University Hospital Southampton NHS
NHS Foundation Trust

Randomised controlled trial comparing molecular Point-of-Care testing for gastrointestinal pathogens with standard clinical care, in adults presenting to secondary care with suspected infectious gastroenteritis (GastroPOC Trial)

Sponsor: University Hospital Southampton NHS Foundation Trust

Chief Investigator: Dr Tristan William Clark

Confidential

Protocol Modification History

Version	Date	Modifications	Author(s)
1.0	11/05/2016	N/A	Tristan Clark, Nathan Brendish
2.0	30/05/2018	Changed study from pilot to full study Samples size increased to 300 participants from 200 Allows three years for participant recruitment Addition of de-isolation measures as secondary endpoints	Nathan Brendish, Kate Beard, Tristan Clark

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Contents

1. Synopsis

Trial location

Single centre. University Hospital Southampton NHS Foundation Trust, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD

Design

Pragmatic randomised controlled trial with 1:1 allocation to intervention or control arm

Population

Adults aged 18 years old and over presenting to the Acute Medical Unit, Acute Surgical Unit or Emergency Department or inpatient wards with acute diarrhoea and or vomiting (<14 days duration)

Sample Size

300 participants (150 per group)

Intervention

Stool sample and/or rectal swab tested for gastrointestinal pathogens using the FilmArray Gastrointestinal Panel (BioFire Diagnostics) with results communicated to clinical team

Control

Standard clinical care alone

Key Assessment

Subsequent retrospective hospital case note evaluation of clinical data

Primary Objective

Clinical impact assessment

Secondary Objectives

Evaluation of performance of FilmArray Gastrointestinal Panel

Timing

Three years or enrolment of 300 participants, whichever occurs soonest, with a minimum duration of one year (Participant Recruitment)
(With a further five years for laboratory analysis only).

Study Overview

This pragmatic randomised controlled trial will examine the clinical impact of a point-of-care diagnostic test for gastrointestinal pathogen detection (FilmArray Gastrointestinal Panel, BioFire, Salt Lake City, Utah, USA, CE IVD marked) in adults presenting with acute diarrhoea and/or vomiting, compared to routine clinical care. Screened and consented adults with acute diarrhoea and/or vomiting in the Acute Medical Unit, Acute Surgical Unit and Emergency Department and inpatient wards of Southampton General Hospital will recruited and randomised to have a stool sample and/or rectal swab taken and tested for gastrointestinal pathogens by POCT, or to routine clinical care alone. In the event of a pathogen being detected, the clinical team responsible for patient care will be immediately informed of the result. The infection prevention and control team will be notified of results in real time.

The clinical impact of this rapid molecular gastrointestinal pathogen detection test will be assessed by measures including, but not limited to, isolation facility use, antibiotic use, duration of hospital stay, time to diagnosis and diagnostic yield.

The study recruitment period will be across at least one year to include the typical peak periods for seasonal pathogens such as norovirus and campylobacter. The study was originally designed as an internal pilot study, and then amended to be the full randomised controlled trial and health economic evaluation.

2. Abbreviations

AE: Adverse Event
AMU: Acute Medicine Unit
ASU: Acute Surgical Unit
CI: Chief Investigator
CRF: Case Report Form
CV: Curriculum vitae
ED: Emergency Department
GCP: Good clinical practice
GI: Gastrointestinal
GP: General practitioner
HRA: Health Research Authority
ISF: Investigator Site File
MHRA: Medicines and Healthcare Products Regulatory Agency
NHS: National Health Service
PCR: Polymerase Chain Reaction
PI: Principal Investigator
POCT: Point-of-Care-Test
qPCR: Quantitative real time PCR
R&D: Research and Development
REC: Research Ethics Committee
SAE: Serious Adverse Event
UHS: University Hospital Southampton NHS Foundation Trust

3. Background and Rationale

Gastrointestinal pathogens: Epidemiology

There are up to 17 million cases of acute infectious gastroenteritis in the community each year in the UK, excluding outbreaks. Norovirus accounts for about 3 million of these cases and *Campylobacter* for around 80,000 cases.[1]

In acute gastroenteritis cases presenting to primary care in the UK, a wide range of pathogens have been found including *Campylobacter* (13% of cases), norovirus (12%), sapovirus (8.8%), rotavirus (7.3%), adenovirus (3.4%), astrovirus (2.5%), *E.coli* (2.4%), *Cryptosporidium* (1.4%), *Salmonella* (1%), *Giardia* (1%), and in at least half of all cases no pathogen is found.[2] Therefore, testing for a wide range of gastrointestinal pathogens, including bacteria, viruses and parasites, is important to identify the aetiology in acute gastroenteritis.

Infectious gastroenteritis aetiology in adults admitted to secondary care is under-reported. In one small Dutch study, pathogens were identified in about 60% of cases, with rotavirus being the most common pathogen detected. Of note, nearly a third of patients developed renal failure, underscoring the potentially severe nature of gastroenteritis in this group.[3] Another small study, from Germany, found a range of 13 different pathogens in hospitalised adults with gastroenteritis, that 82% had an identifiable causative pathogen. About one-fifth of positive cases had two or more organisms found.[4]

Vaccines that target causes of acute gastroenteritis are only available commercially for rotavirus and *vibrio cholerae* (which cross protects with Enterotoxigenic *E.coli*) meaning that the vast majority of gastroenteritis cases are not currently preventable by vaccination.[5,6]

Gastrointestinal Pathogens: Economic burden

Globally, norovirus infection alone causes around US$4 billion of direct health system costs and US$60 billion in society costs each year. Costs per norovirus illness are highest in adults over 55 years old.[7]

Specific to the UK, norovirus costs, to patients and the health service, about £80 milllion per year and rotavirus about £25 million. *Campylobacter* costs about £50 million per year to the UK, with *Campylobacter*-related Guillain-Barré syndrome hospitalisation about £1.26 million.[8]

Conventional testing

A stool sample from a patient with acute diarrhoea presenting to hospital in England would conventionally undergo a range of laboratory testing methods for different pathogenic organisms. The key methods include microscopy, culture, enzyme immunoassay and PCR.[9] These methods vary in their turnaround times but culture takes several days to generate a result to clinicians. These slow turnaround times mean that patients presenting with suspected gastroenteritis are isolated and often treated with antibiotics empirically leading to unnecessary isolation facility use in those without infection and antibiotic usage in those without bacterial aetiology.

In addition, conventional diagnostic testing is not comprehensive and is insensitive for many pathogens leading to many missed diagnoses and lack of confidence in a negative result.

Potential benefits of molecular point-of-care testing in acute gastroenteritis

<u>Improved pathogen detection compared to conventional testing</u>

A pan-European observational study showed the pathogen detection rate in stool samples from patients with gastroenteritis could be improved from 18% using traditional methods, to 54% using the multiplex rapid PCR FilmArray Gastroenteritis Panel. Site selection bias, a mix of adult and paediatric and inpatient and outpatient populations and heterogeneous local testing methods weaken the findings. Nevertheless, this multicentre, cross-sectional study clearly shows a wide spectrum of pathogens were detected; 20 different GI pathogens were detected, out of 22 tested for, in around 700 stool samples.[10]

Other studies have suggested similar improvements in diagnostic yield, including a US-based study of 230 prospectively collected stool samples, with 8.3% positivity with conventional diagnostics compared to 33.3% positivity with the FilmArray GI panel. Again, a broad range of pathogens were observed.[11]

A paediatric-based study comparing the aetiologic yield of standard-of-care microbiologic testing ordered by physicians with that of the multiplex FilmArray GI panel, showed that identification of a pathogen increased from 46% to 65%.[12] This suggests a syndrome-based approach to testing may be clinically beneficial.

Decreased length of hospitalisation

In a single pre-and post-implementation observational study in a hospital setting, a rapid molecular test for *Clostridium difficile*, led to faster turnaround times compared with conventional toxin assays and a decrease in the length of hospitalisation.[13]

Reduced and directed use of antibiotics

A single-centre non-randomised study in France demonstrated that rapid molecular testing for *C.difficile* results in faster results compared to conventional tests and speeds up initiation of appropriate antimicrobial therapy.[14]

More appropriate use of isolation facilities

With the lack of diagnostic yield and slow turnaround time of current testing methods, patients with infectious gastroenteritis may not be appropriately isolated, risking spread of disease. One US study suggested that 60% of patients with gastroenteritis, who were eventually found to have had an infectious aetiology, were never placed in appropriate isolation during hospitalisation. In addition, just over 20% of isolated patients ultimately had negative test results and so could have been removed from isolation facilities.[15] A UK study suggested that half of all inpatients with potentially infectious gastroenteritis may have the opportunity for earlier de-isolation if tested with a rapid multiplex molecular test compared to routine care.[16] There was a significant large cost saving associated with this from health economic modelling.[17]

A systematic review has noted that POCTs for norovirus have the potential to improve infection control measures but that mediocre sensitivity of the tests and a small number of studies limit this conclusion.[18]

Multiplex PCR therefore has the potential to rationalise and improve the use of valuable isolation facilities, improved the flow of patients through acute areas and potentially to reduce costs.

FilmArray Gastrointestinal Panel

The FilmArray Gastrointestinal (GI) Panel is FDA-cleared and CE-marked multiplex rapid PCR (molecular) system. Specific targets and operating information are listed in a dedicated section in this protocol below. Specificity and sensitivity for a wide range of pathogen detected is close to 100%.[11,19,20]

Point-of-care testing in the wider context

The UK Prime Minister commissioned Review on Antimicrobial Resistance, supported by the UK Government and Wellcome Trust, described point-of-care tests as “a central part of the solution,” and recommends governments, regulators and other health system leaders to support the uptake and use of these tests in primary and secondary care.[21]

The Department of Health commissioned Carter report into UK pathology services noted the importance of developing clinically relevant point-of-care diagnostic tests to reduce turnaround times and improve patient pathways.[22] The MHRA document 'Management and Use of Point-of-Care Test Devices' sets out the context in which POCT should be

considered for use and gives some guidelines for their successful and safe implementation[23]. The objectives of this study are in line with these documents and it aims to examine the initial phase of a POCT programme; establishing a clinical need for the test, validating diagnostic accuracy and evaluating potential clinical and health economic benefits.

Conclusion

This study is designed to prospectively evaluate the clinical impact of point-of-care testing for gastrointestinal pathogens using the FilmArray GI Panel compared to standard clinical care, in adult patients presenting to the secondary care with acute diarrhoea and/or vomiting. Potential benefits include improved use of side rooms, antibiotics and a reduced length of stay. Principally this is a randomised controlled trial examining in detail clinical outcomes and including a health economic evaluation.

4. Objectives

The primary objective of the study is to measure the clinical impact of point-of-care diagnostic testing for gastrointestinal pathogens in adults presenting to secondary care with acute diarrhoea.

The secondary objectives are to evaluate the ease of use and turnaround time of the FilmArray gastrointestinal panel point-of-care diagnostic test kit compared with standard laboratory-based methods and to inform the design of a larger full randomised controlled trial and health economic analysis.

5. Study Design and Methods

This is a pragmatic, single-centre, parallel group, open-label, randomised controlled superiority trial.

Consent is discussed and agreed as per the separate consent form and participant information sheet from potential participants, in-line with the inclusion and exclusion criteria. Blinding is not employed as it is impractical and would obstruct study methods. The study will take place across at least one year to include the peak periods of seasonal pathogens.

<u>Randomisation</u>

Participants will be enrolled and assigned a participant identification number consecutively. Once a patient has been screened and consented a study team member will log onto the password-protected electronic randomisation website (sealedenvelope.com, which uses randomised permuted blocks) to obtain a randomisation code for the patient who will then be allocated to either the intervention or control group.

<u>For those randomised to the interventional arm:</u>

A stool sample will be taken by a member of research staff (doctor or nurse). In the event of the patient being unable to provide a timely stool sample, a rectal swab will be obtained with appropriate chaperone as per hospital policy. The sample is placed in Carey-Blair media and analysed on the FilmArray using the Gastrointestinal Panel, as per training delivered by the apparatus manufacturer. Test results are generated in about 1 hour. In the event of a run failure, the analysis run will be repeated using the same sample; if there is insufficient sample left, further samples may be taken if the participant consents. If a rectal swab was

tested initially, a stool sample will be subsequently obtained at the earliest opportunity and then tested and the results checked to ensure concordance.

The results of the test will be documented in the patient's case notes. In the event of a pathogen being detected, a member of the clinical team responsible for the patient will be directly informed. The participant will also be informed of the result. If a positive result for a bacterial pathogen is detected the clinical team will be directed to the local and national treatment guidelines for that pathogen.

For all positive and negative results the infection and prevention control team and the site managers responsible for side room allocation will be informed in real time.

Further laboratory stool testing will be at the discretion of the responsible clinical team.

For those randomised to the control group

These patients will be managed according to standard clinical care. Laboratory stool testing will be at the discretion of the responsible clinical team and where performed will be by standard laboratory diagnostic testing.

A stool sample and/or rectal swab will also be taken from patients in the control group and stored for testing at a later date using the FilmArray GI panel. Samples will be taken on enrolment, frozen and stored, and run on the FilmArray at least 30 days after collection. The results will not influence patient care but is scientifically important as it will allow a direct comparison of diagnostic yield between the groups.

For both control and intervention groups:

Clinical and demographic data will be collected by the study team at the time of enrolment. After patients have been discharged or after 30 days (whichever is soonest), clinical data may be collected retrospectively from electronic and physical case notes, including electronic prescribing which will allow data relating to outcome measures to be recorded by the study team. These include: antibiotic use, duration of hospitalisation, isolation facility use, time to diagnoses and test turnaround time, in addition to safety outcomes and in line with all pre-specified outcome measures. All data will be entered onto a standardised case report form and entered into a secure database.

Participants may be approached for blood sampling (a maximum of 21mls) and additional stool samples and/or rectal swabs and/or vomitus samples to be stored for further study including immunological and pathogen sequencing. All samples left-over from testing may be used for further study. Participant consent for this is included in the consent form.

A participant experience and/or satisfaction survey may be collected from participants after their involvement in the trial.

6. Recruitment / screening / inclusion / exclusion criteria

Recruitment and Screening

Eligible patients in the acute medical unit (AMU), acute surgical unit (ASU) and emergency department (ED) and inpatient wards will be identified by research staff who will regularly review the comprehensive admissions IT systems daily.

Inclusion Criteria

- Aged 18 years or over.
- Has the capacity to give informed, written consent and is able and willing to adhere to the study procedures
- Is a patient in Southampton General Hospital ED, AMU, ASU or inpatient ward
- Can be recruited to the study
    - within a 48 hour period of first triage by ED staff OR
    - within a 48 hour period of arrival on AMU or ASU or inpatient ward (if admitted directly to an inpatient ward)
- Has an acute diarrhoeal illness and /or vomiting*
- Has a duration of illness less of than or equal to 14 days

*An episode of acute diarrhoea is defined as the passage of at least 3 loose stools for at least 1 day.

Exclusion Criteria

- Patients not fulfilling inclusion criteria
- A palliative approach being taken by the treating clinicians
- Previously included in this study and re-presenting within the last 30 days after hospital discharge
- Declines to give stool sample and/or rectal swab

Involvement in other research trials is not necessarily an exclusion criterion. Concurrent, prior or subsequent enrolment in an observational study is not expected to be an exclusion criterion, except at the discretion of the PI.

7. Further information regarding the FilmArray Gastrointestinal Panel

The FilmArray Gastrointestinal Panel is a rapid diagnostic test that uses nested RT-PCR followed by melt curve analysis to detect 22 targets. The apparatus takes about an hour to give results. It is deployable as a point-of-care test and the units will be housed in the acute areas for this study. The manufacturers are BioFire Diagnostics, 390 Wakara Way, Salt Lake City, Utah 84108, USA and their website is www.BioFireDx.com. BioFire Diagnostics is owned by bioMérieux. The FilmArray Gastrointestinal Panel is CE IVD marked and FDA (USA) approved.

The following targets are detectable:

Bacteria

Campylobacter (jejuni, coli and *upsaliensis)*
Clostridium difficile (toxin A/B)
Plesiomonas shigelloides

Salmonella
Yersinia enterocolitica
Vibrio (parahaemolyticus, vulnificus and cholerae)
Vibrio cholerae
Diarrheagenic *E. coli/Shigella*:
Enteroaggregative *E. coli* (EAEC)
Enteropathogenic *E. coli* (EPEC)
Enterotoxigenic *E. coli* (ETEC) *lt/st*
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*
E. coli O157
Shigella/Enteroinvasive E. coli (EIEC)

Parasites

Cryptosporidium
Cyclospora cayetanensis
Entamoeba histolytica
Giardia lamblia

Viruses

Adenovirus F 40/41
Astrovirus
Norovirus GI/GII
Rotavirus A
Sapovirus (I, II, IV and V)

The company product information sheet states “The FilmArray Gastrointestinal (GI) Panel tests for common gastrointestinal pathogens including viruses, bacteria and parasites that cause infectious diarrhea.*(sic)* The integrated FilmArray system brings sample to results in about an hour, with only 2 minutes of hands-on time.”

The peer-reviewed published evidence relating to the diagnostic accuracy of the FilmArray Gastrointestinal Panel is discussed in the ‘Background’ section.

8. Analysis

The retrospective review of medical records will allow comparison of participants who received the rapid diagnostic test and controls managed with standard clinical care.

Primary outcome measure

- Duration of time in a side room

Secondary and exploratory outcomes

- Duration of time in a side room for pathogen positive patients
- Duration of time in a side room for pathogen negative patients

- Proportion of patients isolated in a side room
- Proportion of pathogen positive patients isolated in a side room
- Proportion of pathogen negative patients isolated in a side room
- Proportion of pathogen negative patients de-isolated
- Proportion of pathogen positive patient de-isolated

- Time to patient isolation in a side room
- Time to de-isolation in pathogen negative patients

- Proportion of patients treated with antibiotics
- Proportion of patients with bacterial gastroenteritis treated with antibiotics
- Proportion of patients without bacterial gastroenteritis treated with antibiotics
- Time to treatment with antibiotics
- Duration of antibiotics

- Duration of hospitalisation

- Proportion of patients with a pathogen detected
- Proportion of patients with bacterial pathogen detected

- Time to diagnosis
- Missed diagnoses

- Other medication use, complications (including acute kidney injury), ICU admissions, 30 day mortality, representation and readmission.

- Concordance between results obtained from rectal swab and stool culture
- Time from sampling to availability of results (Turnaround time)
- Patient satisfaction scores (using the modified NHS Adult Inpatient Survey Questionnaire 2016)

Sample size and power calculation

The initial phase of this study was designed as an internal pilot study with exploratory outcomes and accurate samples size calculations were not possible. After recruiting 100 patients (50 per group) we now have accurate data on the mean and standard deviation of duration of side room use, and withdrawal rate, allowing us to calculate samples size needed for the primary outcome. 141 patients per group will give 90% power at a 0.05 significance level to detect a 1 day reduction in the mean duration of side room use; from 3 to 2 days (with a standard deviation of 6.7 days).[4] This reduction is be considered clinically and economically significant. Allowing for a ~5% withdrawal rate we will recruit 150 patients per group (300 in total).

Recruitment numbers

300 participants will be recruited and randomised 1:1 intervention to routine clinical care, i.e. 150per group

9. Safety

The risks of stool samples, rectal swabs, vomitus samples and additional blood tests being taken are minimal and where occurring are likely to be of very low impact.

An Adverse Event (AE) is any untoward medical occurrence – there does not need to be a causal relationship between the occurrence and the study.

A Serious Adverse Event (SAE) is any adverse event that

- Results in death
- Is life threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Consists of a congenital anomaly or birth defect

As participants in ED are not yet hospitalised but have a reasonable likelihood of being admitted to hospital, patients enrolled in ED who are subsequently admitted to the hospital will not automatically be counted as having experienced a SAE.

Participants who are already admitted to AMU/ASU are already hospitalised however, an adverse event leading to prolongation of their existing hospitalisation would count as a SAE.

In the event of a SAE, the PI will be involved in deciding whether this was a study-related event.

SAEs occurring more than 30 days after the patient has left hospital will not be recorded or reported as SAEs because of the time lapsed in relation to the event and an acute infection or POC testing.

10. Statistical Evaluation

This will be performed by the research team in conjunction with a dedicated medical statistician from the University of Southampton. Patients tested with the rapid molecular diagnostic test will be compared with patients treated using standard clinical care using standard descriptive and comparative statistical methods using Prism (GraphPad Software Inc; La Jolla, California) and SPSS (SPSS, Inc; Chicago, Illinois). The primary outcome measure of duration of time in side room isolation will be compared using the students t-test or Mann-Whitney U test as appropriate. The effect of group (intervention or control) on the primary outcome will be further assessed using logistic regression to control for demographics (age, sex) and relevant clinical variables.

Analysis will be by intention-to-treat. No interim analysis is planned. Trial results will be reported in accordance with the CONSORT statement. Missing data was minimal in the CI's previous molecular POC RCT in secondary care and therefore expected to not be a significant issue.

11. Ethics and Approvals

<u>Declaration of Helsinki</u>

The Investigators will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki.

ICH Guidelines for Good Clinical Practice
The Investigators will ensure that this study is conducted in full conformity with the ICH Good Clinical Practice (GCP) and local regulatory requirements.

Informed Consent
Written, informed consent will be obtained, as per the informed consent form.

Submissions to HRA, REC and local R&D
The protocol, informed consent form, participant information sheet and GP letter (and any other document requested) will be submitted to the Health Research Agency (HRA) for their processes including Regional Ethics Committee (REC) for written approval, and the study will not commence until REC all necessary HRA approvals are in place. The Chief Investigator will submit and, where necessary, obtain approval from the REC for all subsequent substantial amendments to the protocol and informed consent document. Local R&D approval will be confirmed prior to study commencement.

Participant Confidentiality
All data will be anonymised: volunteer participant data will be identified by a unique study number in the CRF and database. A separate confidential file containing identifiable information will be stored in a secured location in accordance with the Data Protection Act 1998. Only the Sponsor's representative and investigators will have access to the information.

Investigator Responsibility
The Chief Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. Responsibilities may be delegated to an appropriate member of study site staff. Delegated tasks must be documented on a Delegation Log and signed by all those named on the list.

Publication Policy
The investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

12. Data management

The study staff will be responsible for entering study data in the CRF. It is the investigator's responsibility to ensure the accuracy of the data entered in the CRFs.

The subjects' anonymity will be maintained. The study team will keep a separate log of each subject's name, hospital number and NHS number, date of birth, and unique participant trial number. The participant details will be recorded on the secure NHS Edge system in a similar manner. The participant trial number is used on documents after screening to maintain confidentiality. Documents that are not anonymous (e.g. signed informed consent forms) will be maintained separately, in strict confidence.

Only the research study team and sponsor's representatives will know the identity of subjects and have access to the list linking participant details to the participant trial number.

Essential Document Retention

Essential documents, as defined by ICH GCP, include all signed protocols and any amendment(s), copies of the completed CRFs, signed informed consent forms from all subjects who consented, hospital records, and other source documents, REC approvals and all related correspondence including approved documents, study correspondence and a list of the subjects' names and addresses.

The investigator and/or sponsor must retain copies of the essential documents for a minimum period following the end of the study. This period is defined by local guidelines where the research is being conducted. For all subjects that are entered into the study, the medical notes and electronic systems may be marked in line with local R&D guidelines to alert other users of the notes and systems to the patient's enrolment in this study.

The chief investigator, with the sponsor, will ensure that documents are archived in accordance with local NHS R&D procedure.

Data monitoring

On the basis of the very low risk of harms associated with the intervention in this non-CTIMP trial no data monitoring committee or interim analysis is planned.

13. Finances and indemnity

This is an NHS-sponsored study. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm.

14. Other personnel

Key study personnel in addition to the Chief Investigator & Co-investigators include:

- Clinical Research Fellows and other doctors in Infectious Diseases, Acute Medicine and Gastroenterology at University Hospital Southampton NHS Foundation Trust

- Research Nurses and Clinical Trials Assistants at UHS

For the sponsor and R&D contact: Christine McGrath, Director of R&D, University Hospital Southampton NHS Foundation Trust; Tel: 02381208215 Fax: 02381208678; christine.mcgrath@uhs.nhs.uk

15. Laboratory analysis plan

Exploratory assays will be carried out on the samples collected in this study at the discretion of the Principal Investigator, with the purpose of studying localised and systemic infection and the human immune responses to infection. The laboratory analysis will continue after participant recruitment has closed for a period of up to five years.

The gold standard assay for viral detection and some bacteria and parasites is quantitative polymerase chain reaction (qPCR). qPCR may be used to detect and quantify pathogen presence in the samples collected. For stool samples, microscopy, culture and antimicrobial sensitivity testing may be performed where PCR is inappropriate or inadequate.

The standard assay of cell-mediated immunity performed in the laboratory is the interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISPOT) assay, using peripheral blood mononuclear cells (PBMCs) obtained from participants. For the ex vivo IFN-γ ELISPOT assay, thawed PBMCs are stimulated with specific pathogen peptides, control peptides (e.g., purified protein derivative) or other appropriate antigens.

Other exploratory cellular immunity assays may be performed including include intracellular cytokine staining (ICS) and flow cytometry (fluorescence−activated cell sorting, FACS) techniques, proliferation assays, cell culture including cultured ELISPOT, ELISPOT for other cytokines and specific assays assessing cell function and response to viral and other pathogen infection and acute gastroenteritis. Multiplexed technologies of antibody measurement such as Luminex may be used to assess levels of many cytokines simultaneously.

Samples may be used for gene expression studies, where messenger RNA (mRNA) from cells is measured to obtain a “snapshot” of which proteins are being produced. 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 gastrointestinal disease will be performed on these samples.

Other exploratory assays potentially include next generation sequencing of samples for detection of possible pathogens that are not conventionally tested for or are novel. In these studies, human genomic material will not be analysed and will be removed computationally by reference-guided mapping.

The samples are anonymised of personal identifiable information, and identified by the participant’s study number. Anonymised clinical parameters collected can be correlated with these results. The consent provided by participants expressly permits further research on these samples. This work will primarily occur within the Academic Unit of Clinical and

Experimental Sciences, Faculty of Medicine, University of Southampton, but the pioneering nature of this work may mean collaboration with other institutions at the discretion of the PI.

16. Role of BioFire

BioFire Diagnostics, (Salt Lake City, UT, USA, a bioMérieux company) will provide the FilmArray machines and FilmArray Gastrointestinal Panels used for this study but have had no role in the conception or design of this study and will not have any role in the conduct of the study, data analysis, interpretation or preparation of manuscript for submission to scientific journals. Only the research team and medical statistician will have access to the data prior to publication.

17. References

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