria were used in six studies. Balato *et al.* and Kasparek *et al.* adhered to all elements of these criteria[23,27], whereas Bonanzinga *et al.*, Suda *et al.* and Sigmund *et al.* adapted the elements of the criteria. Bonanzinga *et al.* and Gehrke *et al.* limited the collection of histology samples to those patients in whom infection was deemed to be likely by multidisciplinary team meeting, and they did not evaluate ESR[25,28]. Suda *et al.* and Sigmund *et al.* did not measure synovial neutrophil percentage, synovial white cell count or serum ESR[22,29].

Index test

Six studies used a laboratory-based ELISA to measure alpha-defensin levels[6,9,12,24,25,28]. Two of these investigations calculated a threshold level[6,21] while four used predetermined threshold values[9,12,24,25]. Six studies used the lateral flow cassette (Synovasure device) to evaluate alpha-defensin[22,23,26,29,28,27].

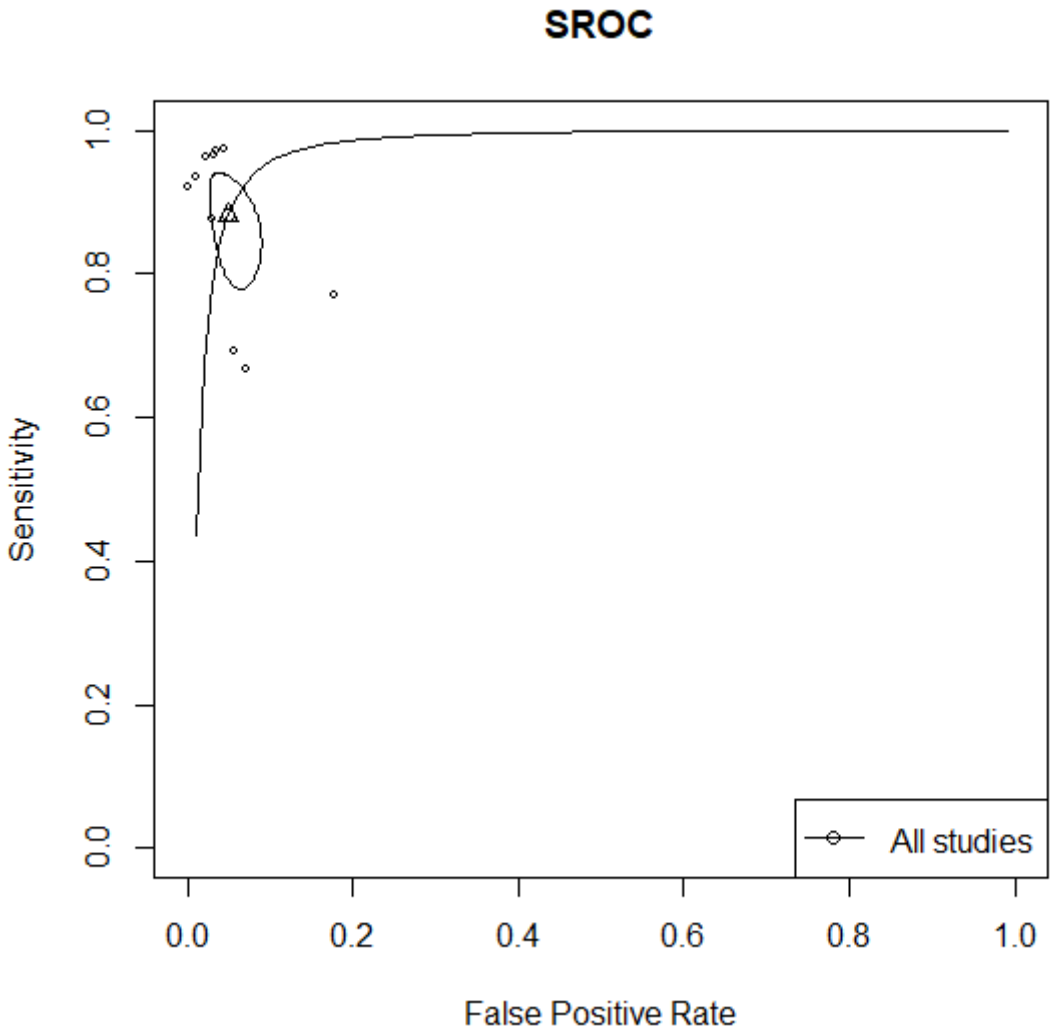
Risk of bias and conflicts of interest

Risk of bias for included studies was low. Median [I.Q.R.] QUADAS score was 13 [13-13] out of 14. Risk of bias scores are shown in Table 4.

Trial kits were provided free of charge by CD diagnostics for four studies[12,25,26,28]. In other studies, trial funding was not described. Five studies reported third-party support, financial incentives or

stock options with CD Diagnostics or another commercial party related to the study for one or more authors [6,9,12,25,26]. Intellectual property directly relating to the study material was identified in one study[9].

Pooled results for all studies



Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Balato 2017 (Lateral Flow)	14	1	2	34	0.88 [0.62, 0.98]	0.97 [0.85, 1.00]
Berger 2017 (Lateral Flow)	33	3	1	84	0.97 [0.85, 1.00]	0.97 [0.90, 0.99]
Bingham 2014 (ELISA)	13	2	0	40	1.00 [0.75, 1.00]	0.95 [0.84, 0.99]
Bonanzinga 2017 (ELISA)	28	4	1	123	0.97 [0.82, 1.00]	0.97 [0.92, 0.99]
Deirmengian 2014a (ELISA)	36	5	1	107	0.97 [0.86, 1.00]	0.96 [0.90, 0.99]
Deirmengian 2014b (ELISA)	23	0	0	66	1.00 [0.85, 1.00]	1.00 [0.95, 1.00]
Frangiamore 2016 (ELISA)	26	2	1	87	0.96 [0.81, 1.00]	0.98 [0.92, 1.00]
Gehrke 2018 (ELISA)	73	1	5	94	0.94 [0.86, 0.98]	0.99 [0.94, 1.00]
Gehrke 2018 (Lateral Flow)	70	0	6	119	0.92 [0.84, 0.97]	1.00 [0.97, 1.00]
Kasparek 2016 (Lateral Flow)	8	2	4	26	0.67 [0.35, 0.90]	0.93 [0.76, 0.99]
Sigmund 2016 (Lateral Flow)	3	2	4	34	0.43 [0.10, 0.82]	0.94 [0.81, 0.99]
Suda 2017 (Lateral Flow)	10	3	3	84	0.77 [0.46, 0.95]	0.97 [0.90, 0.99]

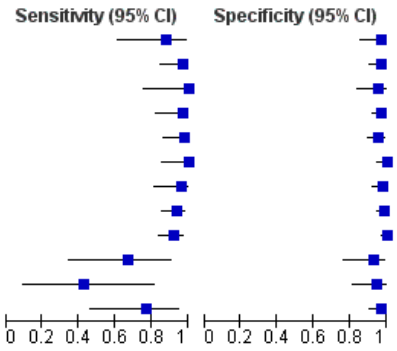


Figure 1 SROC Curve and forest plot for all included studies. SROC graph shows SROC curves and 95% confidence regions

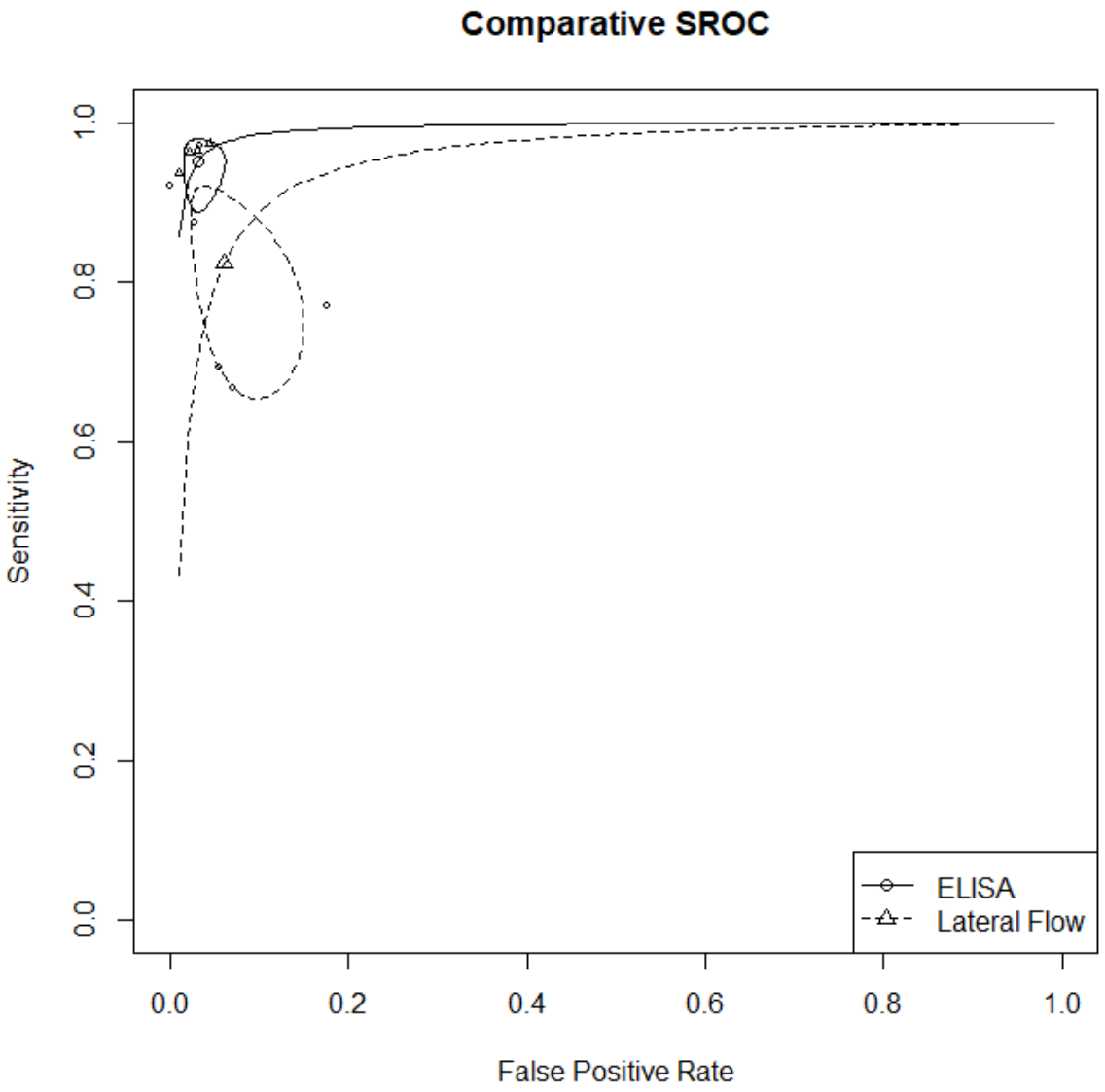
Results from all eleven included studies were pooled. Forest plots and SROC curve are shown in Figure 1. There was high heterogeneity as I^2 was 61% for sensitivity and 50% for specificity. Diagnostic odds ratio was 293 (95% CI 91-952), positive likelihood ratio 21 (95% CI 12-35), and negative likelihood ratio 0.1 (0.04-0.2).

Comparison of lateral flow and laboratory based alpha-defensin tests

Four studies reported the use of a laboratory based alpha-defensin test with a pre-specified threshold value of 5.2mgl^{-1}. Six studies demonstrated results using a lateral flow cassette. Comparative SROC curves and Forest plots for these techniques are shown in Figure 2.

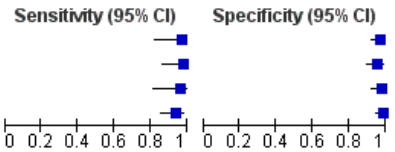
For studies completed using a laboratory test, pooled sensitivity was 0.95 (0.91-0.98) and specificity was 0.97 (0.95-0.98). The heterogeneity was very low with an I^2 for sensitivity and specificity was 0.0%. Positive likelihood ratio was 31 (95% CI 18-54) and negative likelihood ratio was 0.05 (95% CI 0.03-0.1). Diagnostic odds ratio was 1004 (95% CI 326-3087).

The lateral flow cassette had a pooled sensitivity of 0.85 (0.74-0.92) and specificity of 0.90 (0.91-0.98). There was a significant difference in the likelihood ratio test between laboratory-based and lateral flow tests for sensitivity (p=0.019) but not specificity (p=0.47). The heterogeneity was high with an I^2 for sensitivity was 62% and 67% for specificity. Positive likelihood ratio was 17 (95% CI 6-48) and negative likelihood ratio was 0.2 (95% CI 0.1-0.4). Diagnostic odds ratio was 118 (95% CI 24-585).



Lab based alpha-defensin (ELISA)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bonanzinga 2017 (ELISA)	28	4	1	123	0.97 [0.82, 1.00]	0.97 [0.92, 0.99]
Deirmengian 2014a (ELISA)	36	5	1	107	0.97 [0.86, 1.00]	0.96 [0.90, 0.99]
Frangiamore 2016 (ELISA)	26	2	1	87	0.96 [0.81, 1.00]	0.98 [0.92, 1.00]
Gehrke 2018 (ELISA)	73	1	5	94	0.94 [0.86, 0.98]	0.99 [0.94, 1.00]



Theatre based alpha-defensin (Lateral Flow)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Balato 2017 (Lateral Flow)	14	1	2	34	0.88 [0.62, 0.98]	0.97 [0.85, 1.00]
Berger 2017 (Lateral Flow)	33	3	1	84	0.97 [0.85, 1.00]	0.97 [0.90, 0.99]
Gehrke 2018 (Lateral Flow)	70	0	6	119	0.92 [0.84, 0.97]	1.00 [0.97, 1.00]
Kasparek 2016 (Lateral Flow)	8	2	4	26	0.67 [0.35, 0.90]	0.93 [0.76, 0.99]
Sigmund 2016 (Lateral Flow)	3	2	4	34	0.43 [0.10, 0.82]	0.94 [0.81, 0.99]
Suda 2017 (Lateral Flow)	10	3	3	84	0.77 [0.46, 0.95]	0.97 [0.90, 0.99]

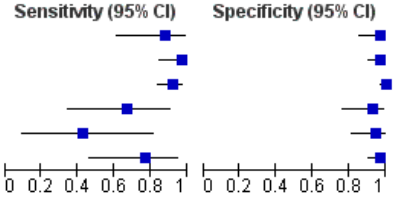


Figure 2 SROC and forest plots curve for studies using a laboratory test (lab test) with pre-specified threshold value of 5.2mgl^{-1} and the lateral flow cassette (theatre test). Shown are SROC curves and 95% confidence regions

Discussion

Summary of results

This review has analysed eleven studies examining the diagnostic utility of alpha-defensin in the diagnosis of periprosthetic joint infection. The summary of results can be found in Table 5.

The results have shown that the sensitivity and specificity of alpha-defensin can be excellent, with a sensitivity and specificity of 0.95 and 0.97 when using a laboratory ELISA with a threshold value of 5.2mgl^{-1}, and negligible study heterogeneity between the four relevant reports[24,25,28,30].

The pooled results from the lateral flow cassette showed lower sensitivity and specificity than results from the laboratory tests. For trials using with the lateral flow cassette, the pooled sensitivity was 0.85 and specificity 0.90[22,23,26–29]. This is contrary to the manufacturer's data sheet, which states the lateral flow cassette has a 100% positive agreement and 96% negative agreement with the laboratory test[31]. In the only study comparing lateral flow results to lab results, Gehurle et al demonstrated 95% agreement[28]. Lateral flow cassettes can be an effective technique for evaluating synovial fluid, as demonstrated by Wouthuyzen-Bakker et al using synovial calprotectin with a sensitivity of 89% and specificity of 95% for periprosthetic joint infection[32].

The reason for this lower sensitivity when using the lateral flow cassette is unclear. It could be due to technical errors in the conduct of the test in theatre compared to the use of a controlled ELISA or test undertaken in a laboratory. Point-of-care users may not be so meticulous in quality control and performing diagnostic tests as trained laboratory staff[33]. Patient selection may also play a role in the difference. Studies by Deirmengian *et al.* were completed in the CD Diagnostic lab[6,9,12] rather in a clinical environment.

A possible further cause for discrepancy is the involvement of industry in supporting many of the most positive studies. These studies did not share a threshold value, so pooled sensitivity and specificity was not appropriate.

The pooled sensitivity and specificity of laboratory-based alpha-defensin testing with a threshold of 5.2mgl^{-1} compares very favourably to other biomarkers for periprosthetic joint infection, as shown in Table 2. The lateral flow cassette performs with a similar sensitivity and specificity to the leucocyte esterase test[8] or synovial CRP[34], but with significant additional cost.

Table 2 Pooled sensitivity and specificity of biomarkers for periprosthetic joint infection from recent meta-analyses. CRP: C-reactive protein.

Reference	Test	Sensitivity	Specificity
Xie[13]	Serum procalcitonin	0.53 (0.24-0.8)	0.96 (0.85-0.99)
Qu[35]	Bacteriological culture	0.72 (0.65-0.78)	0.95 (0.93-0.97)
Wyatt[8]	Synovial leucocyte esterase	0.81 (0.49-0.95)	0.97 (0.82-0.99)
Yuan[34]	Serum CRP	0.82 (0.80-0.84)	0.77 (0.76-0.78)
Wang[36]	Synovial CRP	0.92 (0.86-0.96)	0.90 (0.87-0.93)
Berbari[37]	Serum IL-6	0.97 (0.93-0.99)	0.91 (0.87-0.94)

Limitations

While we are confident that this review has captured all published clinical trials on the use of alpha-defensin for diagnosis of periprosthetic joint infections, the analysis was limited to published studies. We are aware of six conference abstracts where alpha-defensin was evaluated. Two of these studies were then published in full and were included in this review[38,39]. One abstract did not present any results but stated findings would be available at the conference[40]. Martin *et al.* described a

sensitivity of 0.75 (95% CI 0.19-0.99) and specificity of 0.8 (95% CI 0.44-0.97) in 14 patients (4 who had positive cultures) against bacterial culture results[41]. Moore *et al.* showed 100% sensitivity and specificity (95% CI 0.16-1.0 and 0.69-1.0 respectively)[42] as did Refaie *et al.* (95% CI for sensitivity 0.4-1 and specificity 0.84-1)[43]. As these unpublished studies had low numbers of patients, it is unlikely that there would have been a drastic effect on the result from the meta-analysis.

A formal test for publication bias has not been completed due to low numbers of identified studies[44]. Visual inspection of the funnel plot (Figure 3) shows that there is reasonable symmetry about the midline on the log(diagnostic odds ratio) scale. Three studies are outliers with low diagnostic odds ratios.

An additional cause for concern of bias across the published studies is the prevalence of infection. The mean (SD) prevalence of infection in the included studies was 28%. This contrasts to the findings from the U.K. Joint Registry, which identified 130,195 revision hip and knee (single or first stage) replacements between 2003 and 2015. Of these, 13,801 were revisions for infection (11%)[1]. While this should not change the sensitivity or specificity, it may represent a difference between the populations being tested for these studies and the general clinical population in the United Kingdom requiring revision arthroplasty.

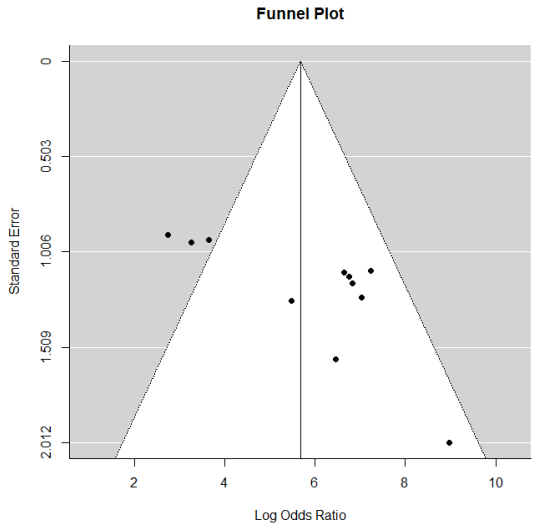


Figure 3 Pyramidal funnel plot of studies identified within this systematic review. Reasonable symmetry is seen around the midline with three outlying studies with negative results. A formal test has not been completed due to low numbers.

Conclusions

Alpha-defensin testing has excellent sensitivity and specificity when performed by ELISA in Citrano Medical Laboratories or using laboratory test kits supplied by the manufacturer. Independent studies have not replicated this high sensitivity and specificity, and this may be due to technical issues, lack of expertise or the wider use of the lateral flow cassette device.

We would advise revision arthroplasty surgeons that the sensitivity of alpha-defensin levels may not be as robust as demonstrated in earlier reviews. It remains a tool to aid diagnosis and needs to be interpreted with the same clinical judgement as other tests.

Further pragmatic studies are required to evaluate the lateral flow cassette in a clinical environment before routine adoption of the device to diagnose periprosthetic joint infection. A sample size calculation using the formula presented by Buderer suggests such a study would require to enrol

1419 patients undergoing revision arthroplasty assuming a prevalence of 10.6% and accuracy of 5%[45]. Such a study would be challenging, but not impossible, with the 7829 revision hips and 5,239 revision knees being undertaken annually in the U.K.

References

1. **National Joint Registry**. 13th Annual Report. London, 2016.

2. **Vanhegan IS, Malik AK, Jayakumar P, Ul Islam S, Haddad FS**. A financial analysis of revision hip arthroplasty. *Bone Joint J* 2012;94–B(5).

3. **Kurtz S, Ong K, Lau E, Mowat F, Halpern M**. Projections of Primary and Revision Hip and Knee Arthroplasty in the United States from 2005 to 2030. *J Bone Jt Surg* 2007;89(4):780.

4. **Workgroup Convened by the Musculoskeletal Infection Society**. New Definition for Periprosthetic Joint Infection. *J Arthroplasty* 2011;26(8):1136–1138.

5. **Parvizi J, Gehrke T**. Definition of Periprosthetic Joint Infection. *J Arthroplasty* 2014;29(7):1331.

6. **Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J**. Diagnosing Periprosthetic Joint Infection: Has the Era of the Biomarker Arrived? *Clin Orthop Relat Res* 2014;472(11):3254–3262.

7. **Wang G, Mishra B, Lau K, Lushnikova T, Golla R, Wang X**. Antimicrobial peptides in 2014. *Pharmaceuticals (Basel)* Multidisciplinary Digital Publishing Institute (MDPI), 2015;8(1):123–50.

8. **Wyatt MC, Beswick AD, Kunutsor SK, Wilson MJ, Whitehouse MR, Blom AW**. The Alpha-Defensin Immunoassay and Leukocyte Esterase Colorimetric Strip Test for the Diagnosis of Periprosthetic Infection. *J Bone Jt Surg* 2016;98(12):992–1000.

9. **Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J**. Combined measurement of synovial fluid α-Defensin and C-reactive protein levels: highly accurate for diagnosing periprosthetic joint infection. *J Bone Joint Surg Am* 2014;96(17):1439–45.

10. **Deirmengian C, Kardos K, Kilmartin P, Gulati S, Citrano P, Booth RE, et al.** The Alpha-defensin Test for Periprosthetic Joint Infection Responds to a Wide Spectrum of Organisms. *Clin Orthop Relat Res* Springer US, 2015;473(7):2229–2235.

11. **Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Booth RE, et al.** The Alpha-defensin Test for Periprosthetic Joint Infection Outperforms the Leukocyte Esterase Test Strip. *Clin Orthop Relat Res* 2015;473(1):198–203.

12. **Bingham J, Clarke H, Spangehl M, Schwartz A, Beauchamp C, Goldberg B**. The alpha defensin-1 biomarker assay can be used to evaluate the potentially infected total joint arthroplasty. *Clin Orthop Relat Res* 2014;472(12):4006–9.

13. **Xie K, Qu X, Yan M**. Procalcitonin and α-Defensin for Diagnosis of Periprosthetic Joint Infections. *J Arthroplasty* 2017;32(4):1387–1394.

14. **Saleh A, Ramanathan D, Siqueira MBP, Klika AK, Barsoum WK, Rueda CAH**. The Diagnostic Utility of Synovial Fluid Markers in Periprosthetic Joint Infection. *J Am Acad Orthop Surg* 2017;25(11):763–772.

15. **Marson BA, Deshmukh S, Grindlay D, Scammell BE**. Synovial a-defensin for diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. PROSPERO 2017:CRD42017069267. PROSPERO. http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017069267 ,.

16. **Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J**. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic

reviews. *BMC Med Res Methodol* BioMed Central, 2003;3:25.

17. **Joanna Briggs Institute**. *The Joanna Briggs Institute Reviewers' Manual 2015: The systematic review of studies of diagnostic test accuracy*. Adelaide, 2015.

18. **Zamora J, Abraira V, Muriel A, Khan K, Coomarasamy A**. Meta-DiSc: a software for meta-analysis of test accuracy data. *BMC Med Res Methodol* BioMed Central, 2006;6:31.

19. **Partlett C, Takwoingi Y**. Meta-analysis of test accuracy studies in R:a summary of user-written programs and step-by-step guide to using glmer. http://methods.cochrane.org/sdt , (date last accessed 26 June 2017).

20. **Doebler P, Münster W, Holling H**. Meta-Analysis of Diagnostic Accuracy with mada. https://cran.r-project.org/web/packages/mada/vignettes/mada.pdf , (date last accessed 2 October 2017).

21. **Frangiamore SJ, Saleh A, Grosso MJ, Kovac MF, Higuera CA, Iannotti JP, et al.** α-Defensin as a predictor of periprosthetic shoulder infection. *J Shoulder Elb Surg* 2015;24(7):1021–1027.

22. **Suda AJ, Tinelli M, Beisemann ND, Weil Y, Khoury A, Bischel OE**. Diagnosis of periprosthetic joint infection using alpha-defensin test or multiplex-PCR: ideal diagnostic test still not found. *Int Orthop* 2017;41(7):1307–1313.

23. **Kasparek MF, Kasparek M, Boettner F, Faschingbauer M, Hahne J, Dominkus M**. Intraoperative Diagnosis of Periprosthetic Joint Infection Using a Novel Alpha-Defensin Lateral Flow Assay. *J Arthroplasty* 2016;31(12):2871–2874.

24. **Frangiamore SJ, Gajewski ND, Saleh A, Farias-Kovac M, Barsoum WK, Higuera CA**. α-Defensin Accuracy to Diagnose Periprosthetic Joint Infection? Best Available Test? *J Arthroplasty* 2016;31(2):456–460.

25. **Bonanzinga T, Zahar A, Dütsch M, Lausmann C, Kendoff D, Gehrke T**. How Reliable Is the Alpha-defensin Immunoassay Test for Diagnosing Periprosthetic Joint Infection? A Prospective Study. *Clin Orthop Relat Res* 2017;475(2):408–415.

26. **Berger P, Cauter M Van, Driesen R, Neyt J, Cornu O, Bellemans J**. Diagnosis of prosthetic joint infection with alpha-defensin using a lateral flow device: a multicentre study. *Bone Joint J* British Editorial Society of Bone and Joint Surgery, 2017;99–B(9):1176–1182.

27. **Balato G, Franceschini V, Ascione T, Lamberti A, D'Amato M, Ensini A, et al.** High performance of α-defensin lateral flow assay (Synovasure) in the diagnosis of chronic knee prosthetic infections. *Knee Surgery, Sport Traumatol Arthrosc* Springer Berlin Heidelberg, 2017;0(0):1–6.

28. **Gehrke T, Lausmann C, Citak M, Bonanzinga T, Frommelt L, Zahar A**. The Accuracy of the Alpha Defensin Lateral Flow Device for Diagnosis of Periprosthetic Joint Infection. 2018;42–48.

29. **Sigmund IK, Holinka J, Gamper J, Staats K, Böhler C, Kubista B, et al.** Qualitative α-defensin test (Synovasure) for the diagnosis of periprosthetic infection in revision total joint arthroplasty. *Bone Joint J* 2017;99–B(1):66–72.

30. **Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J**. Combined measurement of synovial fluid α-Defensin and C-reactive protein levels: highly accurate for diagnosing periprosthetic joint infection. *J Bone Joint Surg Am* 2014;96(17):1439–45.

31. **CD Diagnostics**. Synovasure® Alpha Defensin Lateral Flow Test Kit. https://cddiagnostics.com/instructions/PDF/LF Test Kit

IFUs/EN_M40004B_V3.3_Synovasure_Alpha_Defenins_Lateral_Flow_IFU_(English).pdf , (date last accessed 28 September 2017).

32. **Wouthuyzen-Bakker M, Ploegmakers JJW, Kampinga GA, Wagenmakers-Huizenga L, Jutte PC, Muller Kobold AC**. Synovial calprotectin: a potential biomarker to exclude a prosthetic joint infection. *Bone Joint J* British Editorial Society of Bone and Joint Surgery, 2017;99–B(5):660–665.

33. **Drago L, Toscano M, Tacchini L, Banfi G**. α-Defensin point-of-care test for diagnosis of prosthetic joint infections: neglected role of laboratory and clinical pathologists. *Clin Chem Lab Med* De Gruyter, 2017;0(0).

34. **Yuan K, Chen H-L, Cui Z-M**. Diagnostic Accuracy of C-Reactive Protein for Periprosthetic Joint Infection: A Meta-Analysis. *Surg Infect (Larchmt)* Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA , 2014;15(5):548–559.

35. **Qu X, Zhai Z, Liu X, Li H, Wu C, Li Y, et al.** Evaluation of White Cell Count and Differential in Synovial Fluid for Diagnosing Infections after Total Hip or Knee Arthroplasty. Medeiros R, ed. *PLoS One* 2014;9(1):e84751.

36. **Wang CC-B, Wang Q, Li R, Duan J-Y, Wang CC-B**. Synovial Fluid C-reactive Protein as a Diagnostic Marker for Periprosthetic Joint Infection: A Systematic Review and Meta-analysis. *Chin Med J (Engl)* 2016;129(16):1987.

37. **Berbari E, Mabry T, Tsaras G, Spangehl M, Erwin PJ, Murad MH, et al.** Inflammatory Blood Laboratory Levels as Markers of Prosthetic Joint Infection. *J Bone Jt Surgery-American Vol* 2010;92(11):2102–2109.

38. **Deirmengian C, Kardos K, Kilmartin P, Cameron A, Chung D, Schiller K, et al.** Alpha-defensin in synovial fluid as a new marker for the diagnosis of periprosthetic joint infection. *65th Annu Sci Meet Am Assoc Clin Chem* 2013:A221.

39. **Sigmund IK, Holinka J, Gamper J, Staats K, Böhler C, Kubista B, et al.** Alpha-defensin test for evaluation of periprosthetic joint infection. *35th Annu Meet Eur Bone Jt Infect Soc* Oxford, 2016.

40. **Randelli F, Brioschi B, Favilla S, Maglione D, Pace F**. The role of novel inflammatory biomarkers in the diagnosis and prognosis of periprosthetic joint infection: a prospective cohort study. *Int Comb Meet BHS-SIdA,* 2015.

41. **Martin E, Qamar F, Ng A, Koch L, Shetty A**. 'synovasure' are we really sure? *Int Comb Meet BHS-SIdA* 2015:S48.

42. **Moore P, Kempshall P, Gosal H, Mutimer J**. Early experience with point of care alpha defensin testing in Gloucestershire, UK. *34th Congr Eur Bone Jt Infect Soc* 2015.

43. **Refaie R, Marriott A, Marsh M, Nicolas A, Reed M**. An evaluation of the Synovasure near patient lateral fow test for the diagnosis of periprosthetic joint infection. *5th Annu Oxford Bone Infect Conf* 2015.

44. **Sterne JAC, Sutton AJ, Ioannidis JPA, Terrin N, Jones DR, Lau J, et al.** Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ* 2011;343(jul22 1):d4002–d4002.

45. **Buderer NM**. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Acad Emerg Med* 1996;3(9):895–900.

PRISMA Study flow diagram

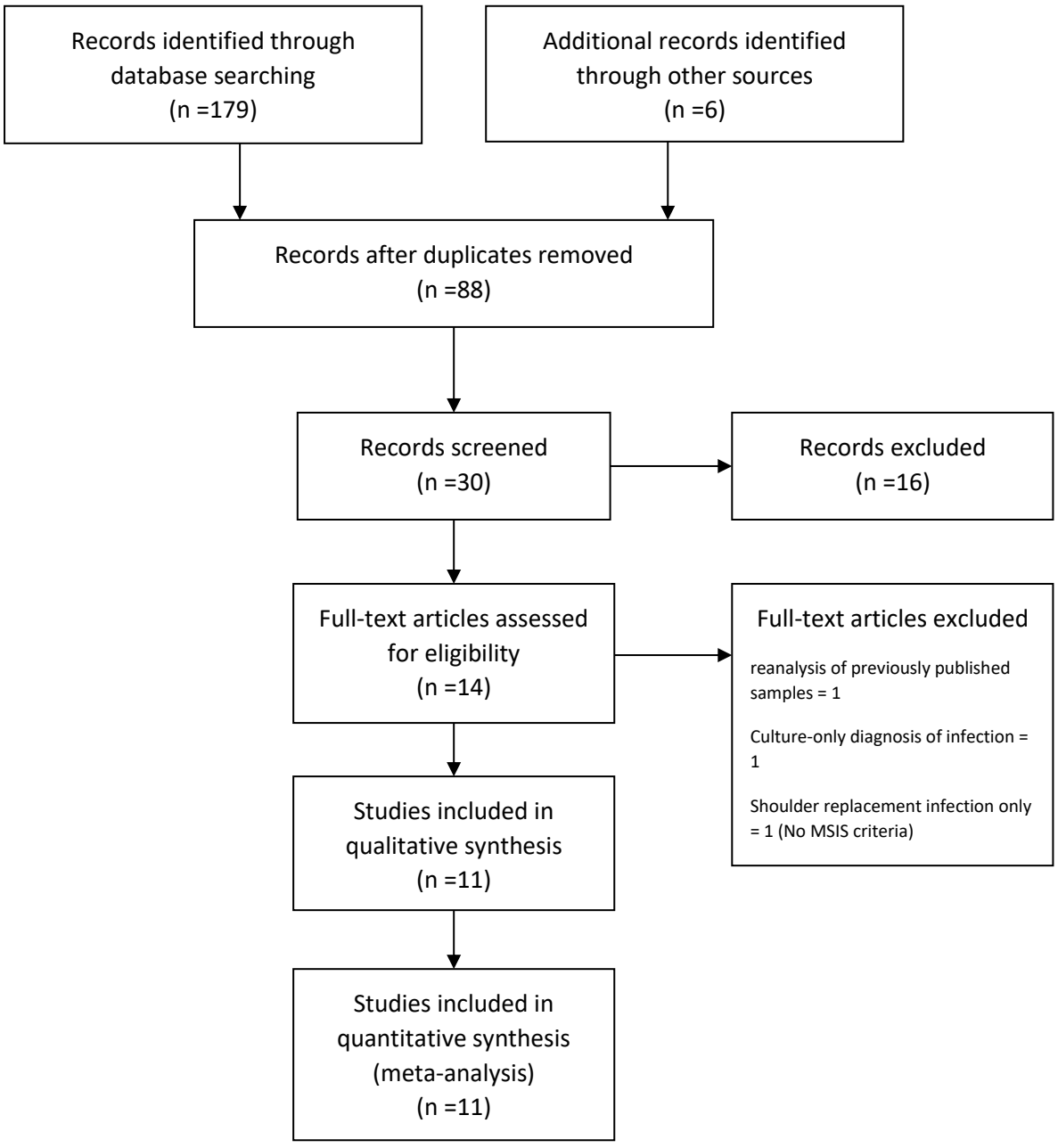


Figure 4 PRISMA Study flow diagram

Characteristics of included studies

*Table 3 Characteristics of included studies. Abbreviations used: MSIS - Musculoskeletal Infection Society; MDT Multidisciplinary team; S/Co Signal/Cut off ratio, TP true positive; FP false positive; FN false negative; TN True negative. *1S/Co was identified as 5.2mgl^{-1} in Deirmengian et al 2014a.*

Study ID	Location	Sample size	Methodology	Index test	Reference test	Threshold	TP	FP	FN	TN
Balato 2017[27]	Italy	51	Prospective, consecutive	Lateral flow	MSIS (2013)	Cassette	14	1	2	34
Berger 2017[26]	Multi-centre, Belgium	121	Prospective, unclear if consecutive	Lateral flow	MSIS (2011)	Cassette	33	3	1	84
Bingham 2014[12]	Mayo clinic, Arizona	61	Retrospective, consecutive	CD Labs kit	MSIS (2011)	7.72mgl^{-1}	19	2	0	40
Bonanzinga 2017[25]	ENDO Klinik, Hamburg	156	Prospective, consecutive	CD Labs kit	MSIS (2013)	1.0 S/Co*	28	4	1	123
Deirmengian 2014a[9]	CD Diagnostics	149	Retrospective, unclear if consecutive	ELISA	MSIS (2011)	5.2mgl^{-1}	36	5	1	107
Deirmengian 2014b[6]	CD Diagnostics	95	Retrospective, unclear if consecutive	ELISA	MSIS (2011)	4.8mgl^{-1}	29	0	0	66
Frangiamore 2016[24]	Cleveland Clinic Foundation	116	Prospective, unclear if consecutive	ELISA	MSIS (2011)	5.2mgl^{-1}	26	2	1	87
Gehrke 2018[28]	ENDO Klinik, Hamburg	195	Prospective, consecutive	Lateral flow and ELISA	MSIS (2013)	Cassette / 1.0S/Co*	73	1	5	94
Kasparek 2016[23]	Vienna	40	Prospective, consecutive	Lateral flow	MSIS (2013)	Cassette	8	2	4	26
Sigmund 2016[29]	Medical University of Vienna	19	Prospective, unclear if consecutive	Lateral flow	MSIS (2013)	Cassette	9	2	4	34
Suda 2017[22]	BG Trauma Centre, Ludwigshafen	30	Prospective, consecutive	Lateral flow	MSIS (2013)	Cassette	10	3	3	14

QUADAS Scores

Table 4 Study quality assessment using QUADAS tool. Studies were rated on the following questions:1) Was the spectrum of patients representative of the patients who will receive the test in practice? 2) Were selection criteria clearly described? 3) Is the reference standard likely to correctly classify the target condition? 4) Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the 2 tests? 5) Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis? 6) Did patients receive the same reference standard regardless of the index text result? 7) Was the reference standard independent of the index test (i.e., the index test did not form part of the reference standard)? 8) Was the execution of the index test described in sufficient detail to permit replication of the test? 9) Was the execution of the reference standard described in sufficient detail to permit its replication? 10) Were the index test results interpreted without knowledge of the results of the reference standard?11) Were the reference standard results interpreted without knowledge of the results of the index test? 12) Were the same clinical data available when test results were interpreted as would be available when the test is used in practice? 13) Were uninterpretable/intermediate test results reported? 14) Were withdrawals from the study explained?

	QUADAS Question Number														Total
Study ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Balato 2017[27]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14
Berger 2017[26]	1	1	1	1	1	0	1	1	1	1	0	1	1	1	12
Bingham 2014[12]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14
Bonanzinga 2017[25]	1	1	0	1	1	1	1	1	1	1	1	1	1	1	13
Deirmengian a 2014[9]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14
Deirmengian b 2014[6]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14
Frangiamore 2016[24]	1	1	0	1	1	1	1	1	1	1	1	1	1	1	13
Gehrke 2018[28]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14
Kasparek 2016[23]	1	1	0	1	1	1	1	1	1	1	1	1	1	1	13
Sigmund 2016[29]	1	1	0	0	1	1	1	1	1	1	1	1	1	1	12
Suda 2017[22]	1	0	0	0	1	1	1	1	1	1	1	1	1	1	11

Table 5 Summary of results table for the diagnostic accuracy of alpha-defensin for detection of infection in revision joint replacement

Patients/population		Painful joint replacements being evaluated for revision			
Prior testing		Nil specified except clinical examination			
Settings		Hospital departments with revision surgery facilities			
Index test		Alpha-defensin ELISA or lateral flow cassette			
Reference test		MSIS consensus criteria or modified MSIS criteria			
Studies		Combination of prospective and retrospective cohort trials.			
Test	Summary accuracy (95% CI)	Number of participants (studies)	Prevalence Median (range)	Implications	Quality and comments
Alpha-defensin ELISA with cut off 5.2mgl^{-1}	Sensitivity 0.95 (0.91-0.98) Specificity 0.97 (0.95-0.98)	594 patients (4 studies)	24% (19-45%)	With a prevalence of 24%, 24 out of 100 patients will have a revision for infection. Of these, one will be missed by the ELISA and two will be unnecessarily diagnosed as infection.	One retrospective study and three prospective study. three of the four studies had financial links to CD Diagnostics.
Alpha-defensin lateral flow cassette	Sensitivity 0.85 (0.74-0.92) Specificity 0.97 (0.0.91-0.98)	486 patients (6 studies)	31% (26-43%)	Of the 24 in 100 patients with infection 4 will be missed and 2 will be unnecessarily diagnosed as infection.	Six prospective trials. Two trials had devices provided by CD Diagnostics.

Appendix 1:

Search terms

OVID MEDLINE

1. exp Defensins/
2. defensin.mp.
3. defensins.mp.
4. antimicrobial peptide.mp.
5. antimicrobial peptides.mp.
6. 1 or 2 or 3 or 4 or 5
7. exp arthroplasty, replacement/
8. exp joint prosthesis/
9. arthroplasty, subchondral/
10. arthroplasty.mp.
11. arthroplasties.mp.
12. joint replacement.mp.
13. joint replacements.mp.
14. joint prosthesis.mp.
15. joint prostheses.mp.
16. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15
17. exp bacterial infections/
18. surgical wound infection/
19. prosthesis-related infections/
20. prosthesis-related infection.mp.
21. prosthesis-related infections.mp.
22. prosthesis infection.mp.
23. prosthesis infections.mp.
24. prosthetic joint infection.mp.
25. prosthetic joint infections.mp.
26. periprosthetic joint infection.mp
27. periprosthetic joint infections.mp
28. peri-prosthetic joint infection.mp
29. peri-prosthetic joint infections.mp
30. 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
31. 6 and 16 and 26

OVID EMBASE

1. exp Defensin/
2. defensin.mp.
3. defensins.mp.
4. antimicrobial peptide.mp.
5. antimicrobial peptides.mp.
6. 1 or 2 or 3 or 4 or 5
7. exp orthopedic prosthesis and orthosis/
8. exp arthroplasty/
9. exp arthroplasty,prosthesis/
10. arthroplasty.mp.
11. arthroplasties.mp.
12. joint replacement.mp.

13. joint replacements.mp.
14. joint prosthesis.mp.
15. joint prostheses.mp.
16. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15
17. exp bacterial infection/
18. exp surgical infection/
19. exp prosthesis infection/
20. exp periprosthetic joint infection/
21. prosthesis-related infection.mp.
22. prosthesis-related infections.mp.
23. prosthesis infection.mp.
24. prosthesis infections.mp.
25. prosthetic joint infection.mp.
26. prosthetic joint infections.mp.
27. periprosthetic joint infection.mp
28. periprosthetic joint infections.mp
29. peri-prosthetic joint infection.mp
30. peri-prosthetic joint infections.mp
31. 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 29 or 29 or 30
32. 6 and 16 and 31

PUBMED

("Defensins"[MESH Term] or "defensin" or "defensins" or "antimicrobial peptide" or "antimicrobial peptides") AND ("arthroplasty, replacement"[MESH Term] or "joint prosthesis"[MESH Term] or "arthroplasty, subchondral"[MESH Term] or "arthroplasty" or "arthroplasties" or "joint replacement" or "joint replacements" or "joint prosthesis" or "joint prostheses") AND ("bacterial infections"[MESH Term] or "surgical wound infection"[MESH Term] or "prosthesis-related infections"[MESH Term] or "prosthesis-related infection" or "prosthesis-related infections" or "prosthesis infection" or "prosthesis infections" or "prosthetic joint infection" or "prosthetic joint infections" or "periprosthetic joint infection" or "periprosthetic joint infections" or "peri-prosthetic joint infection" or "peri-prosthetic joint infections")