University Hospital Southampton NHS
NHS Foundation Trust

Comparison of the performance of 3 Host Immune ResPonse tests for distinguishing bacterial and viral acute respiratory infection (CHIRP study).

Chief & Principal Investigator:

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This protocol has regard for the HRA guidance

1 Protocol overview

1.1 Research reference numbers

Sponsor's Reference number: RHM MED2094

REC Reference: 23/NW/0060

SRB Reference number: RHM SRB0044

HTA Licence number: 12009

ERGO submission ID: 101633

ISRCTN: 16512683

1.2 Protocol version number and date

1.1 16th May 2025

1.3 Sponsor

University Hospital Southampton NHS Foundation Trust

1.4 Title of Study

Comparison of the performance of 3 host immune response tests for distinguishing bacterial and viral acute respiratory infection (CHIRP study).

2 Signature page

The undersigned confirm that the following protocol has been agreed and accepted, and that the Chief Investigator (CI) agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's (and any other relevant) SOPs, Good Clinical Practice (GCP) guidelines, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I confirm that I will make the findings of the study publicly available through publication, or other dissemination tools, without any unnecessary delay, that an honest accurate and transparent account of the trial will be given and that any discrepancies from the study (as planned in this protocol) will be explained.

For and on behalf of the Trial Sponsor:

Signature:

..... Date: ...../...../.....

Name (please print):

.....

Position: .....

Chief Investigator:

Signature: ...

Date: 03-12--2024

Name: (please print): PROFESSOR TRISTAN W CLARK

3 Key trial contacts

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4 Study summary

Title	Comparison of the performance of 3 Host Immune response tests for distinguishing bacterial and viral acute respiratory infection (CHIRP study).	
Short Title	Performance of 3 host response tests	
Design	Observational, retrospective, diagnostic accuracy study	
Participants	Adults ≥ 18 years old, presenting to ED or AMU with acute respiratory illness (ARI)	
Planned Sample Size	200 patients	
Planned Study Period	Recruitment period: Up to three years from data of ethical approval (10th January 2025 - 10th January 2028)	
	Objectives	Outcome Measures
Key	To evaluate the diagnostic accuracy of 3 host response tests for bacterial and viral infection compared to the reference standard of clinical adjudication	Positive percentage agreement (PPA) negative percentage agreement (NPA), Positive Predictive Value (PPV), Negative Predictive Value (NPV), Overall accuracy, AUROC (all with 95% confidence intervals).
Exploratory	To evaluate the equivalence of EDTA blood and PAXgene RNA blood on gene expression values for Cepheid HR test (in development)	Correlation, Spearman's Coefficient, Kappa statistic

5 Funding and resources

FUNDERS	FINANCIAL AND NON-FINANCIAL SUPPORT
NIHR CRN	Clinical fellow (1.0 WTE for 6 months)
BRC MII theme (via CRF)	Research nurse and Laboratory technician for sample processing
Tristan Clark PI fund	For consumables (Paxgene blood tubes)

6 Role of trial sponsor

The Sponsor is University Hospital Southampton NHS Foundation Trust (UHS), which is the organisation that is taking legal responsibility for the trial.

7 Background

Patients presenting to emergency departments are frequently given antibiotics that they do not need

Around 80-90% of adults presenting to emergency departments (ED) with acute respiratory infection (ARI) are treated with antibiotics (1,2), however much of this is unnecessary as around half of episodes of ARI are caused by viruses (3,4). This unnecessary antibiotic use is directly harmful to patients, as 20% of patients experience adverse events including C.difficile infection (5), and also promotes the development of antimicrobial resistance (AMR). AMR is one of the most serious threats to human health and measures to combat it are a global priority (6).

Testing for the presence of viruses in ARI does not reduce antibiotic use

Meta-analyses of trials of rapid molecular testing for respiratory viruses in hospital inpatients and EDs have demonstrated improvements in patient care across a range of outcomes, including antiviral use and infection control measures (7-12). However, the impact on unnecessary antibiotic use has been minimal (10-12). Human factor research suggests this is because the identification of a virus does not rule out concurrent bacterial infection (13).

Single host-response biomarker tests are not able to distinguish bacterial and viral ARI

The blood host-response (HR) biomarkers C-reactive protein (CRP) and Procalcitonin (PCT) are both raised in bacterial compared to viral ARI but neither is sufficiently accurate to distinguish them with confidence (14). Although studies of CRP testing in primary care have demonstrated antibiotic reductions (15), evidence in secondary care is lacking where CRP is routinely used in the assessment of patients with ARI, in whom antibiotic overuse remains common (4,7,8). In addition, studies of PCT have demonstrated that it is insensitive in detecting serious bacterial infection, including bacteraemia (16). Neither CRP nor PCT are widely used to guide antibiotic initiation in ARI in UK EDs.

The ongoing NIHR-funded PRONTO study (17) is evaluating the use of PCT combined with NEWS-2 score in EDs, in patients with suspected sepsis but does not specifically evaluate patients with ARI, where the underlying aetiology and risk-benefit of withholding antibiotics is different. The proposed PROTECT study is a platform study designed to evaluate multiple different diagnostics for infection but does not focus on AR (18).

Combination HR biomarker tests may be able to distinguish bacterial and viral ARI accurately and reduce unnecessary antibiotic use

Combination HR biomarker tests distinguish between viral and bacterial infections using separate immune response biomarkers for each, and have the potential to reduce unnecessary antibiotic use by reassuring clinicians and patients that bacterial infection is absent. There are currently two combination HR rapid tests that have UK regulatory approval, FebriDx and MeMed BV (19,20).

FebriDx (Lumos diagnostics, Carlsbad, US) is a point-of-care test (POCT) that detects 2 host immune response proteins, one raised with bacterial infection (CRP) and one raised in viral infection (Myxovirus resistance protein A) in a finger-pick blood sample. It is an instrument-free, immunochromatographic, lateral flow-based test with results in 10 minutes. The read-out is the presence or absence of 3 coloured lines assessed by visual inspection, with suggested interpretation provided by the manufacturer, figure 1a (19).

MeMed BV (MeMed, Tirat Carmel, Israel) detects 3 host immune response proteins; CRP, Interferon gammainduced protein 10 (raised in viral infection) and TNF-related apoptosis-inducing ligand (raised in viral and reduced in bacterial infection). The levels of these 3 proteins are computationally integrated into a score. The test uses an analyser (MedMed Key, a small-footprint immunoassay platform) that can be located at the point-of-care. Blood samples are loaded into a cartridge which is inserted into the analyser with results in 15 minutes. The read-out is a score from 0-100, divided into bands which indicate the likelihood of a bacterial or viral infection, based on pre-set cut-off values, figure 1b (20,21).

An alternative approach to detecting host immune response proteins testing is the detection of host mRNA (i.e. transcriptomic) to differentiate bacterial from viral infection. The TriVerity Acute Infection and Sepsis Test (Inflammatix, Sunnyvale, CA, USA), uses an isothermal reverse-transcribed loop-mediated amplification (qRT-LAMP) assay to measure levels of 29 host mRNAs in blood and incorporates machine learning to calculate 3 separate scores predicting the likelihood of bacterial infection, viral infection and illness severity. This 29 gene set classifier has shown good levels of accuracy at distinguishing bacterial and viral infection across a range of clinical infection syndromes. The test is not yet FDA approved or CE marked but this is expected early in 2025.

Why is this research important?

ARI is the commonest reason for antibiotic use in ED and around half of this is unnecessary. Antibiotics are very frequently prescribed to patients presenting to the ED with ARI (1) but around half of these prescriptions are thought to be inappropriate (24) with adults being more likely to be prescribed inappropriate antibiotics than children (25). Recent UK trial data suggest that 80-90% of adult patient presenting with unselected ARI receive antibiotics in ED (7,8).

Unnecessary antibiotic use is driven by aetiological uncertainty

Diagnostic uncertainty regarding the aetiology of ARI has been found to play major role in this (26) and in particular the inability to rule out a bacterial infection in the rapid timeframe required in EDs (13,1). In addition, previous studies have shown that provision of antibiotic prescriptions may be associated with increased patient satisfaction for ED visits for ARI (27) and that ED physicians are more likely to prescribe antibiotics if they perceive that patients want them (28).

AMR is driven by unnecessary antibiotic use and is a global threat

Bacterial resistance develops in response to selective pressure associated with all antibiotic prescribing but is accelerated by inappropriate use (29). A substantial increase in global rates of infections caused by resistant pathogens, in combination with limited new antimicrobial agents in development, has raised concerns of an impending 'post-antibiotic era' with catastrophic consequences for human health (6,30). AMR is now recognised as one of the biggest threats to global health and efforts to preserve the effectiveness of antibiotics through antibiotic stewardship are a priority (5,30).

Diagnostic tests are urgently needed to direct antimicrobials and facilitate stewardship

The need for new diagnostics to help combat AMR has been recognised and the development and assessment of rapid tests that distinguish between bacterial and viral infections have been listed as a priority by the WHO (31). The recently published UK department of health document 'Confronting antimicrobial resistance 2024 to 2029' lists the development and implementation of diagnostic tests

to guide antibiotic use as a key component of its action plan (32). The NIHR's stated aims are to support AMR research including the evaluation of diagnostics to guide antibiotic use

Balancing the risks and benefits of reducing unnecessary antibiotic use

Reducing unnecessary antibiotic uses in ARI needs to be balanced against the risk of undertreating clinically important bacterial infection. Currently used single HR biomarkers (CRP and PCT) lack sufficient accuracy to do this (14). Tests with improved accuracy are therefore needed and must be implemented within a robust and safe testing strategy.

EDs are a priority area for antibiotic stewardships

As the interface between the community EDs are a priority area for antimicrobial stewardship (1) and the impact of antibiotic overuse use in EDs extends far beyond these departments as prescriptions started in ED are usually continued in other departments when patient are admitted, or in the outpatient setting if discharged (1). The use of point-of-care testing in the ED setting could be a critical component of a strategy to reduce AMR (31).

Review of existing evidence

A systematic literature review of published research and trial databases was undertaken using terms related to single and combined HR biomarker tests in terms of diagnostic accuracy, usability and clinical impact. For single biomarker accuracy (CRP and PCT) a recent meta-analysis demonstrated the following levels of accuracy for diagnosing bacterial infection in ARI; CRP (cut off of >50mg/L), sensitivity of 74.9%, specificity of 74.6%; PCT (cut off of >0.1ng/L) sensitivity of 73.6%, specificity of 74%, compared to clinical adjudication (14).

Other than FebriDx and MeMed BV no other combined HR-POCTs with CE marking were found. Excluding studies related only to COVID-19, 5 diagnostic accuracy studies evaluating FebriDx and 6 evaluating MeMed BV were found with 1 recent systematic review and meta-analysis (33) that included all of these studies. 1 NICE MedTech briefing was found that evaluated FebriDx (34). Overall, these studies suggest that both FebriDx and MeMed BV may have improved diagnostic accuracy for diagnosing bacterial infection (~85-95% for both sensitivity and specificity) compared to single biomarker tests. However, all these studies were company sponsored or supported and were assessed as being at high risk of bias, plus estimates of accuracy were imprecise due to small numbers of patient in individual studies. Therefore, the level of certainty of evidence is considered low (33).

Three studies were found evaluating the accuracy of the RNA gene classifier used in the Inflammatix Triverity test, which all demonstrated similar levels of sensitivity and specific for bacterial and viral infection (34-36).

No studies were identified that evaluated the usability of any of the combined HR platforms in ED and only two studies evaluating impact were found (one each for MeMed BV and FebriDX) which were both small, performed in primary care and of very low methodological quality (37,38). No ongoing or planned non-industry studies were found evaluating either the usability or impact of FebriDx , MeMed BV or Triverity on antibiotic use in ED.

The current literature therefore provides a strong rationale for urgent independent studies of the currently available and emerging combined HR-POCTS to confirm company-supported data suggesting higher accuracy compared to single biomarker tests for ruling out bacterial infection, which could facilitate antibiotic stewardship.

Aim, objectives, and outcome measures

7.1 Aims and objectives

To evaluate the diagnostic accuracy for the 2 commercially available combined HR-POCT platforms (MeMed BV and FebriDx) and for the Triverity test, for the diagnosis of bacterial and viral infection, against the reference standard of clinical adjudication.

Outcome measures

Key outcome measures:

Positive percentage agreement (PPA) negative percentage agreement (NPA), Positive Predictive Value (PPV), Negative Predictive Value (NPV), Overall accuracy, AUROC, all with 95% confidence intervals.

Exploratory outcome measures:

To evaluate the equivalence of EDTA blood and PAXgene RNA blood on gene expression values for the prototype Cepheid HR test (Xpert Bacterial vs. Viral assay) .

Study design and methods

We will conduct a fully powered diagnostic accuracy study performed on samples prospectively collected from adults presenting with ARI (7) using the Southampton Research Biorepository (SRB) and including routinely collected clinical data including results of diagnostic testing for viruses and bacteria in addition to baseline clinical, laboratory and radiological data, and outcome data. The reference standard used by regulators for defining bacterial and viral infection is clinician adjudication (as no sufficiently accurate gold standard test exists) which take account of detailed clinical data including diagnostic tests for pathogens, blood test results (eg white cell count, PCT) radiological tests, and other data (37).

In accordance with guidelines, adjudication will be performed independently by three blinded experts, with a system for arbitration where there is discordance (37). Full methods for clinical adjudication are detailed in Tanner A, at al, Journal of Infection 2024 (38) Measures of diagnostic accuracy will be calculated for each HR test separately and compared.

Trial overview for patient-participants

Adult patients with acute respiratory illness in Southampton General Hospital's Emergency Department (ED) and Acute Medical unit (AMU) will be identified and assessed for eligibility for the trial. Potential participants would have been seen and assessed by the triage nurse or other triaging clinician but may not have been fully treated by their clinician. The triage nurse or other triage clinician may highlight potential participants to the study team. Potential participants will receive a participant information sheet, and be invited to ask questions, as part of the informed written consent process.

Once SRB generic consent has been obtained participants will be asked for 2 combined nose and throat swabs (1 sent for immediate respiratory viruses testing and the other to be stored at -80°C), finger-prick capillary blood sampling (for immediate FebriDx testing) and venous blood sampling (for ETDA, serum and RNA PAXgene tubes). Results of the FebriDx test will not be shared with treating clinicians or patients. The other tests will be performed retrospectively on stored samples at least 30 days later and will not influence clinic care.

7.2 Participant eligibility criteria

Inclusion criteria

- Is a patient in the ED or AMU, Southampton General Hospital, UHS
- Aged ≥ 18 years old
- Able to be recruited and sampled within 24 hours of arrival in the ED or AMU
- Has the capacity to consent to the study
- Has at least one of the following acute respiratory symptoms*:
    - Cough
    - Shortness of breath
    - Coryza
    - Sore throat
    - Wheeze
    - Fever (where not definitively explained by another cause)
    - Reported exacerbation of a chronic respiratory condition (e.g., asthma, COPD)

*Acute respiratory symptoms are defined as new symptoms or chronic symptoms with an acute worsening of less than or equal to seven days duration.

We note that patients will have been seen and assessed by a triage nurse or other triaging clinician before being approached to enrol in the trial. However, patients may not have been seen by their treating clinician before being approached to enrol in the trial.

Exclusion criteria

- Not fulfilling all inclusion criteria
- Declines nasal/pharyngeal swabbing, finger prick testing or venesection
- Underlying severe bronchiectasis, cystic fibrosis, severe immune suppression

Patient-participant screening and consent

Participant Identification & Screening

Patients in the ED or AMU who have been seen and assessed by the triage nurse or another clinician may be approached for consideration of enrolment into the trial. Staff in the ED may highlight patients for consideration to the research team. Review of electronic systems and triage notes may assist the research team's identification and assessment of eligibility for potential participants. The research team comprises primarily of doctors and nurses who are familiar with, and often work in, the ED environment. The research team have an established relationship of doing trials in the ED and are integrated into acute clinical care.

Potential participant notes will be compared to inclusion and exclusion criteria to ensure likely eligibility before approaching the patient.

Consent

Discussion of the study will be undertaken with the patient by a research team member and a generic SRB Participant Information Sheet (PIS) provided to the patient. There will be opportunity for the participant to ask questions. Research staff will ensure that only participants that meet all the inclusion criteria and none of the exclusion criteria are enrolled and randomised in the study.

If the patient is willing to participate in the study and fulfils the eligibility criteria, the research team will obtain written informed consent using the generic SRB Informed Consent Form (ICF). In view of

the need for the rapid return of test results to facilitate onward patient care the usual 24-hour consideration period for a participant cannot apply in this study. Given the low-risk nature of sample collection and the brevity of the patient's physical involvement with the study, the research team feels that a shorter time to consider involvement is reasonable.

If the patient is willing to be involved in the study and is able to sign and date the ICF to indicate consent, they will do so. If the patient can provide informed consent but has difficulty writing or otherwise filling in the ICF, informed consent from the patient will be verified by an independent witness (this may be a staff member not associated with the study, or friend or relative of the patient), and the independent witness would then fill in, sign and date the ICF on the patient's behalf. Both the person taking consent and either the patient or independent witness must personally sign and date the ICF. A similar process can be used for patients with an understanding of English that may be insufficient to read and take in the participant information sheet and consent form information, but who retains the capacity to make decisions for themselves. In this instance, the patient must fill in the consent form, but the witness must also sign that they have assisted the person in fully understanding the consent process. Copies of the ICF will be given to the patient (and witness if applicable) and put into the patient's notes. The original ICF is stored securely by the study team.

Each patient will be assumed to have capacity unless it is established that they lack capacity. As this study may not offer any direct benefit to the participants, patients lacking capacity and unable to consent for themselves will not be eligible for this study.

7.3 Procedures for patient-participants

The following samples will be collected from all participants

- A combined nose and throat swab in viral transport media (x 2 as above)
- A finger-prick blood sample for immediate FebriDx testing (patients and clinical teams will be blinded to results as above)
- Venous blood sampling (EDTA, Serum and RNA Paxgene tubes, a total volume of not more than 16mls)

7.4 Withdrawal criteria

A patient-participant may decide to withdraw from the study at any time, without giving a reason, and with no detriment to their medical care or legal rights.

The CI/PI may withdraw a patient from the study in the interests of participant safety or the integrity of the research study, or on the advice of the Sponsor's representative (Research & Development (R&D) department).

Any patient who is withdrawing from the study has the options of withdrawing and having any data and/or samples collected so far retained or withdrawing and having their data and/or samples destroyed (although signed ICFs, minimal personal identifiable information to record the withdrawal, and any completed point-of-care test result will be retained). A note to file would normally be sufficient to record any withdrawal.

8 Safety

8.1 Investigator Responsibilities

The CI is responsible for the overall conduct of the study and compliance with the protocol and any protocol amendments. The CI/PI will be responsible for using medical judgement in assigning seriousness of SAEs and causality.

It is the responsibility of the CI/PI to ensure that all SAEs, as previously defined, are recorded and reported to the Sponsor within 24 hours of becoming aware of the event, and to provide further follow-up information as soon as it is available.

Responsibilities may be delegated to an appropriate member of the study site staff. Delegated tasks must be documented on a Delegation Log and signed by all those named on the list.

9 Statistics and data analysis

9.1 Sample size calculation

Assuming a sensitivity and specificity for the detection of bacterial infection (i.e. bacterial versus non-bacterial aetiology) of ~90%, for the combined HR-POCTs (33), a sample size of 200 samples containing 100 with bacterial (including bacterial/viral co-infection) and 100 patients with non-bacterial aetiology (i.e. viral and non-infected), using the reference standard of clinical adjudication, will allow an assessment of positive percentage agreement (sensitivity) and negative percentage agreement (specificity) with high precision (95%CIs of +/- 5%) (39). In the PIs previous studies around 50% of all unselected patient with ARI have had bacterial infection as their adjudicated aetiology and therefore a sample of 200 patient should provide around 100 with bacterial infection. The samples size is based primarily on the performance of the bacterial component of the HR test as the principal intended use case of these tests is to guide antibiotic use.

9.2 Statistical analysis plan

Test results from the combined HR-POCTs will be compared to adjudicated aetiology using the different adjudication methods. Positive percentage agreement (PPA, used in place of sensitivity to reflect an imperfect reference standard), Negative percentage agreement (NPA, used in place of specificity to reflect an imperfect reference standard), positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (with 95% confidence intervals), will be calculated for both bacterial (vs non-bacterial) and viral (vs non-viral) infection. ROC curves will be generated and AUC with 95%CI calculated. Performance metrics including AUROC will be compared between the tests using Chi Squared test and DeLong test.

10 Data management

10.1 Data Collection Processes

After documentation of the patient's identifiable data in the enrolment log, routinely collected anonymised clinical data will be collected using a secure e-Case Report Forms (eCRF), or in the event the system is unavailable, by paper care report forms and subsequent transcribed to an electronic system.

Baseline demographic and clinical characteristics data will be collected for all patients on or shortly after their enrolment in the study (e.g., age, sex/gender, observations, comorbidities, presumptive diagnosis).

Data collection for outcome measures (antibiotic and antiviral use, length of stay, ICU admission, death) will be collected no less than 30 days after enrolment, by review of electronic systems.

10.2 Source Data

Source data will be recorded and maintained in keeping with GCP principles to facilitate reporting and analysis, and quality control, audit, and inspection.

Baseline demographic and clinical data will be collected on all participants retrospectively from the electronic patient records (EPR). For each participant, these data consists of age, sex, ethnicity, comorbidities, vaccination status where available, observations, and NEWS2, duration of illness, clinical presentation, time of arrival in ED, antibiotic prescribing agent, and time/date of administrations, influenza and COVID-19 antiviral administration, and discharge from the ED.

All data points will be inputted directly to the e-CRF.

10.3 Electronic Case Report Forms

The research team members will be responsible for entering study data in the electronic case report forms (e-CRF). This is intended to an e-CRF. It is the investigators' responsibility to ensure the accuracy of the data entered in the e-CRF. The e-CRF will capture only data required by the protocol and will be an accurate representation of the protocol.

As data will be directly entered into the e-CRFs, they will be considered a source document and copies of the e-CRF will be maintained post-study so that an independent account is available to the CI as well as to the Sponsor.

As all metrics discussed during this protocol are either being dedicatedly assessed by the research team members or will be part of the standard documentation of a hospital attendance, it is expected that the generation of complete e-CRFs should not present obstacles.

10.4 Data handling and record keeping

To maintain participant anonymity, upon enrolment in the study all patients will be allocated a unique participant identification number/code. This unique participant identification number/code will be used on samples and documents after screening and recruitment.

The study team will keep an Enrolment Log of each participant's name, hospital ID number, date of birth, and unique participant identification number/code. Only the CI, co-investigators and relevant members of the research team will have access to the Enrolment Log linking participant details to the participant trial number, which will be securely stored within the CI's institution, behind two locked doors. The participant details will also be recorded on the secure NHS EDGE system.

This unique participant identification number/code is used in documentation following enrolment. Documents that are not anonymous (e.g., signed ICFs) will be maintained separately and securely, in strict confidence, in the ISF within the CI's institution.

After enrolment, anonymised study data will be inputted directly to the ALEA e-CRF. Some of these metrics will be recorded at the time of enrolment and a further retrospective collection of data points will be made from the participant's EPR ensuring outcome measures are assessed over 30 days. Research team members will require their unique UHSNFT usernames and passwords in order to access the participants' EPRs.

All research staff are hospital employees (or have appropriate honorary contracts), and all of the medical research staff on this study have dual clinical and research roles, meaning that they access hospital systems and EPRs on a daily basis as part of their clinical roles.

Due to the inputting of all source data directly to the e-CRFs, physical documentation containing identifiable data will consist of the Screening Log, Enrolment Log and ICFs. These will be stored securely in the CI's institution, in the ISF.

10.5 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audit, or inspection.

10.6 Essential Document Retention & Archiving

Essential documents will include all signed protocols (and any amendments), copies of completed e-CRFs, signed ICFs from all subjects who consented, the Enrolment Log, the Screening Log, REC approvals and all related correspondence including approved documents and study correspondence.

The investigator and/or Sponsor must retain copies of the essential documents in the ISF for a minimum period following the end of the study. This is anticipated to be 15 years after completion of the study. Destruction of essential documents prior to this time will require authorisation from the Sponsor. The CI is responsible for, with the Sponsor, ensuring that documents are archived in accordance with local NHS R&D procedure at the close of the study. All essential documents will be stored securely in the CI's institution.

11 Monitoring

Based on the low risk of harm associated with the intervention in this non-CTIMP pilot study, no interim analysis, or Data Monitoring Committee is planned.

This study may be monitored and audited by University Hospital Southampton NHS Foundation Trust, under their remit as sponsor, and other regulatory bodies to ensure adherence to ICH GCP, UK Policy Framework for Health and Social Care Research, applicable contracts/agreements, and national regulations. All study related documents will be made available on request for monitoring and audit by UHS, the relevant REC or other licensing bodies.

12 Ethical and regulatory considerations

The investigators will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki and in conformity with the ICH GCP and local regulatory Indemnity

13 Further laboratory work on retained samples

The consent provided by participants expressly permits research (which may include validation of molecular and other test platforms for respiratory viruses, and pathogen sequencing as well as the measurement of host response biomarkers) on the stored samples, should any remain after this study has concluded. This work will primarily occur within the Clinical and Experimental Sciences (CES), Faculty of Medicine, University of Southampton, but collaboration with other institutions is possible at the discretion of the Chief Investigator.

Exploratory assays will be carried out on the samples collected in this study at the discretion of the Chief Investigator, with the purpose of studying localised and systemic infection and the human immune responses to infection. The samples acquired from participants and subsequent laboratory analysis will continue after participant recruitment has closed for a period of up to five years. After this period, further application to the relevant ethics committee may be required to continue storing and using the samples, or destroy the samples.

The samples will be stored long-term in appropriate, storage conditions (e.g. -80°C freezers) within the Chief Investigator's institution. Access to these samples is restricted to the Chief Investigator's team and relevant laboratory managers.

The samples collected in this study may be used to evaluate the diagnostic accuracy of other infection-related diagnostic tests including those testing for COVID-19 and other respiratory viruses.

The gold standard assay for respiratory virus quantification is quantitative polymerase chain reaction (qPCR). qPCR may be used to detect and quantify pathogen presence and quantity in the various samples collected.

Samples may be used for host response gene expression studies, where messenger RNA (mRNA) from cells is measured to obtain a "snapshot" of which genes are being expressed. qPCR and whole genome high-density arrays may be used to compare gene expression examining for markers of infection. Techniques such as ELISA and ICS may be used to confirm the results. No studies concerning diseases or traits not connected with infectious diseases will be performed on these samples.

Other exploratory assays potentially include next generation sequencing of samples for detection of possible pathogens, and the development and validation of next generation sequencing and polymerase chain reaction tests. In all studies, human genomic material will not be analysed and, if necessary, will be removed computationally by reference-guided mapping.

Samples may also be tested for antibodies to coronaviruses (serology) and other respiratory viruses, and other immune responses including but not limited to cytokines, C reactive protein, and procalcitonin.

All samples are anonymised of personal identifiable information and identified by participants' study numbers. Anonymised clinical parameters collected can be correlated with these results.

14 Dissemination policy

The investigators and research team will be involved in reviewing drafts of the manuscripts, abstracts and any other publications arising from the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. All data generated in the process of this study is under the control of the CI/PI.

The study results are intended to be disseminated via peer-reviewed journals (which typically have summaries available on the internet) and medical conference posters and presentations. Participants will therefore be able to access the study results via a range of methods. The CI is responsible for the study data.

Funders will be acknowledged in any publications.

We intend for this protocol to be freely available on a public-facing website (e.g., ePrints Soton). The study shall be registered on the ISRCTN Registry, or another international trials registry database.

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