TEST-IT

POINT OF CARE TESTING FOR SEPSIS IN ICU PATIENTS: A DIAGNOSTIC ACCURACY STUDY

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PROTOCOL AUTHORISATION

Protocol Title	Point Of Care Testing For Sepsis In ICU Patients: A Diagnostic Accuracy Study
Protocol Acronym (if applicable)	TEST-IT
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A review of the protocol has been completed and is understood and approved by the following:

RONAN MC MULLAN		05 / 07 / 2016
Co-Chief Investigator Name	Signature	Date
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EVIE GARDNER	Evie Gardner	6 / 7 / 16
Senior Statistician	Signature	Date

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LIST OF ABBREVIATIONS

Abbreviation / Acronym	Full Wording
APACHE	Acute Physiology and Chronic Health Evaluation
BHSCT	Belfast Health and Social Care Trust
CI	Chief investigator
CRF	Case report form
EU	European Union
GCP	Good Clinical Practice
HRA	Health Research Authority
ICH	International Conference on Harmonisation
ICU	Intensive care unit
ISF	Investigator site file
ISRCTN	International Standard Randomised Controlled Trial Number
MHRA	Medicine and Healthcare Products Regulatory Agency
NHS	National Health Service
NICTU	Northern Ireland Clinical Trials Unit
PI	Principal investigator
PCR	Polymerase chain reaction
POC	Point of care
PSSRU	Personal Social Services Research Unit
REC	Research Ethics Committee
RMP	Registered Medical Practitioner
ROC	Receiver operator characteristic
SDV	Source data verification
SOFA	Sequential Organ Failure Assessment
SOP	Standard operating procedure
SSC	Surviving Sepsis Campaign
STARD	Standards for Reporting of Diagnostic Accuracy
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UK	United Kingdom

1 STUDY SUMMARY

Protocol Title	Point Of Care Testing For Sepsis In ICU Patients: A Diagnostic Accuracy Study
Health condition(s) or problem(s) studied	Sepsis
Study Design	A prospective, observational, multi-centre blinded accuracy study of a novel diagnostic.
Study Aim and Objectives	**Aim** To assess the diagnostic accuracy of the Randox point of care (POC) multiplex polymerase chain reaction (Multiplex PCR) test in critically ill patients with suspected sepsis. **Primary Objective**: The primary objective of this study is measurement of the sensitivity, specificity, positive predictive value and negative predictive value of this novel test, in comparison with conventional culture, in critically ill adults with suspected sepsis. **Secondary Objective**: The secondary objectives of this study are: (i) to estimate the true cost per test for conventional blood culture and for multiplex PCR (ii) to estimate the time to result for conventional blood culture and for multiplex PCR
Primary Outcome	The primary outcome measure is diagnostic accuracy of the multiplex PCR test for sepsis in ICU patients, expressed as sensitivity, specificity, and positive and negative predictive values, with uncertainty expressed using 95% confidence limits.
Secondary Outcomes	Secondary outcome measures of the study are based on: (i) economic measures; (ii) evaluation of the primary outcome measure as when multiplex PCR testing is conducted at the point of care (vs. centrally); and

	(iii) the time to result for both the PCR test and its paired blood culture.
Inclusion and Exclusion Criteria	**Inclusion criteria**: 1. Patients with suspected sepsis undergoing blood sampling for culture in the course of routine care. **Exclusion criteria**: 1. Patients aged <16 years old. 2. Patients previously recruited to the study. 3. Consent declined.
Countries of Recruitment	United Kingdom
Study Setting	Adult intensive care units (ICU)
Target Sample Size	4501 samples
Study Duration	21 months

STUDY TEAM

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3. BACKGROUND AND RATIONALE

3.1 Lay Summary

Sepsis is the term used to describe serious infections. Up to half of hospitalised patients with sepsis may die. It is caused by microbes, such as bacteria, and one of the most important things in treating patients with sepsis is to give them the right antibiotics as soon as possible to treat the underlying infection.

Many different microbes can cause sepsis. Currently the only way to find out for sure which one to target in any particular patient is to wait for it to grow in a laboratory from a sample of their blood, or other samples. As it takes at least 24-48 hours to grow in the laboratory, doctors choose 'best guess' antibiotics that can treat a lot of different microbes before they know which one would be the best fit. These are not always the right antibiotics for that particular individual, and sometimes patients only get the right treatment once there is a result from the laboratory.

Randox Ltd has recently developed a new bedside device based on technology that is able to identify bacteria in patients' blood within just one hour. Looking only for characteristic fragments of over 40 different microbes means that doctors' decisions about which treatment to give patients will not need to wait for over a day for the microbe to grow in a laboratory. This will allow treatments to be better targeted from a much earlier stage.

We will evaluate the new test in at least 15 intensive care units (ICUs) across the UK and on 4501 blood samples from patients. Whenever a blood sample is taken for culture as part of routine care, a sample will also be taken for analysis with the new test. An additional sample will also be taken for research to develop future tests. Results will be compared to the laboratory culture each time.

3.2 Background Information

3.2.1 Sepsis Management

The current internationally agreed definition of sepsis relies on the suspicion of infection in the context of a systemic inflammatory host response for which there may otherwise be many causes[1]. Microbiological evidence is, therefore, crucial in confirming sepsis in the critically ill where the diagnosis is otherwise difficult to discern, while rapid decision making and intervention too is important. More than 35% of patients in European ICUs are admitted with, or develop, sepsis[2] and the incidence of sepsis is increasing worldwide[3]. The mortality of severe sepsis is consistently reported around 35% with upper estimates of 50%[2,4-6]. In the UK, sepsis has been estimated to account for between 36,000 and 64,000 deaths annually[4] - more than those caused by breast, colorectal and prostate cancer combined – and represents 46% of all intensive care bed-days[7]. Delay in the diagnosis of sepsis is one of the major barriers to the timely implementation of treatments and, consequently, optimal outcomes for patients with this condition[8].

The Surviving Sepsis Campaign (SSC) and the consortium of 30 organisations 2004-2012[9-11], has delivered international guidelines on the management of sepsis that utilise a bundle approach and emphasise early intervention given the wealth of evidence for its clinical importance. The time to appropriate antibiotics is the single greatest predictor of survival in septic shock[12]. Studies have repeatedly demonstrated an increase in mortality in both septic shock[13] and severe sepsis[14] when antibiotics are delayed beyond even just one hour; with an increase of 7.6% each hour shown in one cohort study[15].

The concept of a 'golden hour' of assessment and intervention, initially described in other clinical settings, is therefore now well established in sepsis management[12]. In SSC guidelines, both blood culturing and the administration of broad-spectrum empirical antibiotic treatment are recommended in the first hour after the recognition of potential sepsis. A 2010 review of the implementation of SSC guidelines across two continents demonstrated a 5.4% improvement in mortality even with only limited compliance[16] although several studies also show that the empirical antibiotics received by 20-40% of patients in ICU are found to be inappropriate when the pathogen is later identified[13,17]. Therefore, a rapid test for pathogen identification offers the opportunity to improve patients' treatment and outcomes.

Microbiological sampling in sepsis is independently associated with patients' survival[18] and the SSC recommends at least two samples for traditional culture prior to the administration of antibiotics, within the first hour[11]. Evidence on the sensitivity of blood cultures is conflicting[19]. The value certainly varies with the pathogen, but it is well accepted that it only approaches 100% by increasing the number of samples taken[20].

The emergence of multi drug resistant pathogens has gone from concerning case report to a challenging global priority over recent years- with particular relevance and incidence in intensive care settings worldwide[21,22]. Empirical antibiotics contribute to resistance[23-25] but remain essential to management with existing microbiological techniques that cannot provide a point of care result to identify a pathogen and guide more appropriate prescription[26]. There is agreement that current diagnostic tools for sepsis are inadequate[27]. The clinical need is clear: new, rapid, diagnostic methods that will facilitate prompt treatment with, effective, antibiotic therapy. To have an impact on patient management and outcomes, a novel diagnostic test in sepsis is required to be truly point of care, to provide ease of use, economy and portability, with a time to result of less than one hour.

3.2.2 Multiplex Polymerase Chain Reaction (Multiplex PCR) in Sepsis

For several years, researchers and clinicians have recognised microarray PCR as a promising technique for the rapid identification of sepsis pathogens[28]. PCR techniques have revolutionised diagnostics in infectious diseases, particularly for viral infections, since their widespread deployment in clinical laboratories[29]. There has been limited extension of this technology to detection of bacterial infections in routine practice, though technical progress and limited clinical evaluations have been undertaken[30-33].

One observational study across 9 ICU units and 529 patients showed that a novel laboratory-based device (combining PCR with electro spray ionisation mass spectroscopy) could improve the diagnostic yield of microbiological sampling up to threefold compared to conventional culture and potentially identify a large number of pathogens within 8 hours[34].

Time to pathogen detection has been related to the length of hospital stay[35]. POC testing may further impact the time to result. Multiplex PCR has the potential to reduce the risk of initial antibiotic treatment being inadequate[36]. It is plausible, therefore, that benefits from adopting this technology may be both clinical and financial. Earlier analyses have predicted an absolute mortality reduction of 2.6% with the introduction of PCR techniques, and modelled that costs would be readily recovered through the savings that might be made in a setting such as intensive care[17]. Therefore, there is an opportunity to address this need, of particular importance in the setting of sepsis, and at the point of care.

3.3 Rationale for the Study

Best practice in sepsis is currently centred around the empirical treatment of patients pending definitive laboratory results which are available around 48 hours later. Delay in prescribing antibiotics that are appropriate for the pathogen causing a given patient's infection is associated with increased mortality and other adverse outcomes for patient.

The optimal management of sepsis is therefore still limited by the time it takes to accurately identify a causative organism. No significant progress for culture-based diagnostics has been implemented for decades.

The introduction of Multiplex PCR has been cited for several years as a possible solution to shortening the time to diagnosis. Multiplex PCR devices for the identification of sepsis pathogens currently show increased diagnostic yield when used to supplement conventional culture and can significantly reduce the time to a result. PCR may reduce delay in the focused management of sepsis; extrapolation from observational studies[12-15] further implies potential for reduction in mortality associated with rapid diagnostic tests.

The emergence of multiply resistant pathogens and the need for improved antimicrobial stewardship are major healthcare issues. Empirical use of broad-spectrum antibiotics contributes significantly to resistance in both the individual and in the population's ecology. Measures to reduce the use of broad-spectrum antibiotics wherever possible are therefore advocated worldwide.

Randox Laboratories Limited, a clinical diagnostic company based in Northern Ireland, have recently developed a point of care (POC) testing device utilising Multiplex PCR for the detection of sepsis pathogens. Point of care testing would provide a result in less time than current laboratory based multiplex PCR techniques and could genuinely advance the focused management of sepsis towards improved patient outcomes. Facilitating early and focused antibiotic therapy for critically ill patients with suspected sepsis, and reducing the use or duration of broad-spectrum antibiotics, would have beneficial consequences for both patient outcomes and antibiotic conservation.

In order to have confidence in the diagnostic accuracy of the new test, it needs to undergo validation across a range of hospital sites and a broad case mix of patients. This will enable the assessment of the accuracy, NHS deliverability and potential for impact of the test in the management of suspected sepsis.

This study will test the use of a novel POC Multiplex PCR platform by assessing its accuracy in comparison with conventional cultures.

4. STUDY AIMS AND OBJECTIVES

4.1 Research Hypothesis

The Randox POC Multiplex PCR test has high diagnostic accuracy, in comparison with conventional culture, for detecting pathogens in critically ill adults with suspected sepsis.

4.2 Study Aim

The overall aim of this study is to assess the diagnostic accuracy of the Randox POC Multiplex PCR test in critically ill adults with suspected sepsis.

4.3 Study Objectives

4.3.1 Primary Objective:

The primary objective of this study is measurement of the sensitivity, specificity, positive predictive value and negative predictive value of this novel test, in comparison with conventional culture, in critically ill adults with suspected sepsis.

4.3.2 Secondary Objectives:

The secondary objectives of this study are:

(i) To estimate the true cost per test for conventional blood culture and for multiplex PCR

(ii) To estimate the time to result for conventional blood culture and for multiplex PCR

5. OUTCOME MEASURES

5.1 Primary Outcome Measure

The primary outcome measure is diagnostic accuracy of the multiplex PCR test, expressed as sensitivity, specificity, and positive and negative predictive values, with uncertainty expressed using 95% confidence limits.

In the primary analysis, these will be calculated by using the paired blood culture as the reference standard (i.e. the blood culture that had been taken contemporaneously). True positivity will be defined as the PCR test detecting the same genus of organism as identified in the paired blood culture.

Acknowledging that there is no perfect reference standard for sepsis, a secondary analysis will be undertaken, using a less stringent reference standard to resolve discrepant results in a 'fair umpire' test[37]. This will be applied when the PCR test is positive and the paired blood culture is negative. In this analysis, true positivity will be defined as either:

(i) culture of the same genus of organism in blood or other sterile site culture within 7 days of the test sample being taken; or

(ii) culture of the same genus of organism from a non-sterile anatomic site within 3 days of the test sample being taken which is judged, by the patient's clinical team, to represent a focus of infection.

Furthermore we will undertake analysis of the primary outcome measure when multiplex PCR testing is conducted at the point of care, vs. centrally, using both the paired blood culture and the fair umpire test. The primary outcome (diagnostic accuracy) will be re-analysed including only those samples tested at the point of care and compared to the same metrics obtained when only those samples tested centrally are included.

5.2 Secondary Outcome Measures

Secondary outcome measures of the study are based on:

(i) Economic measures, including resource use associated with the multiplex PCR testing and conventional blood culture

(ii) The time to result for both the PCR test and its paired blood culture.

5.3 Exploratory Outcome Measures

There are a number of exploratory outcomes which include:

(i) Neutrophil activation biomarkers which may include but are not limited to measurement of plasma MPO and MMP-8.

(ii) Plasma and serum inflammatory response biomarkers which may include but are not limited to measurement of CRP, cytokines (including but not limited to TNFα, IL-1β, IL-6, IL-8), proteases and anti-proteases, adhesion and activation molecule expression (including but not limited to sICAM-1), NETs, coagulation factors (including but not limited to thrombin-anti-thrombin complex, tissue factor, protein C, thrombomodulin and plasminogen activator inhibitor-1), RAGE ligands and vitamin D status specific cellular populations within the blood (using but not limited to cytospins and flow cytometry) and identification of transcriptome changes within these cell populations.

(iii) Pulmonary and systemic epithelial and endothelial function and injury will be assessed by the following: Plasma and serum biomarkers which may include but not be limited to measurement of RAGE, Ang I/II, SP-D, vWF and PCP3 will be undertaken. Urinary albumin/creatinine ratio will also be measured.

(iv) Samples from subjects will also be tested on primary cultures of fresh human neutrophils monocytes and macrophages as well as mesenchymal stromal cells to determine surrogate markers of inflammation which may include but not be limited to the measurement of activation (shape change, CD11b surface expression, superoxide release), adhesion and transmigration, cytokine release and MMP production, rate of apoptosis and their ability to phagocytose.

6. STUDY DESIGN

6.1 Study Design

This is a prospective, observational, multi-centre blinded diagnostic accuracy study of a novel multiplex PCR test. Site research and clinical teams will be blinded to results obtained by multiplex PCR; these results will be compared to those from conventional microbiological methods observed in the course of standard care in ICU.

6.2 Study Setting

At least 15 adult intensive care units (ICU) across the UK will participate.

6.3 Study Schematic Diagram

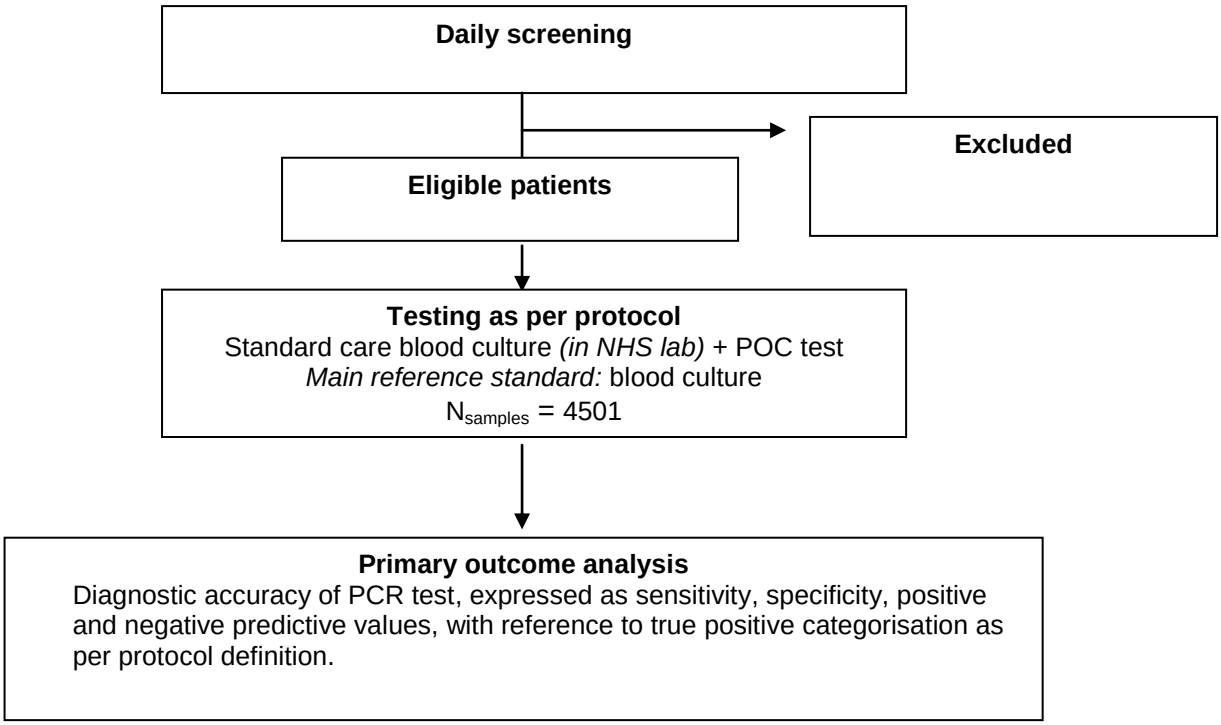

Figure 1: Study Schematic for the TEST-IT trial

6.4 Study Timeline

Table 1: Study Timeline Gantt Chart

Year			1												2											3		
Quarter			Q1			Q2			Q3			Q4			Q5			Q6			Q7			Q8			Q9		
Period end month			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Trial Stage	Pre-clinical	Set up	Lead-in			Main study																		Analysis/report					
Technology Development	X																												
Recruit Staff	X	X																											
Ethics Approval		X																											
Sponsor Approval		X																											
Local R&D Approvals		X	X	X	X	X																							
Site Training & Initiation		X	X	X	X	X	X	X																					
Patient Recruitment			X	r/v	X	X	X	r/v	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Number of Sites Open			1	3	3	6	6	9	9	12	12	>15	>15	>15	>15	>15	>15	>15	>15	>15	>15	>15	>15						
Sample Accrual			4	22	54	113	204	317	471	647	864	1103	1383	1673	1988	2303	2618	2933	3248	3563	3878	4193	4501						
Assessments:																													
Blood sampling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Data Entry			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
TMG Meetings	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
TSC Meetings		X				X						X						X						X					
Site Close Down																							X	X	X	X			
Data Analysis																								X	X	X			
Health Economics Analysis																								X	X	X			
Study Report																										X	X		
Dissemination																											X	X	X

6.5 End of Study

For the purposes of submitting the end of trial notification to the Sponsor and REC the end of trial will be considered to be when database lock occurs for the final analysis.
The trial will be stopped prematurely if:

- Mandated by the Research Ethics Committee (REC)
- Mandated by the Sponsor (e.g. following recommendations from the Trial Steering Committee (TSC)
- Funding for the trial ceases

The REC that originally gave a favourable opinion of the trial will be notified in writing once the trial has been concluded or if terminated early.

7. PATIENT ELIGIBILITY, SCREENING AND RECRUITMENT

7.1 Eligibility Criteria

Patients will be screened for eligibility based on the inclusion/exclusion criteria outlined below.

7.1.1 Inclusion criteria:

1. Patients with suspected sepsis undergoing blood sampling for culture in the course of routine care.

7.1.2 Exclusion criteria:

1. Patients aged <16 years old.
2. Patients previously recruited to the study.
3. Consent declined.

7.2 Co-enrolment Guidelines

Patients enrolled in any other studies are potential candidates for this study. This is at the Principal Investigator's (PI) discretion and should be considered when the burden on participants is not expected to be onerous. Co-enrolment with any studies should be documented in the Case Report Form (CRF).

7.3 Screening Procedure

Adult patients admitted to ICU who undergo blood culture testing for suspected sepsis are eligible for this study and will be screened daily, on the basis of the inclusion/exclusion criteria as specified in the protocol.

The Clinical Trials Unit (CTU) will provide screening logs which must be completed by the PI or designee to document all patients screened for the study and all patients recruited. Patients screened and not recruited on to the study should also be documented on the screening log, including the reason for not being enrolled on the study. The PI or designee will be required to submit screening logs to the CTU approximately every month.

7.4 Recruitment

In order to estimate the overall sensitivity of the test 4501 samples are required and will be tested to assess the diagnostic accuracy of the Multiplex PCR test. This will be achievable given the high incidence of suspected sepsis in the critically ill and the large number of patients expected to meet the eligibility criteria. Each patient can contribute more than one sample to this study; however an interval of at least 5-days must lapse between consecutive samples obtained.

8. INFORMED CONSENT

The consultation and recruitment of patients lacking capacity is regulated by different legal jurisdictions across the sites of this study. Practice will adhere to local regulations as outlined below.

It is the responsibility of the Principal Investigator (PI) (or designee) to ensure that informed consent is obtained for each participant. Consent may be obtained by the PI; an appropriately trained Research Nurse; or medically trained investigator. The PI (or designee) taking informed consent must be GCP trained, suitably qualified and experienced and have been delegated this duty by the Principal Investigator on the delegation log.

A Covering Statement, Patient Information Sheet and Consent Form approved by the Research Ethics Committee (REC) will be provided to each study site. Wherever possible, consent will be taken directly from the patient, however, the incapacitated nature of patients in intensive care units will usually preclude obtaining prospective informed consent from participants.

For patients who are unable to give informed consent due to the effects of sedation, infection, delirium and mechanical ventilation; consent or personal/nominated consultee

opinion will be obtained as outlined below and in line with the legal requirements for patients without capacity in England and Wales (Mental Capacity Act 2005), and in Scotland (Adults With Incapacity (Scotland) Act 2000). Consent processes in Northern Ireland follow common law. For the purposes of the trial, the consent processes used in England and Wales will be used in Northern Ireland.

At all sites other than those in Scotland, a deferred consent process will be applied. Samples will be taken as outlined in section 9 of this protocol and will be held at site pending consent being obtained for inclusion in the study. Patients or their personal/ nominated consultee will have 48 hours to decide whether or not to take part in the study. No sample will be tested or further stored without consent having first been obtained. If after providing consent a participant subsequently loses capacity, personal / nominated consultee opinion will be obtained as outlined below.

In Scotland consent from the patient or nearest relative / welfare attorney may be obtained after admission to ICU but prior to meeting study inclusion criteria.

8.1 Personal Consultee - England, Wales and Northern Ireland

The researcher will seek advice from a Personal Consultee (who may be a relative, partner or friend of the participant). This should normally take place during a face-to-face meeting. An authorised staff member/researcher will describe the trial to the individual, and provide them with a Covering Statement, Information Sheet and Declaration Form for Personal Consultee (England/Wales and Northern Ireland). The researcher will seek their views about whether the patient should take part in the study. They will be asked about their opinion of the wishes and feelings of the patient if they had capacity.

After the researcher has checked that the information sheet is understood, the researcher will invite the Personal Consultee to sign the form and will then countersign it. A copy of the form should be placed in the patient's medical notes and a copy filed in the Investigator Site File (ISF).

If the Personal Consultee is not available at site, the researcher may contact the Personal Consultee by telephone and seek verbal agreement. This verbal agreement will be recorded in the Consultee Telephone Agreement Form. The Consultee Telephone Agreement Form will be signed by a second member of staff who has witnessed the telephone consent. This witness may be a member of the site study team or site medical staff. A copy of the Consultee Telephone Agreement Form should be placed in the patient's medical notes and a copy filed in the ISF. Written agreement will then be obtained as soon as possible.

8.2 Nominated Consultee - Approval by a Registered Medical Practitioner (RMP) - England, Wales and Northern Ireland

In the event that there is no Personal Consultee for sites in England, Wales and Northern Ireland, authorisation to recruit the patient will be sought from a RMP (a doctor unrelated to the study conduct). The RMP will be informed about the trial by a member of the research team and given a copy of the Registered Medical Practitioner Form (England/Wales and Northern Ireland) and a copy of the Information Sheet. If the RMP decides that the patient is suitable for entry into the study they will be asked to complete the relevant authorisation form. A copy of the authorisation form should be placed in the patient's medical notes and a copy filed in the ISF. In the event that a Personal Consultee is identified after the RMP has provided their opinion, the above process for Personal Consultee will be followed and all forms will be filed as instructed above.

For sites in Scotland where no Welfare Guardian / relative is available it will not be legally possible to enroll the patient (specific to the Adults with Incapacity Act Scotland for non-CTIMP trials).

8.3 Nearest Relative / Welfare Attorney – Scotland

In Scotland consent from the patient or nearest relative / welfare attorney may be obtained after admission to ICU but prior to meeting study inclusion criteria.

The researcher will seek consent from a Nearest Relative / Welfare Attorney (who may be a relative, partner or friend of the participant). This will usually take place during a face-to-face meeting. An authorised staff member/researcher will describe the trial to the individual, and provide them with a Covering Statement, Patient Information Sheet and Consent Form for Nearest Relative/Guardian or Welfare Attorney (Scotland). The researcher will seek their views about whether the patient should take part in the study. They will be asked about their opinion of the wishes and feelings of the patient if they had capacity

After the researcher has checked that the information sheet is understood, the researcher will invite the Nearest Relative / Welfare Attorney to sign the form and will then countersign it. A copy of the form should be placed in the patient's medical notes and a copy filed in the ISF.

8.4 Patient Consent to Continue - England, Wales and Northern Ireland

Site research staff as delegated by the PI will assess whether the patient has regained capacity to give informed consent. Patients may be approached either whilst still in ICU or within 48-96hrs after discharge from ICU to obtain permission to continue in the study.

The consent to continue process will include providing the Covering Statement, Patient Information Sheet and Consent Form and allowing sufficient time for the patient to understand the material and ask questions. If the patient agrees to continue in the study they will be asked to sign the Consent Form which will then be counter signed by a member of the research team.

If the participant declines on-going participation in the study no further follow-up will take place. Data collected up until that point will be anonymised before returning to the co-ordinating centre. In the rare event that the patient does not regain capacity or the staff have been unable to obtain consent to continue, the opinion provided by the Personal/Nominated Consultee or consent from the Nearest Relative/ Guardian or Welfare Attorney will continue.

8.5 Withdrawal of Consent

Participants or their Personal Consultee/Welfare Guardian/Registered Medical Practitioner may withdraw the patient from the study at any time without prejudice. In the event of a request to withdraw, only anonymised data recorded up to the point of withdrawal will be included in the study analysis and any stored samples from such participants will be destroyed.

9. SCHEDULE OF ASSESSMENTS AND STUDY PROCEDURES

9.1 Schedule of Assessments

All patients will be evaluated during the study and data collected at each of the following time-points. For routinely collected clinical data the NHS record will be the source document and for study specific clinical measurements the CRF will be the source document.

Baseline

- Patient Demographics (date of birth, gender)
- Date and Time of ICU admission
- Suspected Source of Sepsis
- The Acute Physiology and Chronic Health Evaluation score (APACHE II) at ICU admission

At Sampling

- Site, Date and Time of sampling
- Previous Antibiotic regimens within 3 calendar days of sampling (Yes/No)
- Determinants of SOFA Score at time of sampling

Additional Assessments

- Paired blood culture result
- Multiplex PCR result
- Date and Time Multiplex PCR test completed
- Date and Time clinical team informed of blood culture result
- Results of additional blood and other sterile anatomic site cultures taken within +/-7 days of the research blood sample for suspected sepsis will also be recorded.
- Results of cultures from non-sterile anatomic sites within +/-3 days of the research blood sample for suspected sepsis will be recorded. Whether the treating physician judged this to represent a clinically-suspected focus of infection will also be recorded.

At Discharge

- Date of critical care discharge
- Date of hospital discharge

Mortality

- Date of death (within 28 days of recruitment)

9.2 Study Procedures

9.2.1 Blood Sampling

Blood cultures will be taken in the usual manner, for the participating study site, in the course of routine care. At the time that each blood culture is taken from an eligible patient, a 5ml sample of blood will also be collected for multiplex PCR testing. An additional 10ml sample of blood will also be collected where

(i) it is the first sample
(ii) research staff are available to process and store the sample

In patients meeting these criteria, and where resources permit, a further 3ml sample of blood will be taken into RNA preservation reagent (Tempus) tubes.

The research blood will be obtained from the same sampling event as the matched blood culture (i.e. from the same syringe draw); if this is not possible then research blood should be obtained by the same sampling technique (i.e. venepuncture or accessing the same vascular device).

When multiple sampling sites are being used for routine clinical blood cultures at the same time, the peripheral (venepuncture) sample is preferred for matched sampling in this study. If a peripheral sample is not being obtained in routine clinical care, then obtaining matched research and routine culture samples from a vascular access device is acceptable.

At participating sites in England, Wales and Northern Ireland, patients undergoing blood culture in the course of routine clinical care will have a research blood sample taken at the same time. The research blood sample will be stored pending informed consent/assent being obtained within 72 hours of taking the sample. No research blood samples will be tested until consent/assent for participation in this study has been obtained. In the event that consent/assent is not obtained the entire research sample will be discarded with no research tests being carried out on it.

9.2.2 Sample Processing

The research blood sample will be divided between testing and storage for evaluation of discrepant results and future analyses. The procedure for this will be detailed in the Sample Processing Guideline.

9.2.2.1 Sample for Multiplex PCR Testing

Approximately the first half of the study samples will be initially stored at site and transferred in batches to a central laboratory in Queen's University Belfast, for testing using the Multiplex PCR device, in accordance with the Sample Processing Guideline. Subsequent samples will be analysed using the Multiplex PCR device at the POC by ICU clinical and/or research and/or technical staff at participating sites who have received training in the use of the Multiplex PCR testing device and transferred in batches to a central laboratory in Queen's University Belfast for testing using the Multiplex PCR device.

9.2.2.2 Sample for Storage

The portion of the research blood sample for storage will be transferred to Queen's University Belfast for storage at -80^{o}C and subsequent testing in accordance with the Sample Processing Guideline.

9.2.3 Time to Result

The time required to complete testing will be measured for both Multiplex PCR and the paired blood culture. In the case of the blood culture two measures will be recorded:

(i) the time between sampling and the test first being reported to clinical teams as positive;

(ii) the time between sampling and a final pathogen identification first being reported to clinical teams. It is acknowledged that, for both of these, the result will usually be 'first' reported verbally.

Blood cultures that do not flag positive after 5-days of incubation will be categorised as negative with a time to result of 5-days.

9.2.4 Clinical Management of Patients in the Study

There will be no other change to standard care in ICU.

10. DATA COLLECTION & MANAGEMENT

10.1 Data Quality

Data integrity and study credibility depend on factors such as ensuring adherence to the protocol and using quality control measures to establish and maintain high standards for data quality.

The Co-Chief Investigators (CI) and CTU will provide training to site staff on trial processes and procedures including the case report form (CRF) and data collection.

On-site monitoring visits during the trial will check the accuracy of entries on CRF's against the source documents, the adherence to the protocol, procedures and Good Clinical Practice (GCP).

Quality control is implemented by the CTU in the form of Standard Operating Procedures (SOPs), which are defined to encompass aspects of the clinical data management process, and to ensure standardisation and adherence to International Conference of Harmonisation Good Clinical Practice (ICH-GCP) guidelines and regulatory requirements.

Data validation will be implemented and discrepancy reports will be generated following data entry to identify discrepancies such as out of range, inconsistencies or protocol deviations based on data validation checks programmed in the clinical trial database.

10.2 Data Collection

To ensure accurate, complete and reliable data are collected, the CTU will provide training to site staff in the format of investigator meetings and/or site initiation visits.

All data for an individual patient will be collected by the PI or designee and recorded in source documents/electronic CRF for the study. Patient identification on the CRF will be through their unique trial identifier, allocated at the time of recruitment.

Data should be entered onto the online electronic study database as per the CRF entry timelines.

10.3 Data Management

Following the entry of patient data into the study database, the data will be processed as per the CTU Standard Operating Procedures (SOPs). Data queries will be generated for site staff as required to clarify data or request missing information. The designated site staff will be required to respond to these queries within 2 weeks. All queries will be responded to/ resolved within the study database. Any amended information will then be entered in the study database.

11. STATISTICAL CONSIDERATIONS

11.1 Sample Size

The sample size is based on:

(i) an assumed 10% event rate for true positive results among specimens collected – this is based on pilot data collected in a single centre.
(ii) an estimated overall test sensitivity of 90%, and specificity of 95%.
(iii) the need to ensure that the overall test sensitivity is estimated with a high degree of precision. We have set this precision as +/-3%, at the 95% confidence level.
(iv) the ability to assess performance of the test for detecting pathogens for which true positive results are expected as infrequently as 1 in 500 tests. We have set the precision for this as +/-20%, at the 95% confidence level
(v) Given a 10% event rate for true positive results, a sample size that achieves the proposed degrees of precision for the sensitivity estimate will enable the specificity of the test to be measured with even greater precision.

With an assumed sensitivity of 90% with a 10% true positivity rate, in order to estimate the overall sensitivity of the test with a precision of +/-3% at the 95% confidence level, 3851 samples are required. For uncommon pathogens, with an assumed sensitivity of 90% with a 0.2% true positivity rate, in order to estimate the overall sensitivity of the test with a precision of +/-20% at the 95% confidence level, 4501 samples are required. Therefore, 4501 samples will be tested to assess the diagnostic accuracy of the test using these parameters.

The sample has not been inflated for dropouts or missing data because: (i) research sampling is not necessary after the day of enrolment; (ii) the follow-up period is very short; and (iii) the nature of ICU care presents minimal risk of attrition for follow-up.

11.2 Statistical Methods

Data will be analysed by the Study Statistician. Results will be presented as 2x2 tables for the whole study population. The sensitivity and specificity of POC testing are the main constituents of the primary outcome measure of the study. An assessment of the diagnostic accuracy of the POC device will be based on these measures and on positive and negative predictive values calculated from comparison with conventional culture as the reference standard. The data supporting an assessment of diagnostic accuracy will be reported according to STARD[38] criteria, including explanations of losses of participants. The possible effects of heterogeneity in the study population will be examined and separate sub-analyses for the different types of patients recruited will be performed. These will provide assessments of the validity of POC testing by this method and device in the context of prior antimicrobial exposure, varying underlying diagnoses and by patient condition.

11.3 Missing Data

Where data is incomplete despite the efforts to ensure continuous high quality data collection and reporting as detailed in section 9 of the protocol, information relating to the corresponding participant will be excluded from relevant analyses. Where either the result of Multiplex PCR testing or blood culture is absent, data relating to the corresponding participant will be excluded from all analyses.

11.4 Health Economic Analysis

The health economic analysis will estimate the cost per POC multiplex PCR test and the cost per conventional blood culture. A costing exercise of the resources used to test patients via each route will be undertaken in 5% of samples. The personnel involved in taking the samples will record details of their time and the consumables used. Costs will be obtained directly from the manufacturer for the Multiplex PCR testing equipment and disposables. Unit costs will be applied from national sources such as the NHS reference costs and the Personal Social Services Research Unit (PSSRU) Costs of Health and Social Care.

11.5 Additional Analysis

Planned additional analyses will be described in the statistical analysis plan.

12 DATA MONITORING

12.1 Data Access

Prior to commencement of the study, the PI will give permission for trial related monitoring, audits, ethics committee review and regulatory inspections, by providing direct access to source data and trial related documentation. Consent from patients for direct access to data will also be obtained. The patients' confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

12.2 Monitoring Arrangements

The CTU will be responsible for trial monitoring. The frequency and type of monitoring will be detailed in the monitoring plan and agreed by the trial Sponsor. On-site monitoring visits and central monitoring activities will be conducted in accordance with the trial monitoring plan. On-site monitoring will be an on-going activity from the time of initiation until trial close-out and will comply with the principles of Good Clinical Practice (GCP).

On-site monitoring visits during the trial will check the accuracy of entries on CRF's against the source documents, the adherence to the protocol, study procedures and GCP.

The PI or designee should ensure that access to all trial related documents including source documents (to confirm their consistency with CRF entries) are available during monitoring visits. The extent of source data verification (SDV) will be documented in the monitoring plan.

13. TRIAL COMMITTEES

13.1 Trial Management Arrangements

The Co-CI's will have overall responsibility for the conduct of the study. The CTU will undertake trial management including all clinical trial applications (Ethics and Research Governance), site initiation/training, monitoring, analysis and reporting. The Trial Co-ordinator will be responsible on a day to day basis for overseeing and co-ordinating the work of the multi-disciplinary trial team, and will be the main contact between the trial team (and other parties involved. Before the trial starts site training will take place to ensure that all relevant essential documents and trial supplies are in place and that site staff are fully aware of the trial protocol and procedures. The CTU will assist and facilitate in the setting up and

co-ordination of the trial committees including the Trial Management Group (TMG) and Trial Steering Committee (TSC).

13.2 Trial Management Group (TMG)

A Trial Management Group (TMG) will be established and Chaired by one of the Co-CI's. The TMG will have representation on it from the CTU and other investigators/collaborators who are involved in the study and provide trial specific expertise (e.g. trial statistician). This group will have responsibility for the day to day operational management of the trial, and regular meetings of the TMG will be held to discuss and monitor progress. The discussions of the TMG will be formally minuted and a record kept in the TMF.

A TMG Charter will be drawn up to detail the terms of reference of the TMG including roles and responsibilities.

13.3 Trial Steering Committee (TSC)

The conduct of the trial will be overseen by a TSC. The TSC is a group that act as the oversight body for the trial on behalf of the Sponsor/Funder. Throughout the trial the TSC will take responsibility for monitoring and guiding overall progress, scientific standards, operational delivery and protecting the rights and safety of trial patients.

The TSC will include an independent Chair, not less than two independent clinicians/trialists, a patient representative and the Co-CI's. Representatives of the Sponsor/Funder, Co-investigators and CTU may attend TSC meetings as observers and at the discretion of the Chair. The TSC Charter will document the membership of the committee and outline the terms of reference of the TSC including roles/responsibilities,, organisation of meetings, reporting, decision making and the relationship with the other trial committees. An inaugural meeting will be held prior to recruitment commencing. Subsequent meetings will be scheduled within 6 months of commencing recruitment and approximately annually thereafter.

14. REGULATIONS, ETHICS AND GOVERNANCE

The trial will comply with the principles of GCP, the requirements and standards set out by the applicable regulatory requirements in the UK and the Research Governance Framework.

14.1 Sponsorship

The Belfast Health and Social Care Trust (BHSCT) will act as Sponsor for the study and the Co-CI's will take overall responsibility for the conduct of the trial. Separate agreements will be put in place between the Sponsor, CI and each organisation who will undertake Sponsor delegated duties in relation to the management of the study.

14.2 Funding

This study is funded by Innovate UK. Funding provided by Innovate UK covers the costs for staff based at the Clinical Trials Unit (CTU) the trial co-ordinating centre for the study.

14.3 Indemnity

The BHSCT will provide indemnity for any negligent harm caused to patients by the design of the research protocol through the Clinical Negligence Fund in Northern Ireland.

14.4 Competing Interests

The co-CIs and members of the TMG have no financial or non-financial competing interests. The study is funded by Innovate UK and McMullan and McAuley are co-investigators.

14.5 Ethical Approvals

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The protocol will be approved by a Research Ethics Committee.

14.6 Good Clinical Practice

The trial will be carried out in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines (www.ich.org). All members of the trial team will be required to have completed GCP training.

14.7 Protocol Compliance

A protocol deviation is defined as an incident which deviates from the normal expectation of a particular part of the trial process. Any deviations from the protocol will be fully documented on the protocol deviation form in the CRF.

A serious breach is defined as a deviation from the trial protocol or GCP which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial

The PI or designee is responsible for ensuring that serious breaches are reported directly to the CTU within one working day of becoming aware of the breach.

Protocol compliance will be monitored by the CTU who will undertake site visits to ensure that the trial protocol is adhered to and that necessary paperwork (e.g. CRF's, patient consent) is being completed appropriately.

14.8 Protocol Amendments

The investigators will conduct the study in compliance with the protocol given approval/favourable opinion by the Ethics Committee. Changes to the protocol may require ethics committee approval/favourable opinion prior to implementation. The CTU in collaboration with the sponsor will submit all protocol modifications to the research ethics committees for review in accordance with the governing regulations.

14.9 Patient Confidentiality

In order to maintain confidentiality, all study reports and communication regarding the study will identify the patients by the assigned unique trial identifier only. Computers where information will be stored will be password protected. Patient confidentiality will be maintained at every stage and will not be made publicly available to the extent permitted by the applicable laws and regulations.

14.10 Record Retention

The PI will be provided with an Investigator Site File (ISF) by the CTU and will maintain all trial records according to GCP and the applicable regulatory requirements. The Trial Master File (TMF) will be held by the CTU within the BHSCT and the essential documents that make up the file will be listed in an SOP. On completion of the trial, the TMF and study data will be archived by the CTU according to the applicable regulatory requirements and as required by the BHSCT Sponsor. Following confirmation from the Sponsor the CTU will notify the PI when they are no longer required to maintain the files. If the PI withdraws from the responsibility of keeping the trial records, custody must be transferred to a person willing to accept responsibility and this must be documented in writing to the CTU and Sponsor.

15. DISSEMINATION/PUBLICATIONS

15.1 Trial Publications

The final study report will be provided by the Trial Statistician; it is anticipated that the study findings will be published in national and international peer review journals which will be led by the Co-CI's. This will secure a searchable compendium of these publications and make the results readily accessible to the public and health care professionals. In addition study findings may be presented at both national and international meetings and also to appropriate patient groups.

15.2 Authorship Policy

An author will be considered to be someone who has made a substantive intellectual contribution to the study. All investigators, Trial Statistician and relevant members of the Trial Management Group are potential co-authors. Collaborators will be acknowledged.

15.3 Trial Registration

The trial will be registered with the UK Medicines and Healthcare products Regulatory Agency (MHRA) as a diagnostic accuracy study of a medical device and with the International Standard Randomised Controlled Trial Registry (ISRCTN).

15.4 Data Sharing Statement

Requests for data sharing will be reviewed on an individual basis by the Co-CI and TMG.

15.5 Data Access

Following the publication of the primary and secondary outcomes there may be scope to conduct additional analyses on the data collected. In such instances formal requests for data will need to be made in writing to the Co-CI's who will discuss this with the TMG. In the event of publications arising from such analyses, those responsible will need to provide the CI with a copy of any intended manuscript for approval prior to submission. Authorship will need to take the format of "[name] on behalf of the TEST-IT Clinical Trial Group" or something similar which will be agreed by the TMG.

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