An evaluation of the High Frequency Digit Triplet Test as a screening tool for early detection of hearing loss in individuals with Cystic Fibrosis

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SYNOPSIS

Title	An evaluation of the High Frequency Digit Triplet Test as a screening tool for early detection of hearing loss in individuals with Cystic Fibrosis
Short title	An evaluation of the High Frequency Digit Triplet Test in Cystic Fibrosis
Chief Investigator	Professor Alan Smyth
Objectives	(1) To validate the High Frequency Digit Triplet Test (HFDT) against the Pure Tone Audiogram (PTA) in adults and young people (aged over 11 years) with cystic fibrosis (CF) in a period of clinical stability. (2) To explore the use of the HFDT test immediately prior to aminoglycoside treatment in the same population (again compared with the PTA) to see if the test identifies those who should not proceed to aminoglycoside treatment. This work stream will assess feasibility and test validity in a patient who is unwell. We will perform both tests pre-treatment and again 6-8 weeks later. (3) To define the lower age limit of the HDFT test in children. We will test each age group from 5-10 years. The lower age limit for routine use will be the age with at least 80% test completion and where the correlation between the HFDT test and PTA is at least half that of adults. (4) To assess the prevalence of hearing loss in people with CF. (5) To look for factors which may explain differences in the prevalence of hearing loss in a population with variable exposure.
Study Configuration	A multi-centre observational study.
Setting	Cystic Fibrosis Secondary Care Clinics, Acute hospital admissions to the CF service and local primary school children
Sample size estimate	**Work stream 1:** In clinical practice the High Frequency Digit Triplet Test (HFDT test) will be used as a screening tool to identify those patients with CF in whom a full PTA is indicated. These patients will have a Speech Reception Threshold (SRT) with a less negative value than the threshold for hearing impairment of -16.5dB. We expect that the sensitivity and specificity of the HFDT test, in patients with CF, are similar to the reported sensitivity (95%) and specificity (98%) of a similar speech-in-noise test in the general population[1]. However, we have designed the study to have 80% power to detect a lower confidence limit of 80% sensitivity i.e. there is an 80% chance that the lower confidence limit for sensitivity is 80% or greater. For work streams 1 and 2, we have used the method for calculating sample size described by Flahault *et al* [2] with the statistical package GPower 3.1.3. Using this method the sample size depends on the prevalence of hearing loss. In a previous review[3] we have identified

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	those studies which measure the prevalence of hearing loss in CF and which are of acceptable quality (study population is defined and the definition of hearing loss is clearly described). However, the studies show considerable heterogeneity which may be related to the population described (less aminoglycoside use in earlier studies from the 1970s and 1980s) and the precise definition of hearing loss used. They give a range of prevalence values from 1% to 51%. We have used a weighted mean of the prevalence values from the individual studies (27% prevalence), weighted by the number of participants in the study. This gives a sample size of 111 participants for work stream 1. We aim to enrol 117 participants in work stream 1 in order to have 111 who will complete both the HFDT test and PTA test successfully (5% drop out). A low drop out is expected as the tests are simple and quick to undertake and they will be performed during a routine clinic visit. **Work stream 2:** For work stream 2 the test is performed under more challenging conditions. The patient may be tired and short of breath. Coughing may prevent participants responding to the speech or tone stimuli. This will affect the feasibility of the test (some patients will not be able to complete it) and also its specificity (patients may "fail" the HFDT test due to coughing or fatigue, in spite of having normal hearing). The same factors may lead to patients being unable to perform a PTA. We aim to detect the same lower confidence limit for the HFDT test as in work stream 1 (80% sensitivity) with the same power (80%). We will increase the number of patients enrolled by 20% to allow for more patients not completing the test (compared to work stream 1) and so we will approach 133 patients to achieve 111 participants successfully completing both tests. **Work stream 3:** We will assume the test is not feasible, due to limited performance, in 20% of children in both the CF and control groups. (Feasibility will vary with age. Most 10 year olds but only some 5 year olds will complete the test). We expect that few children in this young age group have hearing impairment and so we have powered this work stream on the feasibility of the HFDT test, rather than the sensitivity of the test to detect abnormal hearing. The sample size required to test for a difference in test completion rates from 95% for older children to 80% for younger children with 80% power is 134 (including those who complete the test and those who do not, as both groups contribute data). The overall sample size will be 134. Sample size was calculated using GPower 3.1.3. Therefore, for work stream 3, we will study 11 children with CF and 12 control children for each age from 5 to 10 years (66 CF and 72 controls). This gives a total sample size of 138 participants.
Number of participants	Work stream 1: 117 participants Work stream 2: 133 participants Work stream 3: 138 participants

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Eligibility criteria	**Eligibility for Work stream 1** Inclusion criteria: • A diagnosis of CF, confirmed by genotype or sweat test, with characteristic clinical features[4]. • Aged 11 years and over. • Informed consent. Consent will be sought from the parent (11-15, and the young person may give their assent if they wish) and young person (16-18) will consent for themselves. Exclusion criteria: • None. In individuals with a hearing aid we will perform PTA and HFDT tests without the aid. **Eligibility for Work stream 2** As above but patient requires intravenous antibiotics for a pulmonary exacerbation. **Eligibility for Work stream 3** Inclusion criteria: • As for work stream 1 but CF patients aged 5-10 years and healthy control children in the same age range. Informed consent from parent with assent from the child. Exclusion criteria: • None. In individuals with a hearing aid we will perform PTA and HFDT tests without the aid. **Eligibility for Genetic Testing** Inclusion Criteria • Informed consent. • Diagnosis of CF as confirmed above. Exclusion Criteria • None • If participants are found to have an ear infection or discharging ears this will be investigated and treated prior to continuing with the study.
Description of interventions	**Work stream 1** The participant will attend for a routine clinic appointment. During this visit the participant and/or their parent will be asked questions about their hearing. They will undergo an examination of the outer ear, otoscopy and tympanometry. The participant will have the new, High Frequency Digit Triplet Test (HFDT) first then the standard tests (a Pure Tone Audiogram (PTA), High-Frequency PTA (HF-PTA) and Distortion-product Otoacoustic Emissions (DPOAE)). They will then have a second HFDT-test to ensure there is no intra-individual variability. **Work stream 2** On arriving to clinic or the ward to start a course of IV antibiotics the participant will undergo the same test procedure as described above (though modifications may be necessary to reduce the test burden where clinically necessary and they will only have the HFDT-test once). Follow-up tests will be carried out in clinic at the follow-up visit 6-8 weeks later.

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	Work stream 3 The participant will undergo a tympanogram and questions about their hearing, an HFDT-test then a PTA (with a modified high frequency PTA if the test burden permits this), DPOAE, and a repeat HFDT test, during a routine clinic appointment or at the end of a hospital admission. They will have the HFDT-test twice to ensure there is no intra-individual variability. Control children from local primary schools will undergo the same tests ideally during school hours. **Genetic Analysis** Participants with CF in work streams 1, 2 and 3 will be asked to provide a sample of saliva and consent will be requested for a blood sample at the next clinically required opportunity. These samples will be analysed for the presence of genetic mutations known to be associated with hearing loss including the mitochondrial DNA mutation m.1555A>G. **Data Collection** Notes for all CF participants will be reviewed to collect data including demographic data, current markers of wellbeing, microbiological data and a list of current and past treatments including exposure to aminoglycosides.
Duration of study	Data collection will start in Autumn 2014 and finish in Autumn 2016. Per participant Work stream 1: One half day Work stream 2: 6-8 weeks (one half day at beginning and end) Work stream 3: One half day
Randomisation and blinding	The study will not be randomised or blinded.
Outcome measures	The correlation of the results of the HFDT test with a PTA. The feasibility of the HFDT in unwell patients. The youngest age at which the HFDT is clinically useful. The prevalence of hearing loss in a CF population. The prevalence of genetic mutations associated with hearing loss in a CF population.
Statistical methods	**Work stream 1:** We will calculate the sensitivity and specificity of the HFDT test treating PTA as a 'gold standard' and determine confidence intervals (using Wilson score method). In addition, in order to compare estimated sensitivity and specificity of the HFDT test with PTA, a latent variable model will be fitted and receiver operating characteristic curves obtained, from which sensitivity and specificity of the two tests can be compared. Prevalence rates of hearing loss and the m1555A>G mutation as well as correlations between the HFDT test SRT and PTA (including HF-PTA) thresholds and between the HFDT SRT and DPOAE amplitudes will also be calculated for reporting. **Work stream 2:** We will report the number of patients and the number of tests where the HFDT test was feasible in the patient about to receive intravenous antibiotics. The feasibility of the HFDT test and PTA at baseline will be

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	compared based on the numbers completing each test using logistic generalised linear mixed effects modelling (GLMM). As for work stream 1 sensitivity and specificity of the HFDT relative to PTA will be provided, with confidence intervals, at both baseline and follow up. Reliability of the HFDT test and PTA between baseline and follow up will be compared using a random intercept mixed-effects model. Additionally the latent variable model outlined in work stream 1 will be extended by modelling the pre-/post-treatment effect allowing receiver operating characteristics of the two tests to be compared both at baseline and follow up. Finally the correlation between HFDT SRT and PTA thresholds will also be reported at baseline and follow-up. **Work stream 3:** As with work stream 2, logistic regression methods will be used to assess the feasibility of each of the tests for CF patients *vs*. controls, and as age varies. This analysis will be based on the numbers completing each test and will allow us to estimate the minimum age at which 80% are able to perform the test. Comparisons of correlations between groups and by age will be performed using Fisher's *z*-transformation of Pearson's correlation. These correlations will also be compared with those reported from work streams 1 and 2. Finally, the receiver operating characteristics will be further explored using the latent variable approach described under work stream 2, but extended to model age effects in addition to modelling patient group effects. We will use the R statistical package (http://www.r-project.org/) except for the latent variable model where SPSS Amos will be used to fit SEM.

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ABBREVIATIONS

AE	Adverse Event
BSA	British Society of Audiology
CF	Cystic Fibrosis
CI	Chief Investigator overall
CRF	Case Report Form
CRN	Clinical Research Network
DMC	Data Monitoring Committee
DPOAE	Distortion Product Otoacoustic Emissions
GCP	Good Clinical Practice
HFDT	High Frequency Digit Triplet [test]
HF-PTA	High Frequency Pure Tone Audiogram
ICF	Informed Consent Form
NHS	National Health Service
P/GIS	Parent / Guardian Information Sheet
PI	Principal Investigator at a local centre
PIS	Participant Information Sheet
PTA	Pure Tone Audiogram
REC	Research Ethics Committee
R&D	Research and Development department
SAE	Serious Adverse Event
SNR	Signal to Noise Ratio
SRT	Speech Reception Threshold
YPAG	Young Person's Advisory Group

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STUDY BACKGROUND INFORMATION AND RATIONALE

Cystic fibrosis (CF) is the commonest, life limiting, autosomal recessive condition in the UK. There are over 9000 people with CF in the UK[6]. Affected individuals have respiratory problems including chronic pulmonary infection, bronchiectasis and respiratory failure[7]. Most individuals will have pancreatic malabsorption and may be underweight. Later complications include liver disease, diabetes and male infertility[7]. Life expectancy is currently into the early forties[8] and is dependent on early treatment of respiratory infection, airway clearance and nutritional management.

Most CF patients in the UK have chronic pulmonary infection with *Pseudomonas aeruginosa* by early adulthood[6]. Repeated courses of intravenous antibiotics create a great therapeutic burden and expose individuals to cumulative adverse effects. This is a particular problem with aminoglycoside antibiotics which can cause both acute kidney injury[9], chronic kidney disease[10] and bilateral sensorineural hearing loss[11] in CF patients. In spite of these adverse effects, aminoglycosides are recommended because of their clinical effectiveness[12].

Estimates of the prevalence of sensorineural hearing loss in CF, measured using a pure tone audiogram (PTA), vary from 1-51%[3]. The wide range of estimates is determined by the definition used and increased exposure to aminoglycosides in successive decades. We have calculated a weighted mean estimate of 27% for the prevalence of hearing impairment in CF patients. Teenagers and young adults with CF are more likely to suffer hearing loss, compared to the general population, where hearing loss is relatively uncommon in younger individuals (6-10% for a comparable age group)[13]. Even mild sensorineural hearing loss can adversely affect school performance in children[14]. People with hearing impairment are more likely to be unemployed and, if they do find work, are likely to earn around 15% less than those with normal hearing[15]. Furthermore, both hearing loss and CF affect young people's ability to make friends and form relationships[15].

Middle ear disease, causing conductive hearing loss, is less common in CF than in the general population[16]. Rarely, individuals may possess a mitochondrial DNA mutation which predisposes to severe deafness following minimal exposure to aminoglycosides[17]. The commonest mutation is m1555A>G which has a prevalence of around 0.2% in both children[18] and adults[19].

Current UK CF guidelines make no firm recommendation on screening for hearing loss: *"Screening is not currently advocated but audiological assessment may be necessary in some patients."* [20] In contrast, generic, US guidelines for patients receiving aminoglycosides state *"...weekly or biweekly monitoring is recommended, ideally. Because aminoglycosides can also cause delayed hearing loss, follow-up testing should also be scheduled a few months after drug discontinuation."*[21] For CF patients, who may receive a 2 week course of intravenous antibiotics, including aminoglycoside, every 3 months, the intensive monitoring recommended in the US is impractical.

The gold standard hearing test is the pure tone audiogram (PTA) which requires a trained clinical audiologist, a suitable environment, less than 35dB(A) of background noise)[22] and takes about 15-20 minutes. The PTA measures hearing loss in the frequency range 0.25-8 kHz[22], which is important for conversational speech. The High-Frequency Pure Tone Audiogram (HF-PTA) tests for frequencies above these thresholds, typically 9-16 kHz. Distortion Product Otoacoustic Emissions (DPOAEs) are responses generated by the cochlea when it is simultaneously stimulated by two pure-tone frequencies whose ratio is between 1:1 and 1:3. [23]. OAEs typically disappear after damage to the inner ear. Recent

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work has shown that DPOAE is a sensitive test for picking up abnormal hearing in people with cystic fibrosis and it may be more sensitive to subtle changes in outer hair cells than conventional PTA[24].

Aminoglycosides may initially cause hearing loss at higher frequencies (10-16 kHz)[25], which are outside the range of the standard PTA. However, an effect on speech, in the real life setting, is only seen if progression of hearing impairment to the 0.25-8 kHz frequency range occurs. It is possible that identifying hearing loss earlier, while it is confined to higher frequencies, will allow therapeutic changes to reduce the risk of progression to clinically significant hearing impairment.

The increased risk of hearing loss in CF patients, together with the burden (cost and inconvenience) of the PTA test, means that there is a need for a simple screening test. This should be suitable for use in the clinic, at the bedside, or in the patient's home, without the need for a clinical audiologist. The ideal test would be quick, accurate and could be used repeatedly to detect toxicity early, allowing a change from an aminoglycoside to an alternative (such as colistin). The risk of hearing loss is estimated to be 1.7% per course of aminoglycoside in CF[11] and changing the antibiotic regimen will reduce this risk.

Speech-in-noise testing, involves the participant correctly identifying recorded words or digits, played back by a computer programme, against a background noise. The test is therefore a functional assessment of hearing, in a real life setting. Digit Triplets test is a form of speech-in-noise test that uses spoken digits instead of words. It is a well-established screening test for hearing impairment. The test has been adopted by UK Biobank in its mass population study where 200,000 people have completed the test so far[26]. The test is available to complete online at
http://www.researchmyhearing.org/HearingPortal/Live/Tests/InternetSpeechinNoiseTest

The NIHR Nottingham Hearing Biomedical Research Unit (BRU) has licensed the Digit Triplets test and has produced a modified version – the High Frequency Digit Triplet (HFDT) test. The HFDT test has been compared to the PTA in adults with and without hearing loss and accurately identifies loss at the higher end of the PTA frequency range (2-8 kHz)[27]. A Dutch version of this test, optimised for the same frequencies, has a high sensitivity (95%) and specificity (98%) when compared to the PTA for detecting noise induced hearing loss[1]. Both noise[28] and aminoglycosides[11] cause hearing loss in the 4-8KHz range. The HFDT test differs from the Dutch test only in the use of digits rather than words and in the language used. The HFDT test (on the NIHR Nottingham Hearing BRU servers) is being used in the London Life Sciences Prospective Population Study (LOLIPOP).
https://sites.google.com/site/lolipopstudy/

The Nottingham HFDT test has been validated against the PTA in a population of adults, including (both hearing impaired and normal hearing)[27]. The test asks the participant to correctly identify twenty-five sequences of three digits. If the user is correct, the speech to noise ratio decreases (harder test) and if the user makes a mistake the ratio increases (easier). The first five tests are discarded. The test output is the speech reception threshold (SRT). This is difference between signal and noise which yields 50% intelligibility. Hearing impairment is classified as an SRT that is between -16.5 and -10.0dB (hearing a little low) or between -9.9dB and 0dB (hearing low).

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STUDY OBJECTIVES AND PURPOSE

PURPOSE

To develop a practical screening test for hearing impairment for individuals with Cystic Fibrosis

PRIMARY OBJECTIVE

To compare the High Frequency Digit Triplet test with the Pure Tone Audiogram in individuals with Cystic Fibrosis in the following settings:

(i) In a period of clinical stability
(ii) During a respiratory exacerbation
(iii) In children of primary school age.

SECONDARY OBJECTIVES

- To assess the prevalence of hearing loss in a population with cystic fibrosis.
- To look at factors which may explain the difference in prevalence in a population with variable exposure. This may include genetic and environmental factors.

DETAILS OF PRODUCT(S)

Description

A portable audiometer capable of performing standard and high frequency PTA.

An otoscope

Sennheiser HDA 200 sound-attenuating headphones

A tympanometer with capacity to assess DPOAE amplitudes

All equipment will be purchased through Interacoustics or PCWerth through the Division of Child Health, Obstetrics and Gynaecology. Funding for the equipment was included in the project grant. All devices will be used in accordance with their marketing authorisation.

STUDY DESIGN

STUDY CONFIGURATION

This is a multi-centre observational study with the novel and standard comparator tests performed in random order.

Primary endpoint

- Validation of the HFDT test with the PTA
- Feasibility of performing the HFDT test in symptomatic participants
- Establishment of the youngest age at which the HFDT test is clinically useful

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Secondary endpoint

- The prevalence of significant hearing impairment in a population with CF
- The prevalence of mutations associated with hearing loss in a population with CF.

Safety endpoints

The HFDT test and the PTA are non-invasive tests which are highly unlikely themselves to cause an adverse event. However we recognise that an unexpected finding of hearing loss may cause distress.

In the event of this outcome we will liaise with the participants' usual medical practitioners to enable an expedited referral to formal hearing services.

Stopping rules and discontinuation

Participants may be withdrawn from the study either at their own request or at the discretion of the Investigator. Participants will be removed from the study if they withdraw consent, for example in young children if the testing procedure becomes distressing.

Participants found to have evidence of an ear infection or discharging ears will be investigated and treated for this prior to continuing with the study.

RANDOMIZATION AND BLINDING

Participants will not be randomised as in a real-world setting they would undergo the HFDT-test first. In order to re-create these conditions the HFDT-test will be performed first. The study will not be blinded.

Maintenance of randomisation codes and procedures for breaking code

As the study is not blinded these are not required.

STUDY MANAGEMENT

A Research Management Group, chaired by Prof. Smyth, will meet monthly to review progress against agreed milestones, identify delays and put in place any corrective measures needed. Other members of the group will be the co-applicants from the Hearing BRU and the Research Fellow appointed to undertake data collection. Consultants from collaborating CF centres will be invited to dial in when necessary as will the lay co-applicant. The Research Fellow will also have fortnightly supervision meetings with Prof Smyth, as is the normal practice with Prof Smyth's postgraduate students.

There will be an Independent Study Steering Committee, with a lay member and an independent chair (to be appointed) which will meet six-monthly. The lay advisory group will

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also meet six-monthly. Prof Smyth and the Research Fellow will attend or dial in for part of these meetings, as invited by the chair of each group.

The Chief Investigator has overall responsibility for the study and shall oversee all study management.

The data custodian will be the Chief Investigator.

DURATION OF THE STUDY AND PARTICIPANT INVOLVEMENT

Study Duration: Recruitment began in Winter 2015 for work streams 1 and 2. Recruitment for work stream 3 began in Spring 2015. Data collection has taken longer than originally planned. We now expect recruitment to last until summer 2018. This includes the period of maternity leave for the clinical research fellow. Statistical analysis for each work stream will commence once all data is available.

Participant Duration: For work streams one and three the participant duration will be approximately one hour at the end of a clinic appointment (usually one morning or afternoon). For work stream 2 the duration will be six to eight weeks with one morning or afternoon at the beginning and end of the study period. There will be no obligations on the participant in between these times.

End of the study

The end of the study will be the last paired PTA and HFDT test of the last participant in whichever work stream is the last to complete recruitment.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

Participants will be recruited from paediatric and adult CF clinics at Birmingham Children's Hospital, West Midlands Adult CF Centre, Nottingham Children's Hospital and The Wolfson Cystic Fibrosis Centre (Nottingham). These provide secondary care for all adults in each region and shared care (with local hospitals) for paediatric patients for whom the centre is not the local hospital. Information about the study will be on display in the relevant clinical areas.

The initial approach will be made by letter from the principal investigator at each site following identification of potential participants by review of clinic lists by the clinical research fellow, CF Nurse Specialist or clinic co-ordinator depending on the site.

Two weeks prior to a clinic these patients will be sent an invitation letter and an age-appropriate information leaflet. In the case of paediatric patients a parental information leaflet will also be sent. The leaflets will include contact details for the study team.

For patients who receive their primary care as inpatients, or who are admitted before they are seen in clinic, approach will be made by the clinical team on the ward. The patient (and parent/legal guardian) will have sufficient time to consider the patient information sheet before consent is sought.

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For the control children permission will be sought from the head teachers of local schools to approach parent/legal guardians for consent. Our group have experience of successfully involving children attending local schools in research [29].

The investigator or their nominee (listed on the delegation log), e.g. from the research team or a member of the participant's usual care team will inform the participant or their nominated representative (other individual or other body with appropriate jurisdiction), of all aspects pertaining to participation in the study.

If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the study, the participant information sheets and consent forms but the consent forms and information sheets will not be available printed in other languages.

It will be explained to the potential participant that entry into the research study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

If a potential participant declines we will ask for consent to review their medical notes to ensure that there is no difference in risk factors for hearing loss in the group which declines compared to the participants.

Eligibility Criteria

Inclusion criteria

Work stream 1

- A diagnosis of CF, confirmed by genotype or sweat test, with characteristic clinical features[4].
- Aged 11 years and over.
- Informed consent. Consent will be sought from the parent/legal guardian (11-15, and the young person may give their assent if they wish) and young person (16-18) will consent for themselves.
-

Work stream 2

- As above but the participant has a pulmonary exacerbation requiring intravenous antibiotics.

Work stream 3

- As for work stream 1, defined above.
- CF patients aged 5-10 years
- Healthy control children aged 5-10 years.
- Informed consent from parent/legal guardian with assent from the child.

Genetic Testing

- Informed consent
- Diagnosis of CF as above

Exclusion criteria

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- None. In individuals with a hearing aid, we will perform PTA and HFDT tests without the aid.
- Participants found to have an ear infection or discharging ears will have this investigated and treated prior to continuing with the study.

Expected duration of participant participation

Study participants in work streams 1 and 3 will be participating in the study for up to half a day. For work stream 2 this will be half a day at the beginning and end of a six-to eight week period. There will be no obligation on the participants in between those times.

Removal of participants from therapy or assessments/Participant Withdrawal

Participants may be withdrawn from the study either at their own request or at the discretion of the Investigator. Participants will be removed from the study if they withdraw consent, for example in young children if the testing procedure becomes distressing. The tests themselves however are non-invasive and we do not anticipate a high drop-out rate, particularly as the participant will only be needed for one half-day at most in work streams 1 and 3.

In work stream 2 we anticipate a higher drop-out rate at baseline – if the participant feels too unwell to continue the test. At follow-up there is a reasonably high non-attendance rate for CF clinics. If a participant does not attend their follow-up clinic they will be contacted by letter and by text message (if this is the normal contact method for the participant in question) to ascertain whether they will have the test at their next appointment.

The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Informed consent

All participants will provide written informed consent. For participants aged 5-15 years, parental/legal guardian consent will be sought – they may give their assent if they wish. For participants 16-18 they will provide their own consent. If there is conflict between the participant and the person with parental responsibility the young person will not be entered into the study.

The Investigator will explain the details of the study and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. Where the Participant is a child under age 16 an age appropriate Participant Information Sheet will be provided. The Investigator will answer any questions that the participant has concerning study participation.

The Informed Consent Form will be signed and dated by the participant (or parent/legal guardian) before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one will be kept by the Investigator, and a third will be retained in their hospital records.

Should there be any subsequent amendment to the final protocol, which might affect a

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Participant's participation in the study, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

The process for obtaining participant informed consent or assent and parent / guardian informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant or other legally authorised representative shall both sign and date the Consent Form before the person can participate in the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to the participant that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

We will inform the participant's General Practitioner of their inclusion in the study, and will specifically seek consent for this.

For patients who decline to take part written consent will be requested to review their notes to assess for any risk factors for hearing loss, including aminoglycoside exposure. This is to assess whether there are any systematic differences between participants and non-participants.

Those approached to participate in the study, whether they consent or decline, will be given the contact details for the Lay Representative with an invitation to provide feedback on the recruitment procedure. This information will be fed back anonymously at the Study Steering Group Meeting.

STUDY TREATMENT AND REGIMEN

Identifying and Approaching Participants

Clinic lists will be reviewed two weeks in advance by the clinical research fellow (where they are part of the clinical team), the CF Nurse Specialist or the Clinic Co-ordinator. Appropriate patients will be sent a letter of invitation an information leaflet (or in the case of child participants an age-appropriate sheet with a parental information sheet) and a consent form. This will give potential participants time to read the information and consider it before coming to clinic.

When the patient arrives at clinic they will be asked if they wish to participate in the study. If an interest is expressed the clinical team will introduce the patient to the research team.

For participants in work stream 2 the initial approach will be in clinic as described above with confirmation of the wish to participate at the start of a course of intravenous antibiotics. Where patients are admitted to the ward without previous clinic discussion the clinical team will discuss the study and if the patient is interested provide an information sheet. The patient/legal guardians will have sufficient time to consider the information prior to consent.

For work stream 3 participants will be approached in clinic as above or on the ward if they come in for an admission. For participants approached during an admission the approach will be made at the beginning of the admission, to allow time to consider the patient

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information sheets. Testing would then be performed towards the end of the admission when the participant is clinically better.

Healthy control children will be recruited from local schools. The Headteacher will be asked for consent to approach children. Information will be given to the children at school. They will then be given a pack to take home containing a letter of introduction, a parent and an age-appropriate information sheet, a hearing questionnaire and a consent form. The parents will have an opportunity to contact the research team for further information prior to the return of the research team to the school for testing. Return of the signed consent form will be required for participation. Testing will take place at school during school hours where possible. If this is not feasible testing will take place at Queen's Medical Centre during school holidays and will be done by the clinical research fellow or their nominated deputy.
A dedicated twitter account will be created to provide information about the study (drawn from the patient information sheets), recruitment updates and answer questions where required.

Participant Testing

This will be performed by the clinical research fellow or their nominated deputy.

Preparation
The participant will be taken into a quiet room and any further questions answered. A measurement of background noise will be taken at the beginning and end of testing using a calibrated sound level meter. For children a parent may come into the room and help the participant understand what they have to do but they will not be able to help with the performance of the test itself.

Questionnaire and Examination
A baseline questionnaire (part of the CRF) will be asked of the participant and/or someone with parental responsibility to look for any prior risk factors for hearing impairment beyond exposure to aminoglycosides.

The participant's external ear will then be examined and otoscopy performed to ensure there is no blockage of the ear canal with wax and that the tympanic membrane appears healthy. Participants found to have discharging ears or evidence of an ear infection will have this investigated and treated prior to continuing with the study in accordance with standard NHS clinical care.

Tympanometry
Tympanometry will be performed to differentiate between sensorineural hearing loss (associated with aminoglycoside use) and conductive hearing loss (associated with middle ear disease). The procedure will be done in accordance with the British Society of Audiologist (BSA) 2013 Guidance, "Recommended Procedure – Tympanometry" (BSA 2013 http://www.thebsa.org.uk/wp-content/uploads/2014/04/BSA_RP_Tymp_Final_21Aug13_Corrected24June14.pdf).

Pure Tone Audiogram
The participant will undergo a PTA according to BSA standards[30]. The effects of any background noise will be reduced by delivering auditory stimuli using headphones with effective sound-attenuating cushions (Sennheiser HDA200). The audiometer used will be capable of performing an HF-PTA. High Frequency Audiometry will be assessed in participants in work streams 1 and 2. It may be necessary to modify this in participants in

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work stream 2 to reduce the test burden where clinically appropriate. If possible modified high frequency audiometry will be performed in work stream 3 participants.

High Frequency Digit Triplet Test
Using the same headphones the participant will perform the HFDT on a laptop computer (Lenovo ThinkPad L540). The participant will be presented 25 sets of three digits in a varying level of background noise. The task is to correctly identify each digit. With correct identification the signal to noise ratio (SNR) decreases (test gets harder), if the digits are incorrectly identified the SNR increases (test gets easier). Patients in workstreams 1 and 3 will undergo the HFDT-test twice to look for intra-individual variability. This will not be done in workstream 2 due to concerns about the test burden in unwell patients.

Distortion Product Otoacoustic Emissions
This will be carried out in participants in all work streams according to the manufacturers instructions.

Saliva and Blood sampling
Participants who consent to genetic sampling will be asked to provide a saliva sample in an Oragene pot (DNA Genotek), filling the pot up to the pre-specified line.

Participants who consent to blood sampling will have a 1-2ml sample taken at their next clinically indicated blood test.

Post-testing
Participants will be asked:
(a) Do you find the High Frequency Digit Triplet Test acceptable?
(b) Would you be happy to do the High Frequency Digit Triplet test at your annual review?
(c) Would you be happy to do the High Frequency Digit Triplet test at the start of a course of intravenous antibiotics (IVs)?
A final sound-level recording will be checked then the room and equipment will be cleaned according to local protocols to minimize the risk of cross-infection.

Healthy Control Children
These participants will be reviewed at school during school hours. Their parent/legal guardian will have previously returned the signed consent form and hearing questionnaire (see Appendix 1). They will have an ear examination, tympanometry, the HFDT-test, a PTA (if possible with high frequencies – assessed at time of testing depending on feasibility), DPOAE and a repeat HFDT test performed by the clinical research fellow or their nominated deputy.

Work stream 2
For participants in work stream 2 we will offer testing within the first four days of the admission as after this they are more likely to be clinically improved. A record will be kept of how many doses of antibiotics participants have received and their symptom score on the day of testing. If the numbers are large a planned sub-group analysis of these participants' results will be completed to assess for any differences in their hearing tests.

Follow-up Testing

For work stream 2 a second set of paired tests (also administered in random order – see above) will be performed at their routine follow-up appointment after discharge (usually 6-8 weeks). These will be done according to the protocols above.

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Review of Notes

This will be done by the clinical research fellow (or their nominated deputy) who will have an honorary contract with each trust and work in the clinical team. Clinical data on prior aminoglycoside exposure will be obtained from review of the clinical notes and the local clinic registry. These data will be recorded on a paper clinical report form (CRF) and later transferred to a password protected database. Demographic data, genotype, antibiotic sensitivities and characteristics of the organism most recently derived from the sputum and a list of current and past treatments (including other ototoxic treatments) will also be recorded. This data will also be collected for patients who decline to be involved in the main study but consent for review of their notes.

Involvement of the Clinical Team

The results will be fed back to the participant at the time of testing. They will be documented in the clinical notes and a letter will be sent to the clinical team with the results, including a print-out of the PTA. In the case of participants in work stream 2 if hearing loss is found this will be communicated with the clinical team immediately so that antibiotic choice can be altered if this is clinically appropriate.

Concomitant Treatment

There will be no restriction on concomitant treatment as this will not affect the validity of the test. The participant will be treated as per their clinical requirements. The only caveat to this is that antibiotic choice may be altered, at the clinician's discretion, if the tests show any evidence of hearing loss. All concomitant treatment will be documented on the CRF.

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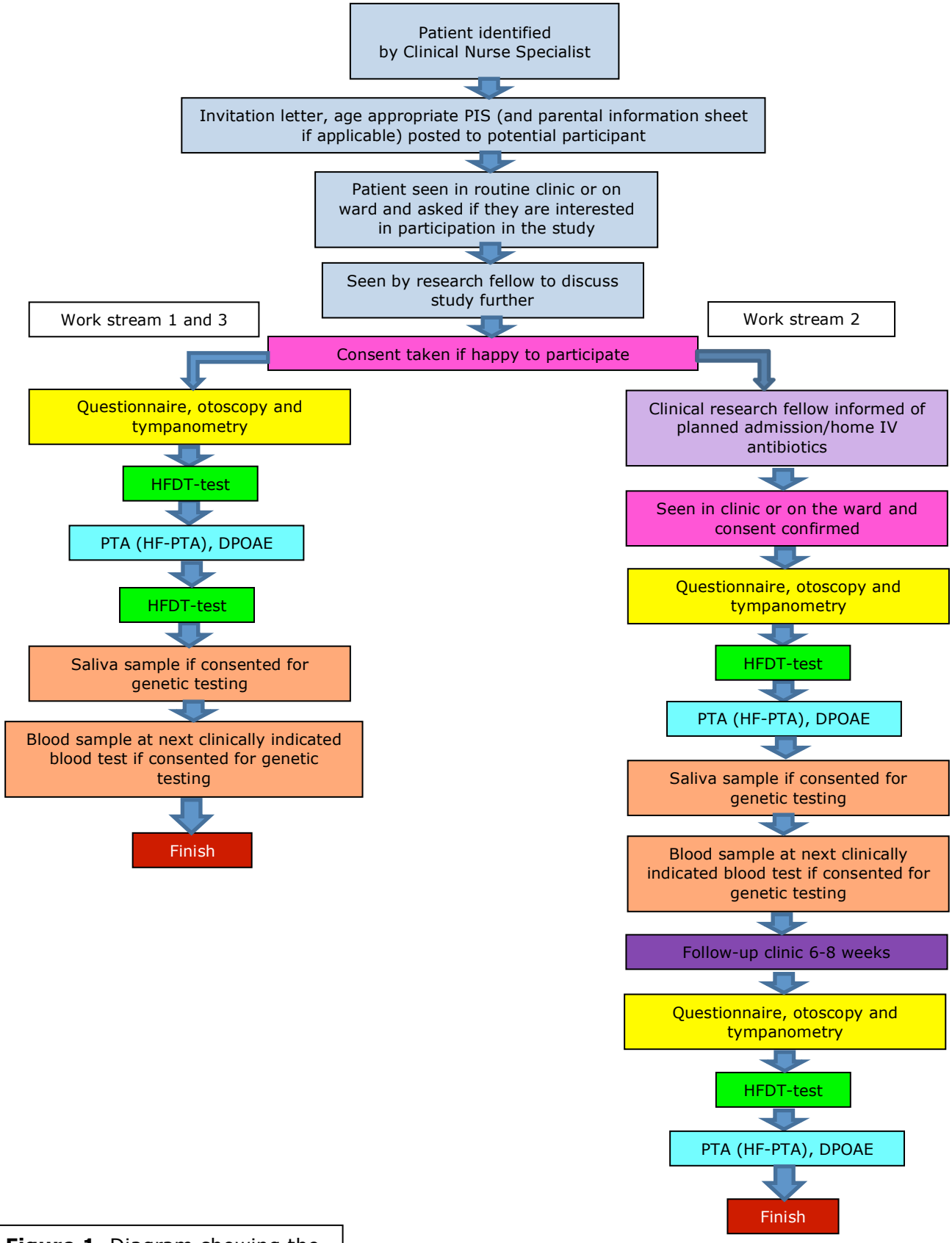

Figure 1. Diagram showing the commitments of CF participants in work streams 1, 2 and 3.

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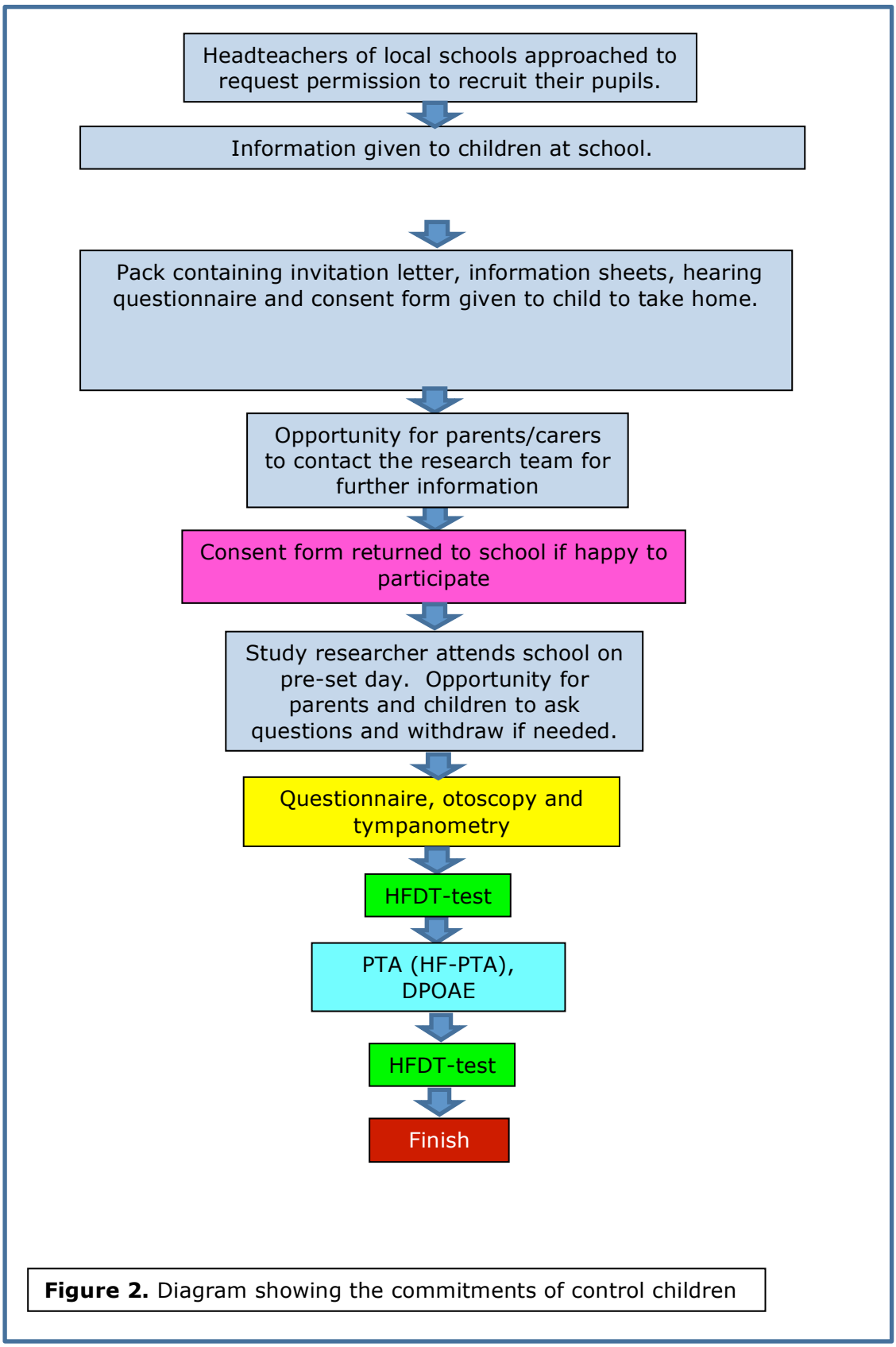

Figure 2. Diagram showing the commitments of control children

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Compliance

As the tests for work stream 1 and 3 will be done on the day of consent with no need for follow-up we do not feel that compliance will be an issue. Should a participant drop out part-way through testing, for example if a child is too distressed, this will be recorded as evidence of test feasibility and data already collected will be analysed.

For work stream 2 there may be difficulties in achieving the follow-up tests if the participant fails to attend the follow-up appointment. In this situation the participant will routinely be sent a further appointment and the follow-up tests will be performed at this visit with a record made on the CRF about any time delay. The participant will be contacted by post and text message (if this medium is routinely used to communicate with the participant) to ask them to come to clinic to undergo their follow-up tests.

Participants who still do not attend will be assessed on their initial tests only.

Criteria for terminating study

Due to the very low chance of any adverse events during the study there will be no interim analysis.

If one centre is struggling to recruit patients then this will be reviewed at the Research Management Group.

For individual participants discontinuation will be reviewed on an individual basis.

TRANSPORT AND STORAGE OF THE TISSUES

Saliva samples for genetic analysis will be collected in self-contained standard salivary DNA sampling kits (Oragene®•DISCOVER from DNA Genotek, Ontario, Canada). This is a non-invasive method that involves spitting into a receptacle. The samples will be collected by the clinical research fellow and transported in a secure manner to HTA compliant laboratory facilities in the Division of Child Health and Obstetrics and Gynaecology. The samples will be stored at room temperature for batch analysis.

Blood samples will be collected in EDTA tubes. One to two millilitres will be collected. These will be transported in a secure manner in sealed containers to the laboratory facilities in the Division of Child Health and Obstetrics and Gynaecology. The samples will be frozen to -80 degrees centigrade and stored in an HTA approved freezer prior to batch analysis.

The samples will be labelled with the study code, the participants' unique study identifier, and DOB in line with HTA guidance. This will allow linkage to the consent form and clinical data. A master database will be held separately by the Clinical Research Fellow in a password protected, encrypted file.

After genetic analysis, any remaining DNA, saliva or blood samples will be stored within the University of Nottingham Research Tissue Bank for future research (DI Prof Jim Lowe-Licence Number 12265) if participants are agreeable and sign the optional clause on the consent form. Where participants do not agree to the future use of the samples they will be destroyed in accordance with the Human Tissue Act, 2004.

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LABORATORY ANALYSES

Mitochondrial DNA will be extracted from the saliva samples using Oragene DNA purifier. From the blood samples it will be extracted using DNeasy Blood and Tissue Kit (Qiagen). The MTRNR1 gene will amplified using classical PCR with primers as previously described [31]. This work will be done in laboratory facilities in the Division of Child Health and Obstetrics and Gynaecology. The DNA will be sequenced at the Biopolymer Synthesis and Analysis Unit at the University Of Nottingham to identify mutations that may be related to hearing loss.

Genetic analysis will be optional and a separate consent clause will be required on the consent form.

STATISTICS

Methods

Work stream 1:
We will calculate the sensitivity and specificity of the HFDT test treating PTA as a 'gold standard' and determine confidence intervals (using Wilson score method). In addition, in order to compare estimated sensitivity and specificity of the HFDT test with PTA, a latent variable model will be fitted and receiver operating characteristic curves obtained, from which sensitivity and specificity of the two tests can be compared. Correlations between the HFDT test SRT and PTA (including HF-PTA) thresholds and correlations between the HFDT test and DPOAE amplitudes will be calculated for reporting. We will report prevalence rates for hearing loss and the presence of genetic mutations known to be associated with hearing loss.

Work stream 2:
We will report the number of participants and the number of tests where the HFDT test was feasible in the participant about to receive intravenous antibiotics. The feasibility of the HFDT test and PTA at baseline will be compared based on the numbers completing each test using logistic generalised linear mixed effects modelling (GLMM). As for work stream 1 sensitivity and specificity of the HFDT relative to PTA will be provided, with confidence intervals, at both baseline and follow up. Reliability of the HFDT test and PTA between baseline and follow up will be compared using a random intercept mixed-effects model. Additionally the latent variable model outlined in work stream 1 will be extended by modelling the pre-/post-treatment effect allowing receiver operating characteristics of the two tests to be compared both at baseline and follow up. Finally the correlation between HFDT SRT and PTA thresholds will also be reported at baseline and follow-up.

Work stream 3:
As with work stream 2, logistic regression methods will be used to assess the feasibility of each of the tests for CF patients *vs*. controls, and as age varies. This analysis will be based on the numbers completing each test and will allow us to estimate the minimum age at which 80% are able to perform the test. Comparisons of correlations between groups and by age will be performed using Fisher's *z*-transformation of Pearson's correlation. These correlations will also be compared with those reported from work streams 1 and 2. Finally, the receiver operating characteristics will be further explored using the latent variable approach described under work stream 2, but extended to model age effects in addition to modelling patient group effects. We will use the R statistical package (http://www.r-project.org/) except for the latent variable model where SPSS Amos will be used to fit SEM.

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Statistical analysis will be conducted by the clinical research fellow with support from Prof Smyth and the study Statistician (M. Edmonson-Jones).

Since the chance of an adverse event is very low and the participants will not undergo an intervention no interim analyses are planned.

Sample size and justification

Work stream 1:
In clinical practice the High Frequency Digit Triplet Test (HFDT test) will be used as a screening tool to identify those patients with CF in whom a full PTA is indicated. These patients will have an SRT with a less negative value than the threshold for hearing impairment of -16.5dB. We expect that the sensitivity and specificity of the HFDT test, in patients with CF, are similar to the reported sensitivity (95%) and specificity (98%) of a similar speech-in-noise test in the general population[1]. However, we have designed the study to have 80% power to detect a lower confidence limit of 80% sensitivity i.e. there is an 80% chance that the lower confidence limit for sensitivity is 80% or greater.

For work streams 1 and 2, we have used the method for calculating sample size described by Flahault *et al*[2], with the statistical package GPower 3.1.3. Using this method, the sample size depends on the prevalence of hearing loss. In a previous review[3], we have identified those studies which measure the prevalence of hearing loss in CF and which are of acceptable quality (study population is defined and the definition of hearing loss is clearly described). However, the studies show considerable heterogeneity which may be related to the population described (less aminoglycoside use in earlier studies from the 1970s and 1980s) and the precise definition of hearing loss used. They give a range of prevalence values from 1% to 51%. We have used a weighted mean of the prevalence values from the individual studies (27% prevalence), weighted by the number of participants in the study.

This gives a sample size of 111 participants for work stream 1. We aim to enrol 117 participants in work stream 1 in order to have 111 who will complete both the HFDT test and PTA test successfully (5% drop out). A low drop out is expected as the tests are simple and quick to undertake and they will be performed during a routine clinic visit. This will also take into account participants who are found to have conductive deafness.

Work stream 2:
For work stream 2, the test is performed under more challenging conditions. The participant may be tired and short of breath. Coughing may prevent participants responding to the speech or tone stimuli. This will affect the feasibility of the test (some participants will not be able to complete it) and also its specificity (participants may "fail" the HFDT test due to coughing or fatigue, in spite of having normal hearing). The same factors may lead to participants being unable to perform a PTA. We aim to detect the same lower confidence limit for the HFDT test as in work stream 1 (80% sensitivity) with the same power (80%). We will increase the number of participants enrolled by 20% to allow for more participants not completing the test (compared to work stream 1) and so we will approach 133 patients to achieve 111 participants successfully completing both tests.

Work stream 3 – sample size:
We will assume the test is not feasible, due to limited performance, in 20% of children in both the CF and control groups. (Feasibility will vary with age. Most 10 year olds but only some 5 year olds will complete the test). We expect that few children in this young age group have

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hearing impairment and so we have powered this work stream on the feasibility of the HFDT test, rather than the sensitivity of the test to detect abnormal hearing. The sample size required to test for a difference in test completion rates from 95% for older children to 80% for younger children with 80% power is 134 (including those who complete the test and those who do not, as both groups contribute data). The overall sample size will be 134. Sample size was calculated using GPower 3.1.3. Therefore, for work stream 3, we will study 11 children with CF and 12 control children for each age from 5 to 10 years (66 CF and 72 controls). This gives a total sample size of 138 participants.

Assessment of efficacy

This study is not a clinical trial.

In both work streams 1 and 2, we will measure the sensitivity and specificity of the HFDT test when compared to PTA as the gold standard. We have designed the study to have 80% power to detect a lower confidence limit of 80% for sensitivity.

Work stream 3, will establish the feasibility of the HFDT test in children aged between 5 and 10 years. We will test for a difference in test completion rates from 95% for older children to 80% for younger children with 80% power.

Assessment of safety

If a patient is found to have abnormal hearing then they will be informed. Contact will be made with their usual CF physician and GP. If hearing loss or other abnormalities are found the patient will be referred for a formal audiological opinion.

In work stream 2, where the HFDT test suggests significant hearing loss, the responsible consultant will be informed and an alternative to an aminoglycoside antibiotic prescribed, if deemed clinically appropriate.

In any work stream, if the participant becomes upset during the testing procedure the test will be discontinued.

If any mitochondrial DNA mutation, known to be associated with sensitivity to aminoglycoside induced hearing loss is found, that individual's consultant will be informed and the information shared with the participant.

Procedures for missing, unused and spurious data

A trained audiologist will review the data generated to identify any outliers which will then be further evaluated. An independent third party will cross-check 10% of the data to ensure accuracy.

Definition of populations analysed

Full Analysis set: All participants, who completed at least one hearing test (work stream 1) or attempted at least one hearing test (work streams 2 and 3).

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Per protocol set: All participants, who completed both the baseline and follow up hearing tests (work stream 2). All study participants who attempted at least one hearing test (work streams 1 and 3).

Protocol violations will be assessed by the clinical research fellow with oversight from the Research Management Group.

ADVERSE EVENTS

As this study is an evaluation of a diagnostic test and not a clinical trial, we do not expect any adverse events, no adverse event data will be recorded. However a number of procedures are in place to ensure the safety of participants (see assessment of safety).

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The study will not be initiated before the protocol, informed consent forms and participant information sheets have received approval / favourable opinion from the Research Ethics Committee (REC) and the respective National Health Service (NHS) Research & Development (R&D) department. Should a protocol amendment be made that requires REC approval the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the Department of Health Research Governance Framework for Health and Social care, 2005.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent or assent and parent / guardian informed consent will be in accordance with the REC guidance and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant or other legally authorised representative shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the study Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

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The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Case Report Forms

Each participant will be assigned a unique study number, allocated at time of consent, for use on CRFs, other study documents and the electronic database. The documents will also use the date of birth (dd/mm/yy).

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number and Participant Study Number (the Study Recruitment Log), to permit identification of all participants enrolled in the study, in accordance with regulatory requirements and for follow-up as required. Access to the CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Study Delegation Log.'

Clinical data including demographics, prior aminoglycoside use, sputum microbiology and sensitivities, allergies, current medical therapies, previous ototoxic treatments and significant illnesses will be collected by the fellow or a delegated member of the research team. These data will be recorded on a paper CRF and later transferred to a password-protected database stored on a University of Nottingham computer.

All paper forms shall be filled in using black ballpoint pen (except audiogram data which will be filled in with blue and red pens as per convention). Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated. The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF. An independent third party will cross-check 10% of the data to ensure accuracy. The paper forms will be stored in a locked filing cabinet in a secure office.

Sample Labelling

Each participant will be assigned a unique study number for use on the samples, other study documents and the electronic database. The samples will also be labelled with the study code and the participants' date of birth (dd/mm/yy) in accordance with HTA guidance.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only study staff as listed on the Delegation Log shall have access to study documentation other than the regulatory requirements listed below.

Direct access to source data / documents

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The CRF and all source documents, including progress notes and copies of laboratory and medical test results, shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (e.g. DH, Human Tissue Authority).

DATA PROTECTION

All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The CRF will only collect the minimum required information for the purposes of the study. CRFs will be held securely, in a locked room, or locked cupboard or cabinet at each site. Access to the information will be limited to the study staff and investigators and relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

Study forms and all other documents (except the consent form) will be anonymised and be identified only by the participants unique study number and date of birth as described earlier. The master database which links the participants' names to the study numbers will be held separately in a password-protected, encrypted file at the University of Nottingham.

Information about the study in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for study participants and study staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

STUDY CONDUCT

Study conduct may be subject to systems audit of the Study Master File for inclusion of essential documents; permissions to conduct the study; Study Delegation Log; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of study materials and equipment calibration logs.

STUDY DATA

Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and

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procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Study Coordinator/Academic Supervisor, or where required, a nominated designee of the Sponsor, shall carry out monitoring of study data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition the subsequent capture of the data on the study database will be checked. Where corrections are required these will carry a full audit trail and justification.

Study data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Study Master File and study documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all study databases and associated meta-data encryption codes.

DISCONTINUATION OF THE STUDY BY THE SPONSOR

The Sponsor reserves the right to discontinue this study at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Study Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

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We will disseminate the findings of this work through presentations at international scientific meetings, publication in peer reviewed journals and through the PhD. thesis of the research fellow. Participants will not be identifiable in any published data.

We have invited key members of the CF patient community to form an advisory group to help us design and implement this work. We will also ask the advice of this group on the most effective ways of disseminating the research findings to the patient community amongst study participants and more widely. We will be guided by the advisory group on the best way of doing this, however we anticipate using some of the following approaches:

- summary written feedback to participants
- publishing results on a study specific website
- publication via the UK CF Trust website and newsletter
- social media (e.g. CF Trust forum)
- feedback at the annual CF parents' conference

Both locally and nationally we will work with the children and young persons group of the Clinical Research Network (CRN) East Midlands. We will explore the use of media such as video clips from research participants. As part of our dissemination strategy we will work with the PPI leads at the CRN Children's Theme co-ordinating centre in Liverpool; together with PPI leads from CRN East and West Midlands. We will also work with leads at the collaborating Trusts.

Our group have pioneered an innovative approach to engaging with the patient community to share the findings of research in the CF field. CF Unite (http://cfunite.org) is a web based programme which allows patients to have a dialog with researchers. This is achieved through online conferencing, without the need for people with CF to meet in person (which would risk cross infection).

USER AND PUBLIC INVOLVEMENT

In the last ten years a small number of patients attending the paediatric CF clinic in Nottingham have suffered sensorineural hearing loss as a result of exposure to aminoglycoside antibiotics. Whilst the numbers are too small for a formal focus group the concerns of these patients and parents have led us to highlight this research topic and develop the current application. Although numbers with severe sensorineural hearing loss (diagnosed because of clinical symptoms) are low we expect that mild to moderate hearing loss in our study will be more common. This should be in line with our estimate of 27% for the prevalence of hearing loss of any severity. It is through concerns expressed by parents of children who have suffered hearing loss that we have included work stream 3 in the study. One of the adult patients on our lay advisory group has personal experience of aminoglycoside-induced hearing loss.

One of our co-applicants (Zoe Elliot) is the parent of twins with CF. Her children have participated in clinical research in CF. Zoe has a career in marketing and business development. She has previously helped with publicity, through news items, describing her experience of clinical research studies which have been run by our group. These news items have featured in television and print news. She will be a member of the planned lay advisory group, though to avoid any conflict of interest there will be an independent chair. Zoe has reviewed the application and has suggested changes and enhancements, particularly to the dissemination section.

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The Young Person's Advisory Group (YPAG) of CRN East Midlands has reviewed the Patient Information Sheets and suggested changes including splitting the 5-10 year old sheet into 5-8 and 9-10 year old sheets, *"Shorten age group for example, 5 to 8 years old, 9-11 years old, 12-15 years old and 16 +," a*s they felt that information sheets designed for five year olds were too babyish for ten year olds. Feedback from adult patients on the adult patient information sheet was also taken into consideration. The GP letters were reviewed by a GP colleague to ensure they were pitched at an appropriate level.

We believe that patient and public involvement has already benefitted this research by helping us to identify and refine the research question and put together a comprehensive and detailed grant application. We will take advice from the lay advisory group which will help with presentation and discussion of our research findings to ensure the patient and parent perspective is not lost. Finally we will rely heavily on the lay advisory group for advice on the best method of disseminating the research findings to parents and patients with CF – both locally among research participants and to the CF community nationally and internationally.

We will provide training and support to the lay advisory group through PPI leads at the CRN Children's Theme co-ordinating centre in Liverpool (Jenny Newman); CRN East Midlands (Kirsty Widdowson) and the CRN West Midlands (Claire Callens). We will also work with PPI leads at the Heart of England NHS Foundation Trust (Simon Jarvis), Birmingham Children's Hospital (Jeanette Vie) and Nottingham University Hospitals' NHS Trust (Jane Flewitt).

STUDY FINANCES

Funding source

This study is funded by an NIHR grant, PB-PG-0213-30055.

Participant stipends and payments

Participants will not be paid to participate in the study. Travel expenses will be offered for any hospital visits in excess of usual care.

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SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name)_____

Signature:_____

Date: _____

Co- investigator: (name) _____

Signature:_____

Date: _____

Study Statistician: (name)_____

Signature:_____

Date: _____

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