Host immune response point-of-care testing for children and adults presenting to primary care with acute upper respiratory tract infection: a mixed-methods feasibility study (RAPID IMMUNE TEST)

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Background

Overview

This study will investigate the feasibility of primary care use of FebriDx®, a host response point-of-care test (POCT^{HR}), for children and adults with acute upper respiratory tract infections (URTIs) in primary care. The study will assess whether FebriDx® changes clinician pre- and post-test diagnostic confidence. Diagnostic accuracy of FebriDx® in primary care will be evaluated, and clinician and patient perceptions of test use will be explored. In combination with results from the current NIHR SPCR funded PREFIX study (from collaborators Francis and Wilcox at the University of Southampton, investigating the value of FebriDx® in children and adults with acute lower respiratory tract infection), results of this study will inform the design of a future randomised controlled trial (RCT) of the same POCT for respiratory tract infections (RTIs) in primary care, with the ultimate aim of establishing whether such POCTs can safely reduce the use of antibiotics in primary care.

Antimicrobial resistance (AMR)

AMR was declared one of the top-10 threats to global health by the World Health Organisation in 2019. Urgent, coordinated transnational and cross-disciplinary action is needed. In 2016, the UK commission 'Review on Antimicrobial Resistance' estimated that by 2050 10 million lives per year would be at risk due to drug resistant infections globally. This is alongside a cumulative cost of $100 trillion[1]. The review predicted that by 2050, AMR will be responsible for more deaths globally than cancer. Recent estimates from the landmark Global Research on Antimicrobial Resistance report confirm that antibiotic-resistant infections are already a leading cause of death worldwide; in 2019, 1.2m people died from antibiotic-resistant bacterial infections[2]. Optimising antimicrobial use is high on the global agenda. This includes both ensuring that those who require antibiotics receive appropriate treatment, as well as reducing the unnecessary use of antibiotics to help combat AMR. Overuse of antibiotics has multiple detrimental effects for both individuals (side-effects and AMR) and the wider population (medicalisation, financial costs and AMR)[3]. Public concern saw AMR voted 'one of the greatest issues of our time' in the ongoing UK Longitude Prize competition (https://amr.longitudeprize.org/).

Antibiotic overprescription in primary care: the problem

The majority of NHS antibiotics are prescribed in primary care[4] and modelling suggests that at least 20% of these prescriptions are unnecessary[5]. There is a critical need to safely reduce antibiotic prescribing, given the established link between antibiotic exposure and the development of antibiotic resistance[5]. This is true for both upper and lower acute RTIs, the problems most commonly managed by health services internationally[6] and the conditions most associated with inappropriate antibiotic prescriptions, including sore throat, cough, sinusitis and acute otitis media[7, 8].

Antibiotic treatment is only effective in susceptible bacterial infection but symptoms and signs are not overly helpful in distinguishing viral from bacterial RTIs; for example, Professor Hay's group has demonstrated that clinical presentation does not differentiate viral from bacterial URTI in children[9]. Acute RTIs commonly arise from viral infection, yet antibiotics are prescribed in up to 67% of UK RTI consultations[10], with prescribing often attributed to clinical uncertainty regarding microbiological diagnosis and concerns regarding disease prognosis, which leads to defensive ("just-in case") care[11]. Increased certainty regarding the microbial aetiology of acute RTIs would help to target antibiotic treatment to susceptible bacterial infections. However, historically diagnostic microbiological services have been too slow to produce results to influence primary care management.

Novel point-of-care tests

The 'Review on Antimicrobial Resistance' placed great emphasis on point-of-care tests (POCTs) that distinguish viral from bacterial infection as a solution to AMR[1], with Chair Jim O'Neill recommending that "no antibiotic should be prescribed without a test"[1] in the initial report and despairing of the lack of progress at the five year review. Manufacturers of POCTs are starting to bring their products to market and the Longitude Prize group is currently seeking to find a POCT that can be distributed across the globe to reduce antibiotic prescribing.

POCTs might be provided to primary care, including general practices, pharmacies and Acute Respiratory Infection (ARI) hubs in the near future[12-15]. This may be accelerated by commercial agendas, NHS pressures and the widespread uptake of testing for SARS-CoV-2 infection during the COVID-19 pandemic and resultant potential change in public attitude towards testing[16]. Most POCTs have not, however, been adequately assessed in primary care, where the patient population is different, with typically less severe infections and thus differing immune responses and spectrum and load of pathogens, hence POCTs must also be evaluated in primary care.

As we describe in a recent editorial[17], there are multiple questions to answer. Fundamentally, we need evidence of clinical effectiveness (such as reduction in antibiotic prescribing or improved/non-inferior patient outcomes), long-term cost effectiveness and safety in practice in primary care before widespread roll-out is even considered. Accuracy, including sensitivity, specificity, positive predictive value and negative predictive value, must be considered in this specific clinical setting. Additionally, *how* POCTs might influence clinician/patient behaviour and antibiotic prescribing in primary care should be explored, given the antibiotic prescribing decision is being made in the context of a conversation between clinician and patient. Use of POCTs in primary care may have benefits, such as reducing antibiotic prescribing and AMR, and reducing individuals' exposure to other antibiotic harms. POCTs may increase clinician and patient diagnostic confidence, potentially reducing repeat consultations during the same illness, and they may increase detection of bacterial infections requiring antibiotics, thus improving clinical outcomes. However, these benefits may not be realised in practice and, indeed, there may be negatives to using POCTs. POCTs may medicalise minor illness, increasing health seeking behaviour and test demands for future illnesses. These medicalisation effects need to be taken into consideration when assessing long-term cost effectiveness. Furthermore, we must ensure that POCTs don't miss significant bacterial infections, worsening clinical outcomes. Additionally, the day-to-day time and cost requirements for an already overstretched primary care system may be prohibitive.

Translating innovative developments from industry into benefits for patient care and wider global issues requires equitable partnerships at the industry/academia interface. Industry is driving pioneering developments in the field of AMR and new diagnostics, yet the academic and clinical community is rightly wary of adopting new technology, such as POCTs, without evidence to support their use – and evidence for the use of POCTs can only be generated from well-designed, independent clinical studies. However, commercial and system pressures may lead to technological implementation, often prior to evidence regarding effectiveness, safety or costs. A recent example is advocacy of POCT^{RM} for sore throat in pharmacies[12, 18] to the concern of professionals in the clinical academic community[19]. A balance must be struck between striving for innovation and the necessity to generate robust evidence.

Broadly speaking there are two types of POCT for diagnosing infections. Firstly, 'microbiological' POCTs (POCT^{RM}), which test for the presence of specific pathogens. Secondly, 'host response' POCTs (POCT^{HR}), which measure and attempt to distinguish host viral from bacterial immune responses.

(i) Tests to identify the pathogen ('microbiological' POCTs, POCT^{RM})
Several antigen and multiplex molecular tests are available to detect a range of upper respiratory tract microbes. They enable clinicians to take a swab from the nose or throat, place the swab into an analyser and, within twenty minutes to two hours, see a list of potential respiratory pathogens which are present or absent in the sample[20-22].

Using funding provided by the NIHR SPCR (grant #391), Professor Hay's group conducted one of the first feasibility studies of a multiplex POCT^{RM} in primary care, testing for 19 viruses and four atypical bacteria via a nose and throat swab. The results, published in *Family Practice*[23], were encouraging, showing clinicians found the POCT acceptable, useful and that it increased their diagnostic confidence and reduced predicted antibiotic benefit. POCT use was limited by time taken for test results and the absence of testing for typical respiratory bacteria (due to being commensally carried in the upper respiratory tract). Findings leveraged £1.85M NIHR EME funding for a primary care randomised efficacy trial of the same POCT (https://fundingawards.nihr.ac.uk/award/NIHR131758, RAPID-TEST RCT, with applicants Brown, Muir and Clarke involved). The trial includes a mixed methods investigation of microbial, behavioural and antibiotic mechanisms influencing the primary and key secondary outcomes: same-day antibiotic prescribing for children and adults presenting to primary care with RTIs, patient reported symptom severity and patient reported antibiotic consumption.

However, there are challenges with POCT^{RM}s. The upper respiratory tract (throat/nose) is easily accessible for sampling, but the clinical significance of detected microbes is not understood, as bacteria and viruses causing common URTIs also harmlessly inhabit the upper respiratory tract ('commensals'). The recent "strep A crisis" in the UK illustrates this well: following reports of increased scarlet fever incidence and deaths from invasive group A streptococcal infection, "strep A" POCTs were suggested as a means to identify children needing antibiotics. However, commensal strep A carriage is present in up to 15% of children[24]. Whilst Professor Hay's group has demonstrated that some microbes swabbed from the throat in children may be aetiological in URTI[25], another recent systematic review[26] has found an absence of evidence as to whether these microbes are related to disease outcomes or response to antibiotics. In addition, it is noteworthy that upper respiratory tract swabs taken from symptomatic people by trained clinicians have zero pathogen detection in up to 28% of cases[27, 28].

(ii) Tests that assess the host immune response ('host response' POCTs, POCT^{HR})
Clinically significant infection triggers a host inflammatory/immune response, which differs depending on whether the infection is bacterial or viral. POCT^{HR} measure host immune proteins ('biomarkers') as proxy markers of infection aetiology. This immune-based approach has a key advantage over POCT^{RM}; results are not confounded by detection of commensal bacteria or viruses that are colonising the respiratory tract, but which are not causative in the current infection. The POCT^{HR} approach is also theoretically robust to rapidly evolving pathogens, which may not be detected by current POCT^{RM}.

Single POCT^{HR} using c-reactive protein (CRP) have been evaluated in randomised controlled trials and shown to reduce antibiotic prescribing for adults with acute RTIs[29]. CRP POCTs have been recommended in the UK by NICE since 2015 for patients with suspected

lower RTIs (LRTIs) in primary care[30]. However, uptake has been low[31]. Reasons may include questions around who funds such tests and because clinicians may be uncertain about the implications of the results (as an elevated CRP does not always mean the infection is bacterial[32])[31].

Diagnostic accuracy of lone host response biomarkers, such as CRP, is likely to be inadequate. CRP levels do not always correlate with bacterial load and can be significantly raised in viral infection[33]. Recently, novel combination POCT^{HR} have been developed, attempting to increase diagnostic accuracy. These have had encouraging results in secondary care but have not been evaluated in primary care[34]. A recent systematic review of novel combination POCT^{HR}s for differentiating acute bacterial from viral RTIs concluded that these show potential clinical utility and that future research should be in primary care, evaluate patient outcomes and use experimental study designs[34].

As a clinical and research community we have no evidence of the 'real-world' impact of POCT^{HR}s in primary care, including feasibility of testing, any clinical or economic advantages and disadvantages, or potential changes in patient consulting behaviour (for example, medicalisation of self-limiting illness or reduction in consulting for future similar illnesses). The work proposed below is vital for the NHS and wider global community to remain responsive to the likely introduction of POCT^{HR}s for acute infections by industry and to understand whether such POCT^{HR}s can improve patient care and outcomes. Furthermore, there is an absence of evidence from the patient perspective for POCTs for acute URTI infection in primary care altogether (e.g. test acceptability, alteration in future consulting behaviour) - something we seek to address in this study.

FebriDx®

The POCT device that we will use in this study is FebriDx® (Lumos Diagnostics), a novel combination POCT^{HR}. FebriDx® has advantages for primary care use; it is dual-marker, hand-held, rapid turnaround (10 minutes) and does not require an additional desktop analyser. It is the only combination POCT^{HR} using a 'finger-prick' (rather than venous) blood sample. It has indicators of both viral (myxovirus resistance protein A, MxA) and bacterial (CRP) host immune response. FebriDx® is CE-IVD (in vitro diagnostic) marked. In the UK, FebriDx® costs approximately £11.80 per test, excluding VAT and does not require the high up-front and maintenance costs associated with a desktop analyser.

Evidence base

FebriDx® has been validated for use in distinguishing viral vs bacterial acute RTI in multiple prospective, multicentre studies in secondary care using combinations of laboratory parameters, microbiological testing and expert opinion as the reference standard[35-38]. NICE has published a Medtech innovation briefing for FebriDx®[39]. A recent systematic review of the diagnostic accuracy of novel combination POCT^{HR}s for differentiating acute bacterial from viral RTIs found that FebriDx® performed better than the single biomarkers of bacterial aetiology, CRP and procalcitonin[34]. FebriDx® has been estimated to have higher specificity for bacterial infection than CRP, procalcitonin and ImmunoXpert (another novel combination POCT^{HR} using a venous blood sample). This is beneficial in outpatient settings where infections tend to be less severe and it is appropriate to prioritise reducing antibiotic prescribing. The systematic review and meta-analysis estimated the average sensitivity and specificity of FebriDx® across studies to be 84% and 93% for bacterial infections, and 87% and 82% for viral infections, respectively[34]. However, no studies included in this evaluation were conducted in primary care. A small retrospective study in UK primary care (21 patients) found that antibiotic prescriptions were reduced by 80% when guided by FebriDx®, with no adverse effects[40]. However, apart from that study, evidence is entirely lacking for primary care[34]. It is important to evaluate FebriDx® further in primary care, where the population is

different, with generally less severe infections meaning data from secondary care cannot necessarily be extrapolated as the sensitivity of a test may vary by disease severity (spectrum bias).

A current NIHR SPCR funded, University of Southampton feasibility study ('PREFIX', led by applicants Francis and Wilcox) is exploring whether FebriDx® could reduce the use of antibiotics for acute LRTI in primary care[41]. PREFIX includes only acute lower RTI, whereas we plan to assess the use of FebriDx® for acute upper RTI. It is important and of value to assess FebriDx® in the context of acute URTI as this group of infections includes the four conditions found to contribute most to inappropriate antibiotic prescribing in English primary care: sore throat (23% of identified inappropriate prescriptions), cough (22%, can be present in both URTI or LRTI), sinusitis (8%), otitis media (6%)[7, 8]. Additionally, data from a recent Trial Steering Committee report for the RAPID-TEST RCT (patients eligible if the clinician and/or patient believes antibiotic treatment is, or may be, necessary) finds 54% of recruited patients have URTIs.

Mechanism

The FebriDx® test is a lateral flow immunoassay using a capillary blood sample. The test is single use and portable, with a built-in retractable lancet, blood collection and transfer tube, and buffer release mechanism. Results are available in 10 minutes (Figure 1). FebriDx® is validated for use in patients one year of age or older.

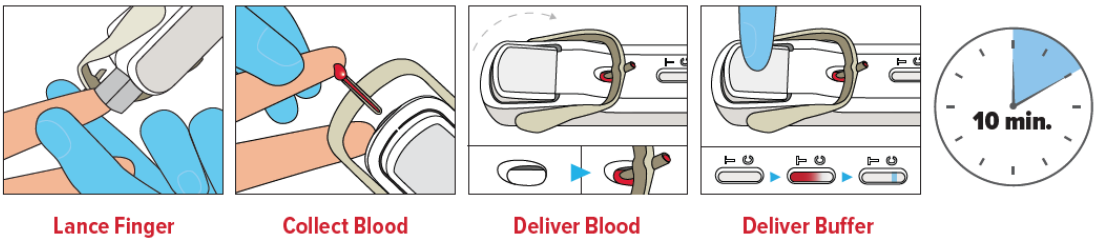


Figure 1: FebriDx® test procedure. Copyright FebriDx®.

The FebriDx® test measures two host immune response proteins:

1. CRP, a non-specific acute phase inflammatory protein. CRP increases within 4-6 hours of bacterial infection and peaks after 36 hours[42, 43]. CRP also increases with some viral infections, including Influenza, Adenovirus and SARS-CoV-2[44, 45]. CRP is detected at approximately 20mg/L serum equivalent in the FebriDx® test.
2. MxA, a derivative of interferon α/β, which is associated with acute viral infection[46-49]. It has an induction time of 1-2 hours and a half-life of 2.3 days. MxA is detected at approximately 40mg/L serum equivalent in the FebriDx® test.

If the blood sample tested has an elevated level of CRP or MxA, at or above the described cut-off levels, the appropriate test line will appear in the result window. A control line indicates correct sample flow and valid results.

FebriDx® results interpretation (Figure 2):

- If MxA is elevated (regardless of CRP result), the test indicates viral infection.
- If CRP is elevated without elevated MxA, the test indicates bacterial infection.
- If neither CRP nor MxA are elevated, the test is negative for infection.

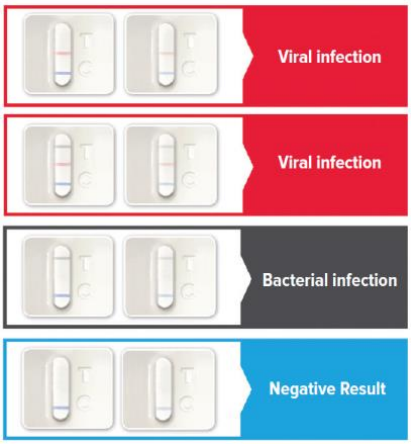


Figure 2: FebriDx® results interpretation. Copyright FebriDx®

The need for improved diagnostic test accuracy assessment

A fundamental challenge in the assessment of the accuracy of these novel POCTs is the lack of an accepted reference-standard for RTI diagnoses[50-52], in part due to the complexity of the respiratory microbiome and the difficulty in distinguishing commensal from pathogenic microbes. Validating novel approaches would help address the diagnostic challenge, which is essential for optimising clinical care and antimicrobial use for RTIs in primary care.

We will use latent class analysis (LCA), an approach to diagnostic test accuracy assessment, which can be used in the absence of an accepted reference-standard. LCA produces estimates of sensitivity and specificity based on a probabilistic definition of disease state, rather than requiring any one test to be treated as a reference-standard[53, 54]. LCA has had encouraging results in other fields where a reference-standard is lacking, such as the diagnosis of TB[55].

Acute Respiratory Infection (ARI) hubs and Pharmacy First

ARI hubs were first set up in winter 2022-23 to assess patients presenting with acute respiratory infections and reduce the burden of acute respiratory illness on general practice[14]. ARI hubs were stepped up again for winter 2023-24. If ARI hubs are stepped up again in winter 2024-25 then we will consider recruiting from them. This allows us to remain responsive to changes in the delivery of community assessment of acute respiratory infections.

The ‘Pharmacy First’ initiative is part of the UK government’s 2023 ‘Delivery plan for recovering access to primary care’(1). The Pharmacy First initiative launched in January 2024 and sees community pharmacists able to prescribe antibiotics for seven common conditions, including three URTIs (sinusitis, sore throat, earache). If this initiative continues, we will consider recruiting from Pharmacy First pharmacies. This allows us to remain responsive to changes in the pathways for acute respiratory infections in the wider primary care setting.

Research aim and objectives

Aim

To investigate the feasibility and value of FebriDx® use for children and adults with acute URTIs in primary care, and inform the design of a future randomised-controlled trial (RCT).

Objectives

Quantitative

1. To investigate if FebriDx® changes clinician pre- and post-test diagnostic confidence for children and adults with acute URTIs in primary care
2. To estimate the percentage of eligible patients in whom FebriDx® is used
3. To describe the clinical and demographic characteristics of patients in whom FebriDx® is used
4. To describe the characteristics of staff members using FebriDx®
5. To describe the distribution of FebriDx® results (viral/bacterial/negative/invalid)
6. To explore if FebriDx® changes clinician pre- and post-test belief that antibiotic treatment is necessary
7. To describe the proportion of patients who are prescribed antibiotics after FebriDx®
8. To explore the diagnostic accuracy of FebriDx® for acute bacterial URTIs in primary care
9. To describe re-consultations and antibiotic prescribing within 30 days following study recruitment
10. To assess adverse participant outcomes

Qualitative

1. To explore clinician and patient (and parent of patients) perceptions of FebriDx® use, including facilitators and barriers to test uptake and to future trial recruitment
2. To better understand the logistics of using FebriDx® in primary care and how it fits within existing care pathways
3. To inform the design of a future RCT

Study methods

Design

Prospective feasibility cohort study with qualitative evaluation and exploration of diagnostic accuracy.

Population

Children and adults aged 12 months or older presenting with symptoms of acute URTI to participating GP practices or Acute Respiratory Infection (ARI)[14] hubs or Pharmacy First pharmacies in the NIHR South West Central Research Delivery Network area. Study site recruitment will be facilitated via the NIHR South West Central Research Delivery Network. Of the interested study sites, we will purposefully sample between six and 12 that vary in their list size and socio-demographics.

Study duration

The study will run for 12 months from September 2024. Prior to this, Dr Emily Brown (the Principal Investigator) will prepare the study materials, complete the ethics application and start to recruit study sites. Study sites will start recruiting patients from autumn 2024 (anticipated during of recruitment approximately six months).

Setting

GP practices in the NIHR South West Central Research Delivery Network area. Recruitment will also be considered from any Acute Respiratory Infection (ARI)[14] hubs or Pharmacy First pharmacies in existence in the same area at the time of the study.

Eligibility criteria

Inclusion criteria

Inclusion criteria for GP practices/ARI hubs/Pharmacy First pharmacies are (all of):

1. Located in the NIHR South West Central Research Delivery Network
2. Served for routine microbiology services by Southmead Hospital (North Bristol). GP practices out of this area are eligible if served by an NHS Microbiology or pathology service that can accept study samples within 24 hours and then transfer samples to Southmead Hospital via DX courier service or equivalent overnight courier within 24 hours of receipt
3. Clinicians managing acute URTIs are willing to consider use of the POCT (min. 2 per practice)
4. Assessing clinician or another staff member willing to perform the test

Inclusion criteria for participants are (all of):

1. Age ≥12 months being assessed (face-to-face or remote, but willing to attend in person for study tests) for symptoms of acute (≤21 days) URTI as identified by the recruiting clinician, including sore throat/pharyngitis/laryngitis, acute middle ear infections (acute otitis media), sinusitis or cough but without symptoms or signs localising to the lower respiratory tract (shortness of breath, wheeze, sputum, chest pain)
2. The clinician has decided that they are likely to prescribe antibiotics in the absence of further diagnostic testing (to prevent over-medicalisation of URTIs)
3. Clinician and patient willing to wait for POCT result before finalising treatment plan

Exclusion criteria

Exclusion criteria for GP practices/ARI hubs/ Pharmacy First pharmacies are (any of):

1. Located outside of the specified recruitment area
2. Clinicians managing acute infection are not willing to consider use of the POCT
3. Assessing clinician or another staff member not willing to perform the test

Exclusion criteria for participants are (any of):

1. Previously participated in this study
2. Age <12 months
3. Symptoms or signs of lower respiratory tract involvement, such as new shortness of breath, wheeze, sputum, chest pain
4. Present with symptoms >21 days
5. Patient unable to receive study tests from the GP practice before a prescribing decision is made
6. Current use of antibiotic or antiviral medication
7. Patients who are immunosuppressed
8. Live viral immunisation within the last 30 days
9. Adults lacking capacity to consent for themselves
10. Prisoners or young offenders in the custody of HM Prison Service or who are offenders supervised by the probation service
11. Study samples cannot be transported to Southmead Hospital (North Bristol) to be received within 48 hours of being taken

Site recruitment

GP practices within the NIHR South West Central Research Delivery Network area (and perhaps ARI hubs and Pharmacy First pharmacies in existence in the same area at the time of the study) will be invited to participate. GP practice invitations will be facilitated via the NIHR South West Central Research Delivery Network, who have already indicated that they

would be willing to provide study support and assist with the identification and recruitment of suitable GP practices to this study. We have already established links with GP practices potentially interested in participating. We will identify ARI hubs and Pharmacy First pharmacies through the NIHR South West Central Research Delivery Network and our own local networks.

Potential sites will be sent detailed information about the study, and those interested will be invited to contact the study team by email, phone or text message to express their interest, check eligibility and discuss availability.

Of the sites that agree to take part, within the limits of recruiting research-active sites, we will purposefully sample between six and 12 sites that vary in their list size and the socio-demographics of their patient populations. Study sites will start recruiting patients from autumn 2024 (respiratory infections are seasonal and peak during the autumn/winter). The anticipated during of recruitment is approximately six months.

Patient recruitment and consent

Patients contacting a study site with a suspected URTI will be individually consented and recruited to the study, following clinical assessment (either remotely or face-to-face). Access to potential participant's medical records prior to study involvement will only ever be undertaken by those in their direct healthcare team.

Study site staff will identify potential participants, and provide them with verbal information about the study, as well as a participant information sheet (PIS) in either paper or online form. Potential participants will be given additional time that day (if wished) to consider whether or not to take part, unless urgent treatment is deemed clinically necessary, in which case study site staff may decide to proceed with ‘usual care’ only if the participant feels they would need more time to decide.

Participants who are still interested in taking part will undergo screening by a study site clinician to confirm eligibility, before asking them to complete a consent form (either online or paper), and taking part in the study. In the case of written consent, a copy of the signed consent form will be given to the participant and a copy kept in the investigator file.

Patients recruited will also be asked to express interest in taking part in a follow-up interview and provide consent for their contact details to be shared with the research team by recording them onto a secure online GDPR-compliant database, which will then be used to transfer the details to a secure password-protected University of Bristol server.

Data collection process

Data will be collected via online case report forms (CRFs). Minor amendments may be made to these following initial trialling. In total, we anticipate that patients will be involved for approximately 20 minutes following completion of the consent form.

1. Completion of initial CRF (5 minutes)**.** Study site staff will be asked to record basic data for each participant using an initial online case report form prior to conducting FebriDx® testing. This data will include patient demographics, clinical features of the presenting illness, their rationale for considering use of antibiotics, and their perceived likelihood of prescribing antibiotics prior to conducting the test.

2. FebriDx® testing and nasopharyngeal swab collection (5 minutes)
   Testing using FebriDx® will be performed. Any invalid tests may increase the length of time required, but we expect based on prior experience that the majority of tests

will be valid. A nasopharyngeal swab will be taken by study site staff. Swab samples will be transported to Southmead Hospital using standard NHS sample transport services.

3. Wait for FebriDx® result (10-minutes)

4. Communication of result and completion of second CRF (5-10 minutes)**.** Following completion of the FebriDx® test, study site staff will be asked to record further data on a second online CRF. This will include the test result, their views on the validity of the test result, time to result, their subsequent management, and the reasons for prescribing or not. The CRF will also have an open-text box available for staff to record any additional data and/or comments they feel pertinent.

   During initial study training we will remind clinicians where NICE guidance and antimicrobial stewardship tools can be found, including evidence-based materials for patients. This is to ensure that patients who are not prescribed antibiotics, who might otherwise have been prescribed antibiotics, are provided with adequate information and advice on symptomatic treatment strategies and safety netting advice.

Additional data collection and follow-up

Patients will be asked to consent to allowing access to their medical records, and at the end of the study the medical records of participants will be reviewed and subsequent healthcare contacts (general practice in-hours, out of hours, A&E, walk-in centres, and hospital admissions), use of antibiotics, and serious complications such as sepsis or death documented.

We will also ask sites to collect anonymous data on reasons for clinicians not recruiting patients who met the inclusion criteria, reasons for non-participation, and to provide practice medical record search results about the number of patients with URTI seen during the recruitment period, and the proportion prescribed antibiotics.

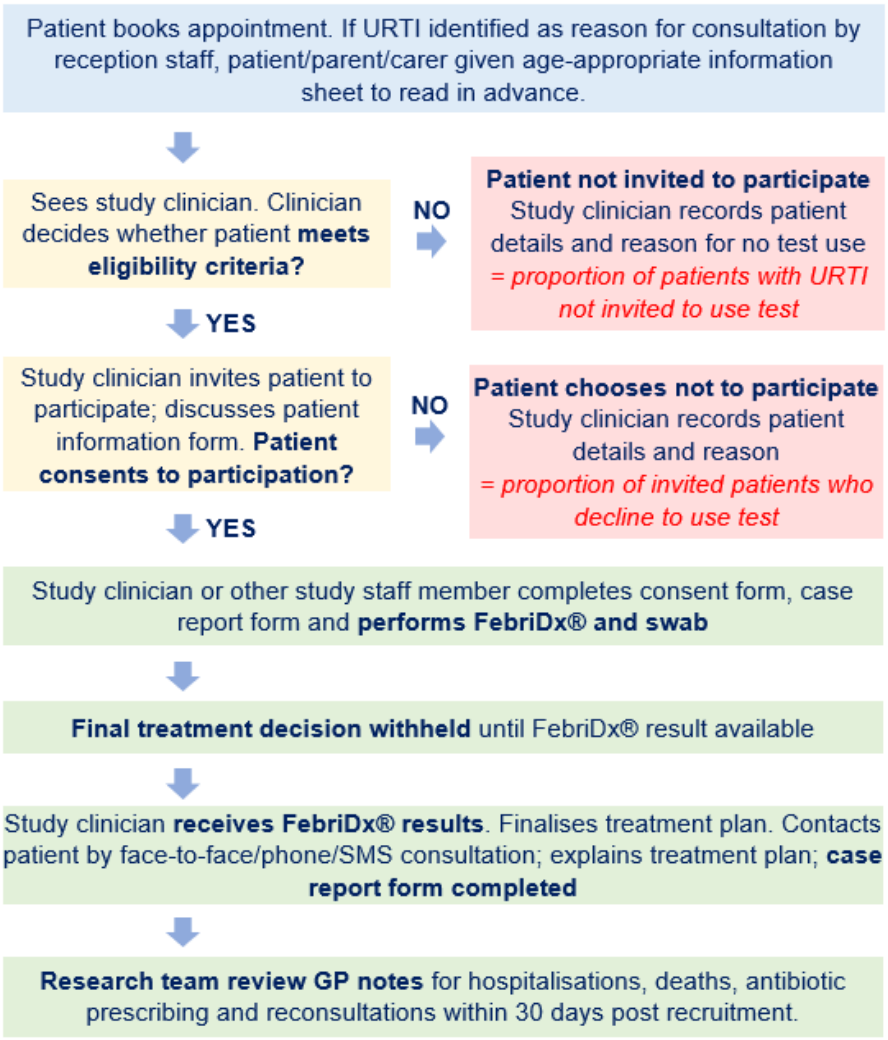
Contact details for the study team (including both telephone and email address) will be provided to participants in order to allow them to seek further information at a later stage if desired.

Laboratory procedures

Swab samples will be transported to the UKHSA south west regional laboratory at Southmead Hospital, Bristol. The samples will be analysed by TaqMan Low Density PCR array card assay. Study samples and derived nucleic acid extracts will be stored in dedicated specimen freezers within the Pathology Sciences Building at Southmead Hospital. The freezers are accessible to staff members of North Bristol Trust Pathology and UKHSA Infection Sciences who have card key access to laboratory spaces. All staff undertake mandatory training with regard to security, patient confidentiality, data governance and sample integrity and storage.

Remaining nucleic acid samples following the above processing will be transported to Professor Preston's laboratory at the University of Bath for metagenomics analysis. The samples will be stored in a freezer at -80 degrees Celsius in a laboratory to which access is restricted to authorised research group members by a card system (Professor Preston and authorised research group members will have access). A third party commercial sequencing company may receive parts of the samples, but these will be destroyed immediately after sequencing by the company, so there will be no long term storage.

Participant flow diagram



Quantitative evaluation

Objective	Outcome Measure	Source
Primary objective		
To investigate if FebriDx® changes clinician pre- and post-test diagnostic confidence for children and adults with acute URTIs in primary care	Clinician pre- and post-test diagnostic confidence	Case report form*
Secondary objectives		
1. To estimate the percentage of eligible patients in whom FebriDx® is used	Percentage of eligible patients in whom FebriDx® is used	Screen logs completed by study site team
2. To describe the clinical and demographic characteristics of patients in whom FebriDx® is used	Clinical and demographic characteristics of patients in whom FebriDx® is used	Case report form
3. To describe the characteristics of staff members using FebriDx®	Job type and number of staff using FebriDx®	Case report form
4. To describe the distribution of FebriDx® results (viral/bacterial/negative/invalid)	Distribution of FebriDx® results (viral/bacterial/negative/invalid)	Case report form
5. To explore if FebriDx® changes clinician pre- and post-test belief that antibiotic treatment is necessary	Clinician pre- and post-test belief that antibiotic treatment is necessary	Case report form*

6. To describe the proportion of patients who are prescribed antibiotics after FebriDx®	Proportion of patients prescribed antibiotics after FebriDx®	Case report form
7. To explore the diagnostic accuracy of FebriDx® for acute bacterial URTIs in primary care	Sensitivity and specificity for acute bacterial URTI TaqMan array analysis of nasal/throat swabs will enable assessment of microbial prevalence, burden and viral/bacterial diversity in the URT.	Comparison to reference standard of nasal/throat swab PCR. Latent Class Analysis using results from clinician diagnosis, FebriDx® POCT, and TaqMan Array Card PCR.
8. To describe re-consultations and antibiotic prescribing following study recruitment	Re-consultation and antibiotic prescribing events within 30 days following study recruitment	GP notes review
9. To assess adverse participant outcomes	Hospitalisations and death within 30 days following study recruitment	GP notes review

*Pre-test diagnosis will be recorded after clinical history and examination are complete, but before the FebriDx® test

Statistical analysis

Summary statistics will be used to describe the baseline characteristics of participants (and those not offered the test or declining to participate) and the distribution of FebriDx® results. McNemar's test will be used to compare diagnostic confidence pre- and post-test (primary outcome). This test is appropriate to use to determine differences between a dichotomous dependent variable (test confidence) between two related or paired groups (pre- and post-test). All statistical analysis will be conducted using STATA software.

Diagnostic tests

1. POCT: FebriDx®

Following written informed consent, participants will have a capillary ('finger prick') blood test. The test will be operated in accordance with the FebriDx® User Guide. Full training will be provided to study site staff. Staff will be supported in test use and evaluation by the members of the study team, who will also be trained in operation of the machine. The FebriDx® test does not require loading chemical reagents, consumables or waste handling. Results will be returned to a clinician who will record the result on the Case report form and patient notes, then contact the patient regarding their treatment plan.

2. TaqMan Low Density PCR array card assay (TAC)

The nasal and throat swabs will be tested for an extended array of respiratory viral and bacterial pathogens[1] using TAC, as used in previous studies[56-58], which provides quantitative data. We will pre-define thresholds for the diagnosis of bacterial and viral infection. We will perform run controls (Zeptometrix respiratory virus and bacterial panels) for the majority of organisms prior to use and during the study. This analysis will be conducted at the UKHSA South West Regional Laboratory, Bristol.). Results will be used as the reference-standard for evaluation of diagnostic accuracy and also enable assessment of microbial prevalence, burden and viral/bacterial diversity in the URT, which will help to contextualise the FebriDx® results.

[1] The organisms detected by TAC are: Coronaviruses (SARS-CoV-2, 229E, HKU1, NL63, OC43), Adenoviruses, Bocaviruses, Enteroviruses (untyped, also EVD68), Influenza A (subtypes H1 and H3), Influenza B, Human metapneumovirus, Parainfluenzavirus (types 1, 2, 3, 4), Parechoviruses, Rhinoviruses, Respiratory syncytial virus (types A, B), *Bordetella pertussis* (IS481, ptxS1), *Chlamydia pneumoniae*, coagulase negative *Staphylococcus* species, *Fusobacterium necrophorum*, *Haemophilus influenzae*, *Moraxella cararrhalis*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *S. aureus* Panton-Valentine Leukocidin, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*.

3. **Metagenomic microbiology**

Metagenomics is a cutting-edge technique, quickly and accurately detecting a wider range of microorganisms than tested for using conventional PCR methods (such as TAC, which tests for a pre-defined list of bacteria/viruses). We will receive the microbial profiles from shotgun metagenomics.

Diagnosis accuracy exploration

Although a recent systematic review has assessed the diagnostic accuracy of FebriDx® for differentiating acute bacterial from viral RTIs, none of the studies in this analysis were based in primary care[34], where the patient population is different, with typically less severe infections and thus differing immune responses. It is therefore reasonable to assume that the sensitivity and specificity of POCT^{HR}s might be different in primary care compared to secondary care, hence the diagnostic accuracy of POCT^{HR}s should be evaluated specifically in primary care.

There is no clearly accepted gold-standard for the diagnosis of acute URTIs in primary care[59], nor in the laboratory[51]. Acknowledging this and the challenges of using URTI swabs as a reference standard due to commensal pathogens, we plan a dual approach:

1. **Comparison to microbiological analysis by nasal and throat swab PCR**

We will explore FebriDx® diagnostic accuracy for viral and bacterial acute URTI by comparison to nasal and throat swab analysis by PCR (TAC), treating PCR as a reference standard for the diagnosis of URTIs, as studies often do[56-58].

2. **Latent class analysis**

In recognition that PCR is not a true gold standard for viral vs bacterial acute URTI due to the presence of commensal pathogens in the upper respiratory tract, we will also estimate the accuracy of FebriDx® using exploratory Latent Class Analysis (LCA). LCA is a technique used to produce estimates of sensitivity and specificity based on a probabilistic definition of disease state, rather than requiring any one test to be treated as a gold standard. The sensitivity and specificity of each test, along with the prevalence of disease in the study population, are simultaneously estimated by fitting a statistical model to the overlap between multiple test results on each individual[53, 54].

Target condition: bacterial acute URTI

The following diagnostics tests will be used in the LCA analysis:

1. Clinician diagnosis. This will be recorded prior to any of the below test results being seen.
2. FebriDx® POCT (capillary blood sample taken at consultation, test performed on-site)
3. Standard microbiology (PCR using TAC, swab taken at consultation, test performed in the laboratory)
4. Metagenomic microbiology (swab taken at consultation, performed in laboratory)

A key challenge in LCA is the number of parameters (quantities) to be estimated – particularly in the presence of “conditional dependencies” between tests. Tests are conditionally dependent if false negative results are correlated across tests among people with bacterial URTI, or if false positive results are correlated across tests among people without bacterial URTI. We will assume that all tests are conditionally independent among individuals without bacterial URTI. Among patients with bacterial URTI, we will also assume that clinician diagnosis is conditionally independent of FebriDx®, TAC and metagenomics. We will further explore the incorporation of external information on plausible parameter values, within a Bayesian statistical framework. We plan to develop our LCA approach for use in the proposed subsequent RCT of this POCT.

Sample size calculation

Primary outcome: clinician diagnostic confidence pre vs post FebriDx®

We consider that an increase in diagnostic confidence of 15% would be clinically important. We note that this is a smaller change than demonstrated in a previous study, which showed an increase in diagnostic certainty of 37.6% (although that study used a different type of POCT and did not restrict to use when the clinician had decided that they are likely to prescribe antibiotics in the absence of further diagnostic testing[23]. To detect an increase of 15% with 90% power at the 5% significance level, a total sample size of 231 is required. We aim to recruit 231 participants (approximately one to three participants per site per week, within the range of six to 12 sites recruited).

Secondary outcome: explore diagnostic test accuracy of FebriDx® for acute bacterial URTI

Approaches to sample size calculation for LCA are complex and under-developed. We therefore consider our use of LCA approaches exploratory, and consider here only sample size calculations for comparison with nasal and throat swab PCR.

We will focus on specificity. We believe it is appropriate for the aim of the FebriDx® test in the community setting to be to improve antibiotic stewardship, given that in the community infections tend to be less severe and it is appropriate to prioritise reducing antibiotic prescribing. This requires a diagnostic test with high specificity for bacterial infection (minimising false positive results).

In the analysis treating PCR as a reference standard, the table shows the required total sample size to achieve a 95% CI of length 10% for specificity:

		Assumed prevalence of bacterial acute URTI**			
		20%	25%	30%	35%
Assumed true specificity*	88%	203	216	232	250
	90%	173	184	198	213
	92%	141	151	162	174
	94%	108	116	124	133
	96%	74	79	84	91

*Carlton et al metanalysis 2021 estimates FebriDx® specificity for bacterial infections as 93% (95% CI 90%-95%, k=4)

**Based on relevant literature[60-62]

A sample size of 231, as per the primary outcome sample size calculations, is suitable for the majority of specificity sample size calculation scenarios above.

Qualitative evaluation

In addition to the inclusion criteria for the overall study, for the qualitative evaluation participants must be ≥12 years old (parents will be invited for participants under this age).

Objectives:

1. To explore clinician and patient (and parent of patients) perceptions of test use, including facilitators and barriers to test uptake and to future trial recruitment
2. To understand logistics FebriDx® use in primary care and how it fits within existing care pathways
3. To inform the design of a future RCT

Sample method:

Within the participating sites, we will purposefully sample patients recruited to the study (≥12 years old, as well as parents of participants <16 years old) and primary care staff (GPs, practice nurses, healthcare assistants and other practice staff). We will ensure those we sample vary in their background and experiences of using the test machines and receiving the results. Information about the qualitative interview will be included on the information sheet given to staff and patients at recruitment to the study. At enrolment into the study, consent will be taken for the research team to contact staff and patients and parents of patients regarding the qualitative interviews. The research team will contact those who consent to invite them to take part in an interview. At the start of each interview, the qualitative researcher will reiterate the purpose of the interview, confirm that the information sheet has been understood and answer any questions. Participants will be reminded they are free to withdraw at any stage without giving reasons and that they can chose not to answer any questions. Consent will be collected in writing prior to the interview, or orally before the interview begins. Consent will be audio-recorded if taken orally.
Up to 20 primary care staff and 20 patients (and parents of patients) will be recruited. This sample size provides scope for comparing experiences within the sample, while permitting participants of a defined identity and allowing a data set that is manageable within the timeframe. Data collection may be stopped earlier if data saturation is reached [63], such that no new information is obtained from interviews that would add to the development of new themes.

Data collection method:
Two trained qualitative researchers will conduct individual, semi-structured interviews (lasting approximately 30 minutes) with primary care staff and patients to explore test experiences and views. The qualitative researcher will arrange a time convenient to the participant to conduct a telephone, online or face-to-face interview. A schedule of semi-structured, open-ended questions will be developed based on the study objectives and existing literature to guide data collection. The following topics will be explored, with flexibility for participants to raise issues that are important to them:

- The logistics of POCT use and how clinicians report results to patients
- How point-of-care testing was perceived to influence the consultation
- The clinical management decision-making process
- Facilitators and barriers to use of the test
- Information and support needs, experiences of other types of point-of-care testing and alteration in future consulting behaviour (patients only).

Qualitative interviews will be conducted face-to-face or remotely (i.e. telephone, Microsoft Teams), according to participant preference. Interviews will be recorded using an encrypted digital audio recorder with consent from participants prior to interviews. The recordings will be transferred to and stored on UoB secure servers at the end of each interview. All data (audio and participant contact details) will be kept on these secure servers in accordance with the Data Protection Act. Clinicians who work at participating sites, but opted not to take part in the study, will be sent a qualitative survey exploring the reasons for this.

Analysis method:
The audio recordings will be transcribed verbatim by an external UoB transcription service who has signed a confidentiality agreement. Transcripts will be anonymised and imported into NVivo to support data management and analysis. Analysis will begin shortly after data collection starts, being ongoing and iterative. Reflexive thematic analysis[64], utilising a data-driven inductive approach, will systematically code data to identify and analyse patterns and themes of particular salience for participants. This approach particularly well suited for multi-disciplinary health research as it permits theoretical flexibility while also allowing exploration of shared meaning across and within the dataset. A sub-sample of transcripts will be independently coded by a second researcher, and our team will meet to discuss different interpretations of data and the development of themes to maximise rigour. Any differences in interpretation will be resolved through discussion.

Patient and public involvement (PPI)

Involvement of patients and the public in developing this protocol
We have established a relationship and discussed patient and public involvement with the PPI coordinators at the Centre for Academic Primary Care (CAPC), University of Bristol.

We held two PPI group meetings during development of this study proposal. The meetings involved discussion with eight group members in total. The group was mixed with a range of age, gender and ethnicities. We discussed:

1. An outline of the proposed study
2. Whether this is an important study and research question
3. Whether the study is likely to be feasible and acceptable to participants
4. The practicalities of the study and participant flow when they consult with an URTI
5. Which infections to include in the study
6. How to ensure the study is inclusive
7. Dissemination of results

The PPI meetings have contributed to the development of this application and led to changes in study design. The meetings have:

1. Enabled the research team to gain a wider range of perspectives on the diagnostic and treatment process for URTIs from the patients' point of view
2. Unanimously confirmed the importance of the research question and proposed study
3. Advised use of 'finger-prick'/capillary (rather than venous) blood samples, informing the choice of point-of-care test (POCT) for the study
4. Refined the practicalities of participant flow, from having the POCT to receiving the results - ensuring the process is efficient and acceptable to patients
5. Enhanced the approach to Equality, Diversity and Inclusion, including agreeing that it was important to try to measure which patients with URTIs are not offered the POCT by clinicians and which patients decline to participate
6. Emphasised the importance of ensuring the results of the study are effectively shared with participants

How will patients and the public be involved in the research?
Effective patient and public involvement (PPI) is crucial to the success of the research we propose here, and for the PI's future doctorate and she will increasingly adopt a 'co-production' approach. Ultimately, the proposed research aims to bring benefit to patients and public at both a personal and global level in terms of reducing antibiotic overuse and AMR. The results may have implications for patient diagnosis and treatment, as well as NHS resource allocation and therefore, embedding PPI throughout the project is paramount, so the patient perspective can meaningfully shape the research. PPI involvement has already positively impacted the development of this research proposal. We plan to build on this, meeting with our PPI group at least six times throughout the study, which will provide an opportunity for the research team to seek PPI on questions arising during the study, from recruitment to interpretation of results and dissemination of findings.

We will recruit and maintain a dedicated and diverse PPI group (~eight members) with a range of perspectives, reflecting the broad recruitment criteria for the study, since most, if not all, members of the public have lived experiences of URTIs. We will aim for diverse and inclusive recruitment to the PPI group. We will select PPI members to ensure the group includes a mixture of ages, genders and ethnicities. We will also ensure the PPI group

contains at least one parent of a young child and one carer of an elderly person. Additionally, we will gain the perspective of younger people through the Bristol Young People's Advisory Group (YPAG), which comprises young people aged 10 and upwards who are interested in healthcare and research. They regularly meet with researchers to provide input to their projects. We aim to have two meetings with approximately six members of YPAG.

The CAPC PPI team has a large, diverse and well-established database of public contributors, including those particularly wanting to be involved in infection research studies. We will recruit the PPI group through this CAPC database, including inviting the PPI members from meetings already held. This includes two people we have an established relationship with through the initial PPI meetings for this study and through working with them on another study.

The Public Involvement Impact Assessment Framework (PiiAF) has been used to help plan PPI. We will hold regular PPI meetings throughout the study (approximately every two months, depending on stage of study and need). These meetings plus offline work will include: reviewing the study design and patient-facing materials pre-ethics application; ensuring inclusive recruitment (with reference to the NIHR INCLUDE Ethnicity Framework); planning qualitative interviews; troubleshooting as recruitment starts; interpretation of results and understanding their meaning from a patient and wider public perspective; planning results dissemination and preparation of materials, and reporting on PPI impact. Regarding dissemination, we will work closely with PPI partners to understand what the study results mean and co-produce key results messaging, ensuring that results are shared in a targeted way with specific audiences including local communities, health care providers and commissioners in the Bristol region, and nationally to key stakeholders. We have costed funding for a PPI member's conference attendance for results co-delivery. Once a PPI group is established, the PiiAF will be consulted again to further develop the PPI involvement in the study.

In terms of evaluating the PPI involvement in the study, the PiiAF will be used to help guide further development of the PPI impact assessment plan. We will keep an impact log throughout the study to record the outcomes of PPI meetings. Our 6th and final PPI meeting will focus on PPI process evaluation. This meeting will take the format of a workshop following the 'cube' framework for evaluation. When we report PPI in this study, we will use the GRIPP2 reporting checklists.

We have included the support of the CAPC PPI advisor and PPI group expenses in the study costings, including reimbursement of the contributors at the NIHR agreed rate of £25 per hour.

We aim to support PPI members throughout the study, ensuring they feel part of the team and able to make a full contribution. We will meet 1:1 with those who express an interest in the PPI group to discuss what involvement would entail and to understand any personal support or access needs. We will plan meetings in advance, taking into account individual's personal circumstances to ensure as many members can attend as possible. we have budget flexibility to host meetings online or in person, depending on the preferences of the group.

We will keep PPI members informed about study developments and ask for feedback on PPI elements regularly. We will have open communication channels between meetings and

further dialogue and feedback would be welcomed. The YPAG members will be supported by the YPAG facilitators in similar ways.

Anticipated risks

Clinical risks

The clinical risks of undertaking the single-use FebriDx® test itself is extremely low. A 'finger-prick' quantity of blood is taken and therefore risks of subsequent bleeding or infection are extremely low. Furthermore, the test is virtually painless, and has been well-tolerated by both adults and young children in previous studies[35, 36, 38, 40, 65].

Clinicians will be advised to incorporate the FebriDx® result into their clinical decision making, but not to replace their clinical judgment. There is a possibility that participants undergoing FebriDx® testing may not be subsequently prescribed antibiotics by their primary care clinician, when they otherwise would have done. There is currently equipoise regarding the risks and benefits of this. Antibiotics cause harms as well as potentially causing benefits in those with susceptible infections. Numerous previous studies have shown that it is safe to reduce antibiotic use through CRP point-of-care testing[62], and previous studies exploring the diagnostic accuracy of FebriDx® have shown good agreement with reference-standard Polymerase Chain Reaction (PCR)[35, 36, 38]. Furthermore, it has been shown that reducing antibiotic prescribing in respiratory tract infections does not increase the risk of serious complications[66-68].

Clinicians will remain responsible for all clinical decision making and patient management. As per usual practice, clinicians will advise the patient about the reasons for doing the test and the expected outcome. This might include advising the patient that they will be contacted about the result of the test and how to expect that contact (e.g. telephone call/ secure text). During initial study training we will provide clinicians with a summary of NICE guidance and links to antimicrobial stewardship tools, including evidence-based materials for patients. This is to ensure that patients are provided with adequate information and advice on symptomatic treatment strategies and safety-netting advice.

Other potential risks

Steps will be taken to ensure confidentiality is maintained, unless there are exceptional circumstances with regards to serious concerns about patient safety (see full details in confidentiality section). We will adhere to GPDR guidance. Any personal identifiable data (such as contact details) will be stored separately to research data to protect anonymity.

Patients will be asked to consent for access to their medical records, and at the end of the study we will review the medical records of participants and document subsequent healthcare contacts (general practice in-hours, out of hours, A&E, walk-in centres, and hospital admissions), use of antibiotics, and serious complications such as sepsis or death.

Patients who express interest in taking part in the qualitative study will also be asked to consent to have their contact details shared with members of the study team. Taking part in interviews themselves will have very low risk to participants and questions asked will not include any sensitive topics. Personal details will not be shared, and any quotes will be completely anonymised.

Regulatory and ethical considerations

FebriDx® was CE-marked as an in vitro diagnostic device in September 2014. This was updated in October 2018. This study does not meet the criteria of a Clinical Trial of an Investigational Medicinal Product (CTIMP).

Study sponsorship

University of Bristol will act as sponsor for study.

Declaration of Helsinki

The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 as revised and recognised by governing laws and EU Directives as well as ICH E6.

ICH Guidelines for Good Clinical Practice

The Investigators will ensure that this study is conducted in full conformity to the guidelines for GCP (CPMP/ICH/135/95) July 1996. This Good Clinical Practices document describes the responsibilities and expectations of all participants in the conduct of clinical trials, including investigators, monitors, and sponsors.

Informed consent process

Each patient's consent to participate in the study will be obtained after a full explanation of the study. Discussion of objectives, risks and inconveniences of the study and the conditions under which it is to be conducted are to be provided to the patient by appropriately delegated staff with knowledge in obtaining informed consent with reference to the patient information leaflet. This information will emphasise that participation in the trial is voluntary and that the patient may withdraw from the trial at any time and for any reason. The patient will be given the opportunity to ask any questions that may arise and provided the opportunity to discuss the trial with family members, friend or an independent healthcare professional outside of the research team and time to consider the information prior to agreeing to participate.

Young people aged 16-17 years will be presumed to be capable of giving consent on their own behalf to participate.

Most children/young people under the age of 16 years will not be able to consent for themselves and we will seek consent from an informed person with parental responsibility. However, we will aim to give the child/young person information about the study which is understandable to them and which explains what is involved and the potential risks and benefits. If the child or young person is capable of assessing the information provided, their wishes will be considered. Assent forms have been created for the purposes of this study and the qualitative interviews (12-15 years). The recruiting healthcare professional will be advised that, whenever practical and appropriate, a child's assent should be sought before including them in the research. This will obviously be inappropriate for very young children.

For children/young people under the age of 16 years who may be capable of consenting for themselves, we will first seek permission from a person with parental responsibility for approaching the child/young person. With the parent's permission, the recruiting clinician will assess whether the child/young person is able to consent for themselves using the principles of 'Gillick competence'. If the parent has given permission to approach the child/young person

and have been assessed as being Gillick competent, then we will only obtain informed consent from the Gillick competent young person.

Confidentiality

Details surrounding maintenance of confidentiality will be described in the participant information sheet, and when taking informed consent. The investigators will take necessary steps to preserve the confidentiality of participants in the study. Only in exceptional circumstances we may be required to break confidentiality. This would only be the case if there was disclosure or evidence of significant abuse/maltreatment/poor care of patients. In these circumstances, we would discuss the concerns raised amongst appropriately qualified team members and, if necessary, notify the appropriate authorities.

Pseudo-anonymous electronic data will be collected on online case report forms via secure University of Bristol-approved online databases, including 'REDCap'. The data collected will be stored on fire-walled University of Bristol servers. Files will be password protected and only accessible to researchers responsible for the running of the study. We will adhere to GPDR guidance. On CRFs patients will only be identified by trial ID code. The information will be available to the study team, safety monitors, sponsor, and external monitors who can ask to audit or monitor the study. The site will retain a patient identification code list which is only available to site staff.

Contact details will be stored securely on a separate database from any other participant data collected to ensure anonymity is maintained. This information will only be accessed by relevant members of the study team. Any written study information will be stored securely in a locked room at study sites, or in electronic form on a secure server. Trial documents will be retained in a secure location during and after the trial has finished. The PIs or delegate will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. All written study documents generated will be kept in the Trial Master File, kept in a secure locked room at each study site. All online data will be retained on a secure online server hosted by the University of Bristol. All source documents and pseudo-anonymous research data will be retained for a period of 10 years following the end of the trial, as per University of Bristol policy.

The nose/throat swabs will be analysed at the UKHSA south west regional laboratory at Southmead Hospital, Bristol. They require the samples to be sent to them with the patient's date of birth, as well as their unique study ID. The ID number, date of birth, date of swab and results of the PCR tests will be held indefinitely on the North Bristol NHS Trust Laboratory Information System.

Qualitative interviews will be audio-recorded and downloaded onto the University of Bristol secure server immediately after the interview. Transcribing will be undertaken by a University-approved professional transcription service, with appropriate confidentiality agreements in place. Transcripts will also be stored on the secure server. Audio recordings will be deleted once they have been transcribed. Personal details will not be shared at any time, and any quotes from participants published in the final study report will be completely anonymised.

Research Ethics Committee
A copy of the protocol, proposed informed consent form, other written participant information and the proposed advertising material will be submitted to the Research Ethics Committee for written approval, using the UK Integrated Research Application System.

Health Research Authority
As this study involves NHS sites, the investigator will seek Health Research Authority (HRA) approval. A copy of the protocol, proposed informed consent form, other written participant information and the proposed advertising material will be submitted to the HRA for written approval, using the UK Integrated Research Application System. The investigator will notify deviations from the protocol, urgent safety measures or SAEs occurring at the site to the sponsor and will notify the HRA and MHRA of these if necessary in accordance with procedures.

Study amendments
The investigator will submit and, where necessary, obtain approval from the University of Bristol Research Governance Office and the HRA for all subsequent amendments to the protocol and associated trial documents as per REC and HRA requirements: http://www.hra.nhs.uk/resources/after-you-apply/amendments/. The investigator is responsible for ensuring that changes to the approved trial, during the period for which regulatory and ethical committee(s) approval has already been given, are not initiated without regulatory and ethical committee(s)' review and approval except to eliminate apparent immediate hazards to the subjects.

End of study
The end of study is considered the date of the last patient recruited into the study has completed the study and data analysis (including laboratory samples) has been completed.

Monitoring

Monitoring and auditing may be undertaken at any time by the Sponsor institution (University of Bristol) or the National Institute for Health Research SPCR as the funding organisation.

Finances and insurance

Financing
The study will be funded by the School for Primary Care Research (SPCR) administered by the National Institute for Health Research by means of a research grant to the Centre for Academic Primary Care, University of Bristol. The research funding will be administered by the University of Bristol.

Insurance
The University of Bristol has a specialist insurance policy in place, which would operate in the event of any participant suffering harm as a result of their involvement in the research.

Participant compensation
Participants in the qualitative interviews (both healthcare professionals and patients) will receive a £40 voucher as a thank you for their time. For adolescents participating in the qualitative interviews, the voucher will be given to their parents.

PPI compensation
PPI members be reimbursed at the NIHR agreed rate of £25/hour.

Publication and dissemination strategy

We intend to publish the results of this study in scientific journals and present the results at scientific meetings. All results in journals and presentations will be anonymous. A summary of findings will be provided to participants, and study progress and results will be made available via appropriate websites and social media feeds. Further avenues for output may also be explored after consultation with PPI members.

Anticipated impact

This mixed-methods study will provide useful data on the feasibility of FebriDx® implementation into UK primary care for acute upper respiratory tract infections (URTIs). This study will provide insights into the acceptability and useability of FebriDx® amongst primary care clinicals and patients for URTIs, as well as the logistics of use in this setting. Further, this study will give an initial indication of whether FebriDx® might impact clinician diagnostic confidence and likelihood of antibiotic prescribing for URTIs. Study results will be influential for patients and the public, clinicians, the research community, POCT manufacturers and policymakers.

The results from this study will support a future grant application for randomised controlled trial of FebriDx® use in UK primary care, from funding programmes such as the NIHR Programme for Applied Health Research or NIHR Health Technology Assessment Programme.

Ultimately, the proposed research programme (including the randomised trial anticipated as the next step) has the potential to bring health, environmental and economic benefit to patients and public at both a personal and global level in terms of reducing antibiotic overuse and reducing antimicrobial resistance, if we find that FebriDx® reduces inappropriate antibiotic prescribing and demonstrates clinical and cost-effectiveness. In addition, the proposed research may bring health benefits by improving identification of patients with bacterial infections and ensuring they receive antibiotics when appropriate. The results from this study are vital to informing the design of a future feasibility trial regarding the clinical effectiveness of FebriDx® and whether its use can reduce same-day antibiotic prescribing in children and adults presenting to primary care with acute URTIs.

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