

Pragmatic randomised controlled trial of molecular point-of-care testing for respiratory pathogens versus routine clinical care in critically ill adults with pneumonia: (SARIPOC)

Sponsor: University Hospital Southampton NHS Foundation Trust

Chief Investigator: Dr Tristan Clark

# Protocol Modification History

|  |  |  |  |
| --- | --- | --- | --- |
| Version | Date | Modifications | Author(s) |
| 1.02.03.03.14.1 | 12th September 201613th March 201910th July 20195th August 20208th February 2021 | N/AChanged from pilot study to full study.Changed definition of severe acute respiratory infection to specifically mean CAP, HAP and VAP exclusivelyChanged from using the Filmarray Respiratory panel to the Filmarray Pneumonia panel.Change of primary outcome measure and some secondary outcome measures to take account of using the Pneumonia panel rather than the respiratory panel.Change of size calculation to account for new primary outcome measure.The introduction section has been changed to reflect the above.Removed inclusion criteria of ‘respiratory illness <72 hours’Changed randomisation software to ALEAAllowed other IVD licenced assays for PCT to be used in analysisChanged sample size to 200 after interim reviewClarified secondary outcome time pointsRemoved mandated requirement for reporting all deaths as SAE, given relative risk of death in trial cohort | Nathan Brendish, Tristan ClarkStephen Poole, Tristan ClarkStephen Poole, Tristan ClarkStephen Poole, Tristan ClarkStephen Poole, Tristan Clark |

# Key information

## Chief and Principal Investigator

**Dr Tristan W Clark**

Associate Professor and Honorary Consultant in Infectious Diseases

University of Southampton and University Hospital Southampton NHS Foundation Trust

Southampton General Hospital

Tremona Road

Southampton

SO16 6YD

Tel: 02381208209

Fax: 02381205023

T.W.Clark@soton.ac.uk

## Co-investigators

**Dr Stephen Poole**

Clinical Research Fellow in Infectious Diseases

University Hospital Southampton NHS Foundation Trust

Southampton General Hospital

Tremona Road

Southampton

SO16 6YD

Tel: 02381204989

Fax: 02381205023

S.Poole@Soton.ac.uk

**Dr Kordo Saeed**

Consultant Microbiologist and Honorary Senior Lecturer

University of Southampton and University Hospital Southampton NHS Foundation Trust

Southampton General Hospital

Tremona Road

Southampton

SO16 6YD

Tel: 02381204989

kordosaeed@nhs.net

**Dr Ahilanadan Dushianthan**

Consultant in Respiratory Intensive Care Medicine

University Hospital Southampton NHS Foundation Trust

Southampton General Hospital

Tremona Road

Southampton

SO16 6YD

Tel: 02381204989

Ahilanadan.Dushianthan@uhs.nhs.uk

**Professor Mike Grocott**

Professor of Anaesthesia and Critical Care Medicine

University of Southampton and University Hospital Southampton NHS Foundation Trust

Southampton General Hospital

Tremona Road

Southampton

SO16 6YD

Tel: 02381208449

Mike.Grocott@soton.ac.uk

## Trial Sponsor

Research and Development (R&D)

University Hospital Southampton NHS Foundation Trust

Southampton General Hospital

Tremona Road

Southampton

SO16 6YD

## Registrations

Internal Study Code: RHM MED 1387

This trial is registered on the ISRCTN international clinical trials database: ISRCTN65693049

IRAS Project ID: 216585

CPMS ID: 34003

## Ethical Approval

Regional Ethics Committee (REC): Southcentral - Berkshire

REC reference: 17/SC/0110

Date of approval: 24th March 2017

Amendment approved: 25th May 2017

Second Amendment approved: 23rd May 2019

Third Amendment approved: 2nd August 2019

Fourth Amendment approved: 18th March 2021

Contents

[Protocol Modification History 2](#_Toc3532007)

[Key information 3](#_Toc3532008)

[Chief and Principal Investigator 3](#_Toc3532009)

[Co-investigators 3](#_Toc3532010)

[Trial Sponsor 4](#_Toc3532011)

[Registrations 4](#_Toc3532012)

[Ethical Approval 4](#_Toc3532013)

[2. Overview 7](#_Toc3532014)

[Background 7](#_Toc3532015)

[Aims 8](#_Toc3532016)

[Methods 8](#_Toc3532017)

[Potential benefits to patients and the NHS 8](#_Toc3532018)

[3. Abbreviations and Definitions 10](#_Toc3532019)

[4. Introduction 12](#_Toc3532020)

[The burden of pneumonia 12](#_Toc3532021)

[The importance of pathogen detection 12](#_Toc3532022)

[Rapid 13](#_Toc3532023)

[Molecular tests for respiratory pathogens 13](#_Toc3532024)

[The potential benefits of a syndromic POCT for pneumonia 14](#_Toc3532025)

[The Wider Context and Global Priorities 15](#_Toc3532026)

[Conclusion 16](#_Toc3532027)

[5. Aim and Objectives 16](#_Toc3532028)

[Aim 16](#_Toc3532029)

[Objectives 16](#_Toc3532030)

[6. Recruitment and Study Processes 17](#_Toc3532031)

[Study Overview 17](#_Toc3532032)

[Screening 17](#_Toc3532033)

[Consent 18](#_Toc3532034)

[Randomisation and allocation 18](#_Toc3532035)

[Procedures for all participants at enrolment 19](#_Toc3532036)

[Procedures for participants randomised to the intervention group at enrolment 19](#_Toc3532037)

[Procedures for participants randomised to routine clinical care at enrolment 20](#_Toc3532038)

[Additional procedures after enrolment for all participants 20](#_Toc3532039)

[Inclusion / exclusion criteria 21](#_Toc3532040)

[Inclusion criteria: 21](#_Toc3532041)

[Exclusion criteria: 21](#_Toc3532042)

[7. Outcomes 22](#_Toc3532043)

[Primary outcome 22](#_Toc3532044)

[Justification of primary outcome measure 22](#_Toc3532045)

[Secondary Outcomes 22](#_Toc3532046)

[Exploratory Outcomes 24](#_Toc3532047)

[8. Sample size 24](#_Toc3532048)

[Proposed sample size 24](#_Toc3532049)

[Recruitment outlook 24](#_Toc3532050)

[9. Data collection 24](#_Toc3532051)

[10. Data Management 25](#_Toc3532052)

[General Data Protection Regulation (GDPR) 25](#_Toc3532053)

[Essential Document Retention 25](#_Toc3532054)

[Data monitoring 26](#_Toc3532055)

[11. Statistical Analysis Plan 26](#_Toc3532056)

[Primary outcome 26](#_Toc3532057)

[12. Safety 26](#_Toc3532058)

[13. Ethics, Oversight and Approvals 27](#_Toc3532059)

[Declaration of Helsinki 27](#_Toc3532060)

[ICH Guidelines for Good Clinical Practice 28](#_Toc3532061)

[Submissions to HRA, REC and local R&D 28](#_Toc3532062)

[Participant Confidentiality 28](#_Toc3532063)

[Investigator Responsibility 28](#_Toc3532064)

[Publication Policy 28](#_Toc3532065)

[14. Finances and Indemnity 28](#_Toc3532066)

[15. Role of BioFire 28](#_Toc3532067)

[16. Laboratory analysis plan 29](#_Toc3532068)

[17. Other personnel and sponsor 30](#_Toc3532069)

[18. References 30](#_Toc3532070)

**1. Synopsis**

**Trial site**

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

**Trial location**

Intensive Care Units (ICUs) including General ICU (GICU) and Neurological ICU (NICU), and Respiratory High Dependency Unit (RHDU) within Southampton General Hospital, which is part of University Hospital Southampton UHS NHS Foundation Trust

**Aim**

To evaluate the clinical impact and safety of molecular point-of-care testing for respiratory pathogens in adults with pneumonia in critical care units

**Design**

Pragmatic, single centre, parallel group, randomised controlled trial.

**Population**

Adults aged 18 years old and over presenting to critical care (GICU, NICU and HDU) with pneumonia.

**Sample Size**

300 participants (150 per group)

**Randomisation**

1:1 allocation using randomisation software

**Intervention**

Lower respiratory tract sample tested for respiratory pathogens and genetic markers of resistance using the FilmArray® Pneumonia Panel with results interpreted and communicated to clinical team by infectious diseases specialist, in addition to standard care

**Control**

Standard clinical care alone

**Key Assessment**

Subsequent retrospective hospital case note evaluation of antimicrobial use and clinical outcomes

**Timing**

Three years for patient recruitment(with a further five years for laboratory analysis)

# 2. Overview

## Background

Acute respiratory tract infections are responsible for over 4 million deaths each year and are the third most common cause of death worldwide. Pneumonia is classified according to where the patient resides when it develops, either in the community or in hospital. This allows clinicians to predict the causative pathogen and hence guide empirical antimicrobial therapy. Patients with hospital-acquired pneumonia (HAP) and ventilator acquired pneumonia (VAP) are frailer and the responsible bacteria are more likely to be resistant to antimicrobials, so empirical antibiotic regimens generally cover a very broad range of pathogens.

Bacteria and viruses are the most common identified pathogens that cause lower respiratory tract infections, but current diagnostic methods only identify an infecting agent in less than 40% of patients. This means clinicians often have little evidence to support antibiotic de-escalation so patients often stay on broad spectrum therapies for longer. Furthermore, the currently used diagnostic tests are time consuming and will generally not provide usable results for at least 48 hours. It has been well established that delay in administration of suitable antibiotics following the onset of illness strongly correlates with mortality. In some cases of pneumonia the infecting organism will not be covered by the empirical regimen and the 48 hour delay in recognising this may be fatal.

Novel rapid syndromic molecular test platforms like the BioFire® Filmarray® can identify a large number of bacterial and viral pathogens in an hour whilst also identifying common antibiotic resistance genes. Observational data shows that PCR based methods are superior to culture in terms of sensitivity and so achieve much higher levels of pathogen detection in respiratory tract samples than current standard methods. This could potentially facilitate the rapid de-escalation of broad spectrum antibiotics to improve stewardship and also to identify highly resistant or unusual organisms that are not covered by empirical antibiotic regimes, leading to changes in therapy earlier in a critical illness. This may translate into an improvement in patient outcomes such as antibiotic associated adverse events, length of critical care stay and mortality.

## Aims

To evaluate the clinical impact and safety of molecular point-of-care testing for respiratory pathogens in adults with pneumonia in critical care units.

## Methods

We will undertake a pragmatic, single-centre, randomised controlled trial in adults with pneumonia admitted to higher level care units (respiratory high dependency unit and intensive care units) at the University Hospital Southampton NHS Foundation Trust. 300 patients over up to three years will be recruited and randomised (1:1) to receive either the intervention of a lower respiratory tract (LRT) sample (sputum, bronchoalevolar lavage sample (BAL) or endotracheal (ET) aspirate) immediately tested on the FilmArray® Pneumonia Panel and results communicated to the clinical team with specialist infection advice, in addition to standard care, or standard clinical care alone. Those allocated to standard clinical care will have a LRT sample taken and stored for later analysis on the FilmArray pneumonia panel to allow direct comparison of detected pathogens between the groups.

The clinical outcomes assessment will be by retrospective case note analysis. The primary outcome measure is the proportion of patients treated with results directed antimicrobial therapy (this is defined as the use of antimicrobial agents that are started or continued on the basis of appropriateness (or the optimal choice) for a detected pathogen(s), where a putative pathogen(s) considered by the investigators to be plausibly causative, is identified, or appropriate de-escalation or discontinuation of antimicrobials when no pathogen is identified). Secondary outcome measures include, time to results directed antimicrobial therapy, total duration of antibiotic use, length of stay in critical care, length of stay in hospital and mortality.

## Potential benefits to patients and the NHS

We hypothesise that the use of a molecular POCT for respiratory pathogens in adults with pneumonia in critical care units will improve antibiotics use and may also improve patient outcomes. We expect the rapid and highly sensitive detection of pathogens and resistance genes will improve antibiotic use by facilitating results directed and organism directed therapies which will reduce consumption of broad-spectrum antimicrobial agents. Pathogen directed antibiotic therapy using POCT molecular diagnostics was a key recommendation of the O’Neill report 2016, a large-scale review of the threat posed by antimicrobial resistance commissioned by the UK Government.

We also anticipate that the POCT will identify more resistant pathogens earlier in the course of illness and hence allow early appropriate escalation of antibiotics when required. This may lead to decreased mortality and reduced time in the intensive care unit.

We expect earlier detection of antibiotic resistance genes and respiratory viruses should improve the use of isolation facilities by allowing rapid identification of organisms requiring isolation, for example Methicillin Resistant Staphylococcus aureus (MRSA) or Carbapenem Resistant Enterobacteriaceae (CRE).

Furthermore, we expect that increased and timely detection of influenza and other respiratory viruses will lead to improved use of neuraminidase inhibitor (NAI) treatment for influenza infection. Rapid and appropriate use of NAI treatment in influenza may lead to decreased mortality and shorten patient length of stay in critical care and in hospital. The identification of respiratory viruses at point-of-care may identify patients where antibiotic use can be reduced, further promoting antibiotic stewardship.

This study will help to inform the design of future clinical trials of respiratory POCT in adults with severe acute respiratory illness.

# 3. Abbreviations and Definitions

AE: Adverse Event

AMR: Antimicrobial resistance

ATS: American Thoracic Society

BAL: Bronchoalveolar Lavage

BRC: NIHR Southampton Biomedical Research Centre

BTS: British Thoracic Society

CAP: Community Acquired Pneumonia

CES: Clinical and Experimental Sciences academic unit, University of Southampton

CI: Chief Investigator

CRE: Carbapenem Resistant Enterobacteriaceae

CRF: Case Report Form

ESβL: Extended spectrum beta-lactamases

ET: Endotracheal tube

GICU: General Intensive Care Unit

HAP: Hospital acquired pneumonia – occurring >48 hours after admission to a healthcare facility

HDU: High Dependency Unit

HRA: Health Research Agency

ICU: Intensive Care Unit

IDSA: Infectious diseases Society of America

MRSA: Methicillin Resistant Staphylococcus aureus

NAI: Neuraminidase inhibitor

NICE: National Institute for Clinical Excellence

NICU: Neurological Intensive Care Unit

(NIHR) WTCRF: NIHR Wellcome Trust Clinical Research Facility

PI: Principal Investigator

POCT: Point-of-Care Test

REC: Regional Ethics Committee

RHDU: Respiratory High Dependency Unit

SAE: Serious Adverse Event

SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2

SCBR: Southampton Centre for Biomedical Research

UHS: University Hospital Southampton NHS Foundation Trust

VAP: Ventilator Associated Pneumonia

Critical care units, also called intensive care units (ICU) or intensive therapy units (ITU), look after patients whose conditions are life-threatening and need constant, close monitoring and support from equipment and medication to support organ functions. Some hospitals have specialist high dependency units as Southampton does, some incorporate this care into their ICU instead. Levels of care which require critical care management are:

Level 2: Patients requiring more detailed observation or intervention, single failing organ system or close monitoring post-operative care, and higher levels of care.

Level 3: Patients requiring advanced respiratory support alone or basic respiratory support together with support of at least two organ systems

At UHS, RHDU generally cares for level 2 patients, whereas GICU and NICU generally care for level 2 and 3 patients.

Critical care, in the context of this protocol, refers to RHDU, GICU and NICU at Southampton, and to prevent confusion, critical care is the preferred term.

# 4. Introduction

## The burden of pneumonia

In the developed world, the incidence of community acquired pneumonia (CAP) is associated with a huge burden both economically1, and in terms of mortality. Pneumonia caused nearly 30,000 deaths in England and Wales in 20152 and it probably costs Europe around €10 million annually3. It is estimated that 25/10,000 adults are hospitalised with pneumonia each year4 and this is associated with significant additional morbidity5. Complicated pneumonia is associated with advancing age and medical co-morbidity. Around 1 in 5 patients admitted to hospital with CAP will require intensive care management6.

Health-care acquired pneumonia (HAP) is defined as pneumonia occurring >48 hours after admission to a healthcare facility. This definition is relevant as infections arising beyond this time are caused by a different spectrum of bacterial pathogens than those occurring in the community. This information, coupled with local resistance profiles informs the empirical antibiotic treatment regimen. Whilst HAP is common, complicating around 2% of admissions7, no progress has been made at reducing the incidence8.

Ventilator acquired pneumonia (VAP) occurs >48 hours after intubation for invasive artificial ventilation. HAP and VAP combined are the most common nosocomial infection in the developed world8. Roughly 10% of patients requiring invasive ventilation go on to develop VAP and there is no evidence that this rate is declining in spite of a decade of research9. VAP has an attributable mortality of around 10% and this is likely to be higher in patients with multi-organ dysfunction10. A retrospective matched cohort study by Kollef et al11 found patients who developed VAP were intubated for longer, spent longer on ICU, and were in hospital for a greater period of time. They estimated the additional cost of VAP from this cohort to be $40,000 per patient.

## An unmet diagnostic need

Timely administration of appropriate antibiotics has been widely encouraged as a central tenant of care for patients with pneumonic infection12,13 and yet the gold-standard for microbiological diagnosis has not changed from traditional, slow, culture based methods. These take greater than 24 hours to identify an organism and often greater than 72 hours to provide phenotypic antibiotic sensitivity data.

Culture is insensitive, only detecting a pathogen in 23-40% of patients with clinically diagnosed pneumonia4,14–16 and an even smaller proportion after the administration of antibiotics. Additionally, there is a bias created by culture towards organisms that grow readily in laboratory. So called ‘atypical’ organisms including Mycoplasma pneumoniae, Legionella species and Chlamidyophila pneumoniae are all established causes of pneumonia which are rarely detected by culture-based methods.

As a result of the limitations of current diagnostic methods, huge numbers of broad spectrum empirical antibiotics are used which inadvertently promote bacterial resistance. Smarter tests are needed which provide clinicians with information facilitating safer, pathogen directed antibiotic prescribing: the knock on effect being drastically improved antibiotic stewardship.

## Rapid Molecular tests for respiratory pathogens

In recent years, the availability and development of novel molecular assays for infection have expanded, including techniques such as multiplex PCR17. These tests offer the potential to revolutionise the treatment of pneumonia by providing information to clinicians in ‘real-time’ about local pathogens as well as insights into potential antimicrobial resistance.

Observational studies have shown that PCR based platforms are capable of detecting bacterial pathogens in the sputum of a much greater proportion of patients than current standard laboratory methods16,18–20. One of the vanguard of this new age of molecular testing is the FilmArray® Pneumonia Panel (BioFire Diagnostics, Salt Lake City, UT, USA, a bioMérieux company)21.

The FilmArray® is an FDA approved and CE marked platform that uses nested real-time PCR to detect 34 clinically important respiratory targets (15 semi-quantitative bacterial targets, 3 qualitative atypical bacterial targets, 8 resistance genes and 8 viral targets). The semi-quantitative bacterial targets are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae complex*, *Acinetobacter calcoaceticus-baumanii* *complex*, *Klebsiella aerogenes*, *K. oxytoca*, *K. pneumonia group*, *Proteus species* and *Serratia marcescens*. The qualitative bacterial targets are *Chlamydia pneumoniae*, *Legionella pneumophilia* and *Mycoplasma pneumoniae*. Resistance gene targets are 5 carbapenemases (*KPC*, *NDM*, *Oxa-48-like*, *VIM*, *IMP)*, 1 extended spectrum β-lactamase (ESβL) (*CTX-M)* and 2 MRSA genes (*mecA/C* and *MREJ)*. The viral targets are influenza A, influenza B, rhinovirus/enterovirus, adenovirus, respiratory syncytial virus (RSV), coronavirus, human metapneumovirus, and parainfluenza viruses (types 1,2,3 and 4). The assay is run on sputum including expressed sputum and bronchoalveolar lavage fluid. The reported pooled sensitivity is 96.2% and specificity 98.3% compared to culture. Sample preparation takes 2 minutes and has a run time of around an hour.

One shortcoming identified in developing molecular diagnostics of non-sterile samples is the ‘over-detection’ of small numbers of colonising bacteria in a non-sterile site. The Filmarrary pneumonia panel has a novel solution to this. The relative abundance of organism for the 15 bacterial targets is estimated based on the number of copies of target gene detected by real-time PCR and is grouped for reporting into bins. These represent logarithmic groups of approximately 104, 105, 106 and >107 genomic copies of bacterial nucleic acid per millilitre. Concordance with reference molecular testing is good22.

To date there have been no interventional trials using the Filmarray® pneumonia panel. Observational data based on lower respiratory tract assays which preceded the final, FDA approved pneumonia panel suggested change of antibiotics could be supported in >50% of cases of pneumonia23,24.

## The potential benefits of a syndromic POCT for pneumonia

1. Smarter antibiotic usage

Perhaps the greatest potential clinical benefit of a syndromic POCT for pneumonia is being able to better utilise antibiotics.

Antibiotic treatment decisions are highly nuanced and require knowledge and consideration of local resistance patterns, pharmacodynamics and potential drug interactions. As such we believe interpretation of these novel test results will need to be done by a clinical infection specialist: as in the case with this trial.

Empirical antimicrobial regimens currently recommended for treating pneumonia are essentially a ‘best-guess’. The empirical, critical care, antibiotic guidelines for University Hospitals Southampton are shown below.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **CAP – 5 days** | **HAP – 7 days therapy** | **VAP – review 48 hourly** |
| Earlya | Late  | Earlya | Late  |
| **1st line** | Cefuroxime 1.5g IV tds or Co-amoxiclav 1.2g IV qds**PLUS**Azithromycin 500mg IV od (3 days only) | Ceftriaxone 2g IV od | Piperacillin-tazobactam 4.5g IV tds  | Co-amoxiclav 1.2g IV tds | Piperacillin-tazobactam 4.5g IV tds |
| **2nd line** | Levofloxacin 500mg IV bd | Chloramphenicol 12.5mg/kg IV qds | Ceftazidime 2g IV tds 7 days. | Levofloxacin 500mg IV bd | Meropenem 1g IV tds |
| 2nd line |  | Vancomycin **PLUS** Ciprofloxacin 400mg IV tds | Vancomycin **PLUS** Ciprofloxacin 400mg IV tds |  |  |

*UHS Guidelines for management of pneumonia*

a‘Early’ is developing illness less than 5 days after admission to hospital or long term care facility.

The negative percent agreement (NPA) of bacterial detection between culture based methods and the FA pneumonia panel is between 95-100%23,25,26. As a result clinicians can be confident that a negative test truly represents the absence of a pathogen. Furthermore, the pneumonia panel detects pathogens in a much higher proportion of samples than culture. Buchan et al23 reported that the Filmarray detected a bacterial target in 71% more specimens than routine culture, equating to a 108% increase in total bacterial detections. As such both positive and negative (non-detections) FA results can support narrowing of the spectrum of antibiotic therapy when this is clinically appropriate.

The majority of culture positive CAPs are caused by *S. pneumoniae*, whilst a smaller proportion are caused by *H. influenzae*, *S. aureus*, *M. catarrhalis* and atypicals including *M. pneumoniae* and *L. pneumophila27*. As a result UK NICE/BTS guidelines advise first line therapy of co-amoxiclav and clarithromycin for severe CAP28. Gadsby et al16 proposed that 77% of antibiotic prescriptions in CAP could be de-escalated based on results from a multiplex PCR platform. The majority of these potential interventions involved stopping clarithromycin (which would translate to stopping azithromycin in our local guidelines) when atypical organisms were not detected or narrowing co-amoxiclav when a sensitive pathogen had been detected. In the context of CAP, both the IDSA29 and NICE/BTS28 guidelines recommend organism directed therapy when a pathogen is identified and observational data suggests this is safe30.

In HAP and VAP, the most frequently grown bacterial pathogens are: *S. aureus*, *P. aeruginosa*, *Klebsiella* species, *E.coli*, *Acinetobacter* species and *Enterobacter* species31. The IDSA and the American Thoracic Society advise de-escalation in HAP/VAP according to culture results on the basis of expert opinion, citing a high level of confidence that it ‘reduces costs, burdens, and side effects, and that it is very likely that de-escalation also reduces antimicrobial resistance’32. There a small number of interventional studies looking at antibiotic de-escalation based upon microbiological culture results in pneumonia which have suggested this practice is safe33–36. High quality data for outcomes, including length of intensive care stay and antibiotic savings, are lacking. Both the IDSA and the National Institute for Clinical Excellence (NICE) cite an urgent need for well-run RCTs on the impact of de-escalating antimicrobial therapy28,32.

Certain organisms detected by the Filmarray are outside of the spectrum of activity of standard HAP/VAP/CAP guideline antibiotics. Earlier detection should facilitate earlier instigation of effective antibiotics which is correlated with better outcomes. The study on CAP by Gadsby et al16 detected *P.aeruginosa* and *A. baumanii*: both of which would be outside of the expected spectrum of activity of the standard empirical regimen.

MRSA is rare in the UK37 and is rare cause of CAP worldwide38. Rates of MRSA pneumonia are falling globally since 200539. Colonisation is now found in <2% of all admissions to critical care units in Britain40. Early identification by the Filmarray® would allow addition of such an agent. Conversely, studies have shown that stopping anti-MRSA agents is safe in the absence of pathogen detection41 and likely to be associated with a reduction in antibiotic related adverse events.

The number of carbapenemase producing enterobacteraecae (CPEs), being detected in the UK are increasing annually. In 2009 there were <100 positive isolates referred to Public Health England whereas around 3,000 were positive in 2017. Of these >99% were from carbapenemase families detected by the Filmarray® (‘ the big 5’) 42. Knowing that these genes are present would allow much earlier addition of antibiotics that evade the detected resistance mechanism.

*S. Aureus* typically causes pneumonia in older patients with co-morbidities. Panton-Valentin leukocidin is a virulence factor produced by a small proportion of S. aureus strains which can cause a severe necrotising pneumonia effecting younger people43. National guidance when this is clinically suspected involves the addition of a ribosomally acting antimicrobial agent in order to suppress toxin production44. Detection of S. aureus in an induced sputum in a patient with a clinically compatible picture could facilitate this antibiotic change.

1. Infection control

The widespread proliferation of antimicrobial resistance is of global concern. Since the 1990s infection control methods including patient source isolation and deep cleaning with targeted decolonisation have been very successful at reducing the spread of MRSA. Enhanced infection control practices are recommended for several of the targets on the pneumonia panel, including ESβLs (in our hospital only when gentamicin resistant due to limited side-room availability), CPEs, MRSA, S. pyogenes (until had 24 hours antibiotic therapy), Influenza and RSV.

1. Public health

In the UK there is a mandatory requirement to report certain infectious diseases to Public Health England so they can be investigated in the community. *L. pneumophilia* is associated with outbreaks around devices that aerosolize water. There were 532 cases in the UK in 201845, earlier sensitive detection of these would allow outbreak investigation to occur sooner and potentially stop further cases occurring.

1. Antiviral treatment

In adults, respiratory viruses are found in approximately one third of CAP cases4,46,47 and this may be greater in severe pneumonia48. About 10 to 30% of all hospitalised adults with influenza require treatment on the intensive care unit (ICU)49–52. Preceding viral infection is thought to be a key predisposing factor in secondary bacterial infections in the lung and other sites in the respiratory tract53–55. 3-15% of adults hospitalised with laboratory-confirmed influenza die in hospital49,50,56. The main therapy for influenza is neuraminidase inhibitors (NAIs) and the greatest benefit from these is within the first 48 hours. This this is probably even more marked on ICU57 therefore it is hugely important that these patients are identified and treated as soon as possible.

## The Wider Context and Global Priorities

The World Economic Forum has stated that antimicrobial resistance is the arguably the greatest threat to global human health and current high-profile initiatives on combating resistance have focused attention on antimicrobial stewardship, which seeks to preserve existing antimicrobial agents and slow the development of resistance. One of the strategies to achieve this goal is the development of rapid diagnostic tests and biomarkers so that antimicrobial use is result directed and pathogen directed rather than empirical and only used in those where there is clear evidence of benefit.

The UK government and Wellcome Trust sponsored O’Neill Review on Antimicrobial Resistance repeatedly cites the implementation of diagnostic technology as a key step in tackling this problem58.

## Conclusion

Pneumonia is the commonest infection leading to critical care admission in the UK and carries a mortality of the 33% in Britain. Vast quantities of broad-spectrum antibiotics are consumed in treatment much of which is likely to be unnecessary. Molecular platforms such as the FilmArray® Pneumonia Panel offer the potential to significantly reduce this by the early identification of causative pathogens. Beyond this, they offer the potential to improve infection control measures, escalate antibiotics for resistant or unusual pathogens and may lead to cost savings across the wider health service.

Global priorities are now focused on replacing empirical antimicrobial use with result directed and pathogen directed therapy to combat resistance and improve patient care and outcomes. As there is currently no high-quality evidence to support the use of these test platforms in this way, high quality randomised controlled trials evaluating the clinical and heath economic impact of such a strategy are urgently needed.

# 5. Aim and Objectives

## Aim

To evaluate the clinical impact and safety of molecular point-of-care testing for respiratory pathogens in adults with pneumonia in critical care units.

## Objectives

1. To evaluate the impact of molecular POCT on the clinical management of CAP, HAP and VAP including; the proportion of patients treated with results directed antimicrobials, time to results directed antimicrobial therapy, and appropriate use of isolation facilities.

2. To evaluate safety of molecular POCT and the impact of on measures of clinical effectiveness including; time on organ support, time on supplementary oxygen, time in critical care, duration of hospitalisation, mortality, and adverse events including antibiotic associated adverse events.

# 6. Recruitment and Study Processes

## Study Overview

This is a single centre, randomised controlled, study.

Blinding of participants is not used. Clinical teams cannot be blinded as the study requires them to know the results of the POCT in real time. The study will take place across up to three years.

Study processes that patient-participants undergo are summarised in Figure 1.

Check patient meets inclusion and none of exclusion criteria

Consent documented

Randomisation

Randomised to POCT

Randomised to routine care

Respiratory specimen tested on the FilmArray® analyser, urine sample tested for Pneumococcal antigen and blood tested for PCT

Results communicated to clinical team with clinical infectious diseases advice. Infection control team and patient informed if appropriate

Record patients screened but ineligible

For all patients once consent obtained:

Respiratory, blood and urine specimens are collected; (those allocated to POCT will have them tested and reported immediately, those allocated to routine care will have their samples stored at -80°C and tested at a later date).

Figure 1: Participant flow through the study

## Screening

The clinical team shall identify potentially eligible patients in critical care areas by regularly reviewing the patients and electronic admission systems against eligibility criteria and inform the research team.

## Consent

The study team will obtain informed consent for those with capacity, or assent via consultee for those without capacity, as per the dedicated study forms. As noted above, in view of the critical nature of patients’ illnesses and the potential benefits of rapid identification of infecting organism, the usual 24 hour consideration period for a participant or consultee will not apply.

Discussion of the study will be provided to patients, or their consultee for those lacking capacity, by study staff. This includes supply of a participant information sheet for the participant or witness to read and retain.

If the patient is able to, they will sign and date the informed consent document. If the patient is able to provide informed consent but has difficulty writing or otherwise filling in the consent form, informed consent from the patient will be verified by an independent witness (this would usually be a clinical member of staff) and the independent witness would then sign and date the informed consent document on the patient’s behalf. Both the person taking consent and either the patient or independent witness must personally sign and date the form. Copies of the informed consent document will be given to the patient and witness (if applicable) for their records and put into the patient’s notes. The original consent form is stored securely by the study team.

Each patient will be assumed to have capacity unless it is established that they lack capacity. For patients unable to consent for themselves, this study complies with the Mental Capacity Act 2005 and in such cases, the patient’s family member, carer or friend may be asked to act as the personal consultee and provide assent. In the event of a personal consultee not being available a nominated consultee (usually the consultant caring for the patient and independent from the study) will be asked if they would provide assent. Both the person taking assent and the consultee must personally sign and date the relevant form.

The personal / nominated consultee will be advised to set aside their own views and take into consideration the patient’s wishes and interests. Advance decisions made by the patient about their preferences and wishes will always take precedence.

In the event of the patient recovering capacity following enrolment by consultee, they will be asked to read the patient information sheet and provide consent for themselves. The patient may give consent, withdraw but have data collected so far retained, or withdraw and have their data destroyed (but signed assent forms and any FilmArray® results will be retained).

Although the study procedures are very brief, if a patient loses capacity after enrolment but before the study procedures are completed, consultee consent must be sought to continue with any study procedures.

## Randomisation and allocation

Participants will be enrolled and assigned a participant identification number consecutively. Once a patient has been screened and found eligible, and consent has been obtained, a study team member uses electronic randomisation software ALEA randomisation (Provided by FormsVision BV), to obtain a randomisation code for the patient who will then be allocated to either the intervention or control group in a 1:1 ratio. The software is integrated into the case report form and uses random block randomisation to ensure total unpredictability of randomisation.

## Procedures for all participants at enrolment

A lower respiratory tract sample (sputum, broncho-alveloar lavage fluid or endotracheal secretions) will be obtained by research staff. Broncho-alveloar lavage fluid samples are preferred but will only be collected if the procedures to obtain them are part of the patient’s standard care and there are sufficient samples for clinical care foremost.

A blood sample (maximum 15mls) will be obtained from all participants, processed in the closely located NIHR Southampton Clinical Research Facility laboratory and will include serum tube (5mls), PAXgene RNA tube (5mls) and EDTA whole blood (5mls). Additionally, a finger-prick blood sample may be taken, although this will not be stored.

A urine sample (maximum 10 mls) will be obtained from all participants. No invasive procedure will be performed (for example urinary catheterisation) exclusively to obtain this. If patients are catheterised for the purposes of their ongoing clinical care then urinary samples will be taken from this.

All respiratory samples left-over from FilmArray® testing may be frozen and stored for further study. Participant consent for this is included in the consent form. Patients may be approached for additional samples to be collected and stored for further study.

Clinical and demographic data will be collected at the time of enrolment. Outcome data will be collected from case notes and electronic health records retrospectively.

A letter detailing that the patient has been included in this trial will be sent to the patient’s general practitioner for information only.

## Procedures for participants randomised to the intervention group at enrolment

The LRT sample obtained as described above will be taken as soon as possible after recruitment and be analysed promptly on the FilmArray® using the pneumonia Panel as per training delivered by the manufacturer, used at the point-of-care in clinical areas. At least one FilmArray® machine will be located in a critical care area for this purpose. Test results are generated in about 75 minutes. 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. An LRT sample will also be requested of the clinical team for culture as per routine clinical care – see procedures for ‘participants randomised to routine clinical care at enrolment’ section below.

The blood sample taken as described above, will be taken as soon as possible after recruitment and tested promptly at the point-of-care using a CE marked diagnostic device for this purpose [including but not limited to miniVIDAS® (bioMerieux) and AQT90 (Radiometer)] for biomarkers including procalcitonin (PCT).

The urine sample obtained as described above, will be taken as soon as possible after recruitment and tested at the point-of-care for Pneumococcal antigens using the Alere Binax NOW! antigen testing kit.

The results of the investigations will be documented in the patient’s case notes and specialist infection advice immediately given on appropriate/optimal antimicrobial therapy. Additionally, ongoing advice will be provided during the following days. The participant will be informed of the result where appropriate. The infection prevention and control team will be informed of any reportable pathogen in real time (according to local hospital infection guidelines).

## Procedures for participants randomised to routine clinical care at enrolment

These patients will be managed according to standard clinical care as directed by their treating clinicians. Standard microbiological investigation including culture of respiratory tract secretions and viral PCR will be at the discretion of the clinical team and will be reported in the usual way by the electronic results system. In UHS there is ongoing regular Microbiology liaison on ICU and NICU from a consultant microbiologist and this will not change during the period of the study.

The standard locally advised investigation panel for CAP and HAP includes blood cultures and a sputum sample for microscopy, culture (performed as per UK SMI B5759, organisms identified by MALDI-TOF Mass Spectrometry) and sensitivity (disc sensitivity according to EUCAST standard methods and breakpoints). All sputum samples (including BALs and ETT) are mucolysed to homogenise the sample prior to culture. Additionally, BAL samples are centrifuged at 1200rpm for 10 minutes prior to culture and a gram stain is prepared from the centrifuged deposit. The presence (or absence) of organisms, white blood cells and epithelial cells is reported electronically in-hours. All samples are inoculated on chocolate (with bacitracin disc), blood (with optichin disc), CLED and Sabouraud agar plates. Other specific media are added at the discretion of the biomedical scientist. The sample is not diluted prior to inoculation. Plates are read at 24 and 48 hours and relative quantification is provided (+, ++ or +++) in the final report. Additionally, urine tests are advised for pneumococcal urinary antigens (Using Alere BinaxNOW®) in all patients and for legionella urinary antigens (Using Tinity Biotech Uni-Gold™ Legionella Urinary Antigen PLUS) when clinically suspected. Respiratory virus PCR is also recommended in patients where there is a clinical suspicion of influenza. For patients with VAP the recommendation is the same with the exception of performing a BAL at time of diagnosis instead of sputum sample collection.

The lower respiratory tract sample obtained as described above, will be stored at -80°C and subsequently tested by the FilmArray® pneumonia panel at least 30 days after collection. This will allow direct comparison of pathogens between the groups (i.e. an estimate of missed diagnoses or possible antibiotic amendments in the routine clinical care group) but will not influence participant care.

The urine sample obtained as described above, will be stored at -80°C and subsequently tested using the Alere BinaxNOW® antigen testing kit, at least 30 days after collection, and will not influence participant care

The blood samples obtained as described above, will be stored at -80°C and subsequently tested using a CE marked laboratory assay [including, but not limited to, miniVIDAS® (bioMerieux) and AQT 90 (Radiometer)] for biomarkers including procalcitonin, at least 30 days after collection, and will not influence participant care.

## Additional procedures after enrolment for all participants

Further blood samples (maximum 10mls) may be taken from all participates for measurement of biomarkers including procalcitonin at the following time points; 12, 24, 48, 72 hours and 5 days post enrolment. For those randomised to the intervention, blood sample will be tested immediately using VIDAS® (bioMerieux) and performed at the point-of-care with results communicated to clinical teams with interpretation by the infection specialist team. For those randomised to routine clinical care the samples will be frozen at -80C and tested retrospectively, at least 30 days after collection, and so will not influence participant care.

## Inclusion / exclusion criteria

### Inclusion criteria:

- Admitted to the Respiratory High Dependency Unit (RHDU), or an Intensive Care Unit (ICU), or about to be transferred to RHDU, GICU, NICU or under the care of the RHDU or ICU team in another hospital area, within University Hospital Southampton NHS Foundation Trust (UHS)

- Aged ≥18 years old

- Has a working diagnosis of CAP, HAP or VAP\* and physician decides to start new antibiotic treatment or modify existing antibiotic treatment.

\*CAP defined by the BTS as: ‘symptoms and signs of acute lower respiratory tract infection associated with new radiographic shadowing for which there is no other explanation’27. CAP patients who are intubated and ventilated remain classified as CAP.

HAP defined as by the IDSA as: ‘new lung infiltrate plus clinical evidence that the infiltrate is of an infectious origin, which include the new onset of fever, purulent sputum, leucocytosis, and decline in oxygenation... arising >48 hours after hospital admission’60. HAP patients who are intubated and ventilated remain classified as HAP.

VAP defined as by the IDSA as: ‘new lung infiltrate plus clinical evidence that the infiltrate is of an infectious origin, which include the new onset of fever, purulent sputum, leucocytosis, and decline in oxygenation... occurring >48 hours after endotracheal intubation’60

### Exclusion criteria:

- Not fulfilling all the inclusion criteria

- A purely palliative approach being taken by the treating clinicians

- Previously included in this study

- Consent declined or consultee consent declined

- Underlying Cystic Fibrosis or other condition characterised by persistent colonisation with resistant organisms

 - Not expected to survive the next 24 hours in the opinion of the responsible clinical team

Involvement in observational trials may not exclude a participant from this trial, and this is at the CI’s discretion.

# 7. Outcomes

## Primary outcome

The proportion of patients treated with results directed antimicrobials within 48 hours of a lower respiratory tract test result (this is defined as the use of antimicrobial agents that are started or continued on the basis of appropriateness (or the optimal choice) for a detected pathogen(s), where a putative pathogen(s) considered by the investigators to be plausibly causative, is identified; or the appropriate de-escalation or cessation of antimicrobials occurs where no pathogen is identified).

### Justification of primary outcome measure

Observational data in studies trialling similar molecular technologies have suggested that large number of patients being treated for pneumonia could have improved results directed therapy based on the results from molecular tests due to their improved sensitivity and higher pathogen yield compared to culture 16,20,24.

As an outcome measure, results directed antimicrobial therapy is desirable as encompasses both pathogen directed therapy where antimicrobial agent are adjusted based on the detection of a pathogen and also cases where antimicrobial agents are adjusted based on the lack of detection of a pathogen (ie a negative result). This strategy aims to improve clinical outcomes, reduces antibiotic associated adverse events, decrease costs of care and facilitate antimicrobial stewardship. Pathogen directed therapy is specifically mentioned in the O’ Neill AMR report as a strategy to facilitate effective antimicrobial stewardship enabling the preservation of antibiotic agents and reducing AMR.

## Secondary Outcomes

Median time to results directed antimicrobial therapy within 48 hours of a lower respiratory tract test result, days

Proportion with results-directed escalation or de-escalation in antimicrobial therapy within 48 hours of a lower respiratory tract test result. Escalation/ de-escalation defined as addition/ cessation of a second agent or increase/ decrease in antibiotic stewardship ‘ranking’. This is adapted from Trupka et al61

|  |  |
| --- | --- |
| **Rank** | **Broad spectrum agents** |
| 5 | Carbapenems, Polymixins, Novel cephalosporin/ beta-lactamse inhibitor combinations, monbactams, tigecycline |
| 4 | Piperacillin-tazobactam, Respiratory Quinolones (Moxifloxacin, Levofloxacin), Chloramphenicol, Ceftazidime |
| 3 | Ceftriaxone, Ciprofloxacin |
| 2 | Co-amoxiclav, Co-trimoxazole, Temocillin |
| 1 | Amoxicillin, doxycycline, clarithromycin, azithromycin |

Median time to results directed escalation/ de-escalation within 48 hours of lower respiratory tract result, hours

Proportion treated with ineffective empirical antimicrobial therapy (defined by the absence of an antimicrobial agent active against the specific class of microorganisms responsible for the infection or the administration of an antimicrobial agent to which the microorganism responsible for infection is resistant) at recruitment

Median duration of ineffective antimicrobial therapy, up to 14 days

Median duration of all antimicrobial therapy for this episode of pneumonia, up to 14 days

Median number of different antimicrobial agents used for this episode of pneumonia up to 14 days

Number of antibiotic free hours in the 14 days following recruitment

Median number of hours piperacillin/tazobactam in the 14 days following recruitment

Median number of hours of meropenem in the 14 days following recruitment

Median turn-around time for results, hours and days

Proportion of patients with a credible pathogen† identified for this episode of pneumonia

Concordance between pathogen identification between molecular methods (FilmArray) and culture

Concordance between genotypic and phenotypic isolate sensitivities

†Credible pathogen as determined by two independent infection specialists, with a third adjudicating in event of disagreement.

**Safety outcomes\***

Proportionate in-hospital, 30 and 60 day mortality

Median duration of hospitalisation, days

Median time on non-invasive ventilation, days

Median time on invasive ventilation, days

Median time on ionotropic support, days

Median time in critical care, days

Proportion with antimicrobial associated adverse events

\*Unless stated otherwise all outcomes are measured for the duration of hospitalisation or up to 30 days (whichever is shortest) and include medication patients are discharged with.

## Exploratory Outcomes

Relationship between pathogen quantification and clinical outcome measures

Utility of serum biomarkers in differentiating between bacterial and viral infection and predict outcome

Proportion and nature of pathogen detection in pneumonia occurring following macro-aspiration

Proportion and nature of pathogen detection in patients with SARS-CoV-2 infection

Proportion and nature of pathogen detection in relation to preceding duration of antibiotic therapy

# 8. Sample size

## Proposed sample size

The study will recruit a total of 300 patient-participants: about 100 per year for three years. With 1:1 allocation to groups, there will be 150 patients per group. This is based on the assumption that around 25% of patients will have results directed therapy (the primary outcome measure - defined previously) in the routine clinical care (control arm) versus 40% in the POCT arm (according to registry studies 40-50% of patients have a pathogen detected using the FA pneumonia panel62), using a 5% significance level a sample size of 150 per group will have an 80% power to detect this difference. This difference is considered to be clinically relevant in terms of antibiotic use and patient outcome. Based on the authors previous studies the drop-out rate is expected to be very low (<1%)63.

## Revised sample size

An interim review (n=100) of the primary outcome and key secondary outcomes was performed in order to ensure critical care resources were being optimally used during the second peak of the SARS-CoV-2 panedmic. 81% (38/47) of patients in the POCT arm were treated with results directed therapy, in comparison to 30% (14/46) in the control arm (p<0.0001). There was no significant difference in any safety outcomes. A pragmatic decision was therefore made by the study management team to close the study after 200 patients in order to allow adequate sample size to investigate secondary outcomes.

### Recruitment outlook

In the 12 months up to April 2015 in UHS, there were 359 adult GICU patients and approximately 200 adult respiratory HDU patients admitted with pneumonia. The CI’s previously mentioned study in adults successfully recruited 720 patients from 868 approached from the acute medical department and emergency department at the same hospital. Consultee consent for those lacking capacity circumvents a key barrier to recruitment experienced in previous studies.

Overall, these numbers demonstrate that a recruitment rate of 100 participants per year is likely to be realistic and achievable in this hospital setting and with this study team. Recruitment to the study will be throughout the year. The three year duration of the study will allow adequate numbers of patients to be recruited allowing for seasonal variations in respiratory illness.

# 9. Data collection

Demographic and clinical data will be collected for all patients at enrolment including: age, sex, ethnicity, smoking status, vaccination status (influenza and all pneumococcal vaccines), co-morbidities, medication use, symptoms, duration of illness, observations (pulse rate, respiratory rate, blood pressure, oxygenation status), laboratory results, radiology results, antimicrobial use prior to admission to higher level care unit and provisional diagnosis. Patients with VAP will additionally have the duration of invasive ventilation recorded. Data will be recorded on a standardised electronic Case Report Form and transferred to a secure electronic database. Once patients have been discharged or after 30 days (whichever is soonest), clinical data will be collected retrospectively from electronic and physical case notes including: use of antibiotics, duration of antibiotics, antibiotic modifications based on POCT results, use and duration of antivirals, use of side room facilities, time from assessment to isolation facility use, duration of hospitalisation, final diagnosis, mortality and serious adverse event occurrence. The number of diagnostic tests and procedures performed, along with duration of stay in critical care and hospital may be used to consider a health economic analysis. Data will also be collected on the turnaround time of respiratory pathogen test results in each group.

For those allocated to the intervention group, the FilmArray® pneumonia panel will be run on enrolment as soon as a sample is available. For those allocated to standard clinical care, samples will be taken on enrolment, frozen and stored, and run on the FilmArray® pneumonia panel at least 30 days after collection. This will not influence patient management, but will allow retrospective comparison of pathogen detection. For some patients within both groups a lower respiratory tract specimen may be frozen, stored and tested later.

# 10. Data Management

The subjects' anonymity will be maintained. The study team will keep a confidential log of each subject’s name, hospital ID 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, including NHS number. This 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.

The study staff will be responsible for entering study data in the CRF. It is the investigators’ responsibility to ensure the accuracy of the data entered in the CRF.

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

## General Data Protection Regulation (GDPR)

The patient information sheet given to participants will provide information about how their data is collected, stored and used in accordance with GDPR. As such, should participants elect to withdraw from the study, any data collected will be retained with the minimal patient identifiable information possible and no further data collected.

## 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.

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. A trial management committee will oversee the trial.

# 11. Statistical Analysis Plan

Analysis will be by intention-to-treat. The framework is superiority. Trial results will be reported in accordance with the CONSORT statement.

Statistical analysis will be performed by a dedicated medical statistician from the University of Southampton, independent from the study team. No interim analysis is planned. Patients tested with the rapid molecular diagnostic test will be compared with patients managed according to standard clinical care using standard descriptive and comparative statistical methods using Prism (GraphPad Software Inc; La Jolla, California) and SPSS (SPSS, Inc; Chicago, Illinois).

Missing data was minimal (<2%) in the CI’s previous molecular POCT secondary care trials and therefore is not expected to be a significant issue. The use of multiple imputation will be considered should missing data exceed 10% in the primary outcome.

Summaries of all baseline characteristics will be presented using means and standard deviations, medians and interquartile ranges, or frequencies and percentages, as appropriate. The intervention and control groups will be compared using chi-square tests for equality of proportions for binary data and using independent-samples t-tests or a non-parametric equivalent test for continuous data. Pre-planned subgroup analysis will be performed based on clinical group (CAP, HAP and VAP), pathogens detected, and other factors.

## Primary outcome

The proportion of participants receiving results directed antimicrobial therapy as defined previously will be compared using the chi-square test for equality of proportions between groups. 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 other clinical factors.

# 12. Safety

The risks of respiratory tract sampling and additional blood tests being taken are minimal and where occurring are likely to be mild. For many patients in critical care, venepuncture will not be needed as central line or arterial line access can be used to acquire the blood sample.

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

In the event of a SAE, a study doctor will consider the likelihood of the event being related to the study based on the documented evidence from the treating clinicians. SAEs will be reported via the sponsor’s standard forms and processes. SAEs will be reported within 24 hours of the study team learning of the SAE. In the event of a SAE, the PI will be involved in deciding whether this was a study-related event.

As this trial involves participants who are already experiencing life-threatening events by virtue of their admission to critical care, the illnesses, illness progression or complications from the illnesses that they have at the time of recruitment will not be recorded as a life-threatening SAE. Transfer either way between ICU and RHDU will not count as a SAE. Transfer to another critical care facility (including repatriation to a local hospital) will not count as a SAE except when it is for a level of care that cannot be provided at UHS. Similarly, complications or sequelae of conditions that they had at the time of recruitment will not be counted as a SAE of persistent or significant disability or incapacity or a SAE related to prolongation of existing hospitalisation.

Hospitalisation, life-threatening event or death more than 30 days after the original presentation will not count as SAEs because of the time lapsed in relation to the event and an acute viral infection or the POCT.

In England, pneumonia is the most common cause for admission to the ICU and carries a mortality rate of 35%64. A meta-analysis of studies found the mortality rate for patients admitted to ICU with COVID-19 pneumonia (who are also eligible for recruitment) is greater than 40%65. For this reason, death will only be reported as an SAE if it is deemed to be related to the study.

The SAE conditions noted were prompted by a previous respiratory virus molecular POCT study that showed around 60 SAEs for just over 300 patients and came about by discussion with the study sponsor. A large proportion of the patient group had conditions making readmission likely and had a high background likelihood of mortality. No single SAE was in any way related to the study, but significant resources were taken up in reporting of the SAE without any benefit or impact upon safety. Therefore, the caveats to SAE reporting for this trial have been developed to streamline the reporting process, promote efficiency and maximise safety.

# 13. Ethics, Oversight and Approvals

## Declaration of Helsinki

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.

## Submissions to HRA, REC and local R&D

The protocol, informed consent forms, participant information sheet and GP letter (and any other document requested) will be submitted to the Health Research Authority (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 start.

## 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 2018. 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.

# 14. 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.

# 15. Role of BioFire

BioFire® Diagnostics, (Salt Lake City, UT, USA, a bioMérieux company) will provide the FilmArray® machines, FilmArray® Pneumonia Panels and the procalcitonin analyser (VIDAS) and reagents 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.

# 16. Laboratory analysis plan

Exploratory assays will be carried out on the samples collected in this study at the discretion of the Chief/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 respiratory virus detection and some bacteria is quantitative polymerase chain reaction (qPCR). qPCR may be used to detect and quantify pathogen presence in the samples collected. Other PCR-based techniques may be used on the collected samples.

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 study 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 pathogen infection and acute respiratory illness. 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 respiratory 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.

All 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 (CES), 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.

# 17. Other personnel and sponsor

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

• Clinical Research Fellows and other doctors in Infectious Diseases, Acute Medicine, Respiratory Medicine and Critical Care at UHS

• Research Nurses and Research Assistants at UHS

For the sponsor and R&D contact: Dr Mikayala King, research governance and QA manager at UHS; Email: Mikayala.king@uhs.nhs.uk; Telephone: 023 8120 8689

# 18. References

1. Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. *Thorax*. 2012;67(1):71-79. doi:10.1136/thx.2009.129502

2. Mortality from pneumonia (ICD-10 J12 - J18 equivalent to ICD-9 480 - 486): directly standardised rate, all ages, annual trend: NHS Digital. https://digital.nhs.uk/data-and-information/publications/clinical-indicators/compendium-of-population-health-indicators/compendium-mortality/current/mortality-from-respiratory-diseases. Published 2018. Accessed February 25, 2019.

3. Torres A, Peetermans WE, Viegi G, Blasi F. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. *Thorax*. 2013;68(11):1057-1065. doi:10.1136/thoraxjnl-2013-204282

4. Jain S, Self WH, Wunderink RG, et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *N Engl J Med*. 2015;373(5):415-427. http://www.ncbi.nlm.nih.gov/pubmed/26172429.

5. Pick HJ, Bolton CE, Lim WS, McKeever TM. Patient Reported Outcome Measures in the recovery of adults hospitalised with community-acquired pneumonia: a systematic review. *Eur Respir J*. January 2019:1802165. doi:10.1183/13993003.02165-2018

6. Storms AD, Chen J, Jackson LA, et al. Rates and risk factors associated with hospitalization for pneumonia with ICU admission among adults. *BMC Pulm Med*. 2017;17(1):208. doi:10.1186/s12890-017-0552-x

7. Giuliano KK, Baker D, Quinn B. The epidemiology of nonventilator hospital-acquired pneumonia in the United States. *Am J Infect Control*. 2018;46(3):322-327. doi:10.1016/j.ajic.2017.09.005

8. Magill SS, O’Leary E, Janelle SJ, et al. Changes in Prevalence of Health Care–Associated Infections in U.S. Hospitals. *N Engl J Med*. 2018;379(18):1732-1744. doi:10.1056/NEJMoa1801550

9. Wang Y, Eldridge N, Metersky ML, et al. National Trends in Patient Safety for Four Common Conditions, 2005–2011. *N Engl J Med*. 2014;370(4):341-351. doi:10.1056/NEJMsa1300991

10. Nguile-Makao M, Zahar J-R, Français A, et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. *Intensive Care Med*. 2010;36(5):781-789. doi:10.1007/s00134-010-1824-6

11. Kollef MH, Hamilton CW, Ernst FR. Economic Impact of Ventilator-Associated Pneumonia in a Large Matched Cohort. *Infect Control Hosp Epidemiol*. 2012;33(03):250-256. doi:10.1086/664049

12. Dellinger RP, Carlet JM, Masur H, et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med*. 2004;32(3):858-873. http://www.ncbi.nlm.nih.gov/pubmed/15090974. Accessed February 4, 2019.

13. Daniel P, Rodrigo C, Mckeever TM, et al. Time to first antibiotic and mortality in adults hospitalised with community-acquired pneumonia: a matched-propensity analysis. *Thorax*. 2016;71(6):568-570. doi:10.1136/thoraxjnl-2015-207513

14. Chalmers JD, Taylor JK, Singanayagam A, et al. Epidemiology, Antibiotic Therapy, and Clinical Outcomes in Health Care-Associated Pneumonia: A UK Cohort Study. *Clin Infect Dis*. 2011;53(2):107-113. doi:10.1093/cid/cir274

15. Musher DM, Roig IL, Cazares G, Stager CE, Logan N, Safar H. Can an etiologic agent be identified in adults who are hospitalized for community-acquired pneumonia: Results of a one-year study. *J Infect*. 2013;67(1):11-18. doi:10.1016/j.jinf.2013.03.003

16. Gadsby NJ, Russell CD, McHugh MP, et al. Comprehensive Molecular Testing for Respiratory Pathogens in Community-Acquired Pneumonia. *Clin Infect Dis*. 2016;62(7):817-823. doi:10.1093/cid/civ1214

17. Poole S, Kidd SP, Saeed K. A review of novel technologies and techniques associated with identification of bloodstream infection etiologies and rapid antimicrobial genotypic and quantitative phenotypic determination. *Expert Rev Mol Diagn*. 2018;18(6):543-555. doi:10.1080/14737159.2018.1480369

18. Ozongwu C, Personne Y, Platt G, et al. The Unyvero P55 ‘sample-in, answer-out’ pneumonia assay: A performance evaluation. *Biomol Detect Quantif*. 2017;13:1-6. doi:10.1016/J.BDQ.2017.06.001

19. Kaur A, Kumar N, Sengupta S, Mehta Y. Respiratory Multiplex Polymerase Chain Reaction: An Important Diagnostic Tool in Immunocompromised Patients. *Indian J Crit Care Med*. 2017;21(4):192-198. doi:10.4103/ijccm.IJCCM\_2\_17

20. Baudel J-L, Tankovic J, Dahoumane R, et al. Multiplex PCR performed of bronchoalveolar lavage fluid increases pathogen identification rate in critically ill patients with pneumonia: a pilot study. *Ann Intensive Care*. 2014;4(1):35. doi:10.1186/s13613-014-0035-7

21. Biofire Diagnostics - FilmArray Pneumonia Panel. https://www.biofiredx.com/products/the-filmarray-panels/filmarray-pneumonia/. Accessed February 25, 2019.

22. M Buccambuso, L O’Connor, M Brooks, J Manwaring, T Edwards, M Hockin, J Arce, R Lems, J Larsen, A Fratto, D Abbott, J Southwick AJ. Precision of the FilmArray® Pneumonia Panel Considerations for Interpreting Relative Abundance of Bacterial Nucleic Acids in Lower Respiratory Specimens. In: *ECCMID*. ; 2018. https://wa3k33tsiyh3dl4v410mijmu-wpengine.netdna-ssl.com/wp-content/uploads/2019/01/Buccambuso-M-et-al.-ECCMID.-2018..pdf. Accessed February 12, 2019.

23. Buchan B, Windham S, Faron M, et al. Clinical Evaluation and Potential Impact of a Semi-Quantitative Multiplex Molecular Assay for the Identification of Pathogenic Bacteria and Viruses in Lower Respiratory Specimens. In: *ATS*. ; 2018. www.atsjournals.org. Accessed February 11, 2019.

24. A.M. Huang, S.L. Windham, D. Mahmutoglu, J.M. Balada-Llasat, R.F. Relich, R. Humphries, S. Miller, A. Harrington, C. Murphy, A. Leber7, J. Dien Bard8, C. Zimmerman, S. Kerr, C. Graue, N.A. Ledeboer and BWB. Potential Clinical Impact of a Semi-Quantitative Multiplex Molecular Assay for the Identification of Bacteria, Viruses, and Fungi in Lower Respiratory Specimens. In: *CVS*. ; 2017. https://wa3k33tsiyh3dl4v410mijmu-wpengine.netdna-ssl.com/wp-content/uploads/2018/08/Huang-A-et-al.-CVS.-2017..pdf. Accessed February 11, 2019.

25. Iannello A, Dubost C, Weber C, et al. Evaluation of the BioFire® FilmArray® Pneumonia Panel in ICU Patients with Suspected Ventilator-Associated Pneumonia. doi:10.1016/j.cmi.2016.06.013

26. Kerr S, Graue C, Broadbent K, et al. Clinical Evaluation of the Biofire FilmArray Pneumonia Panel plus. In: *ECCMID*. ; 2018. https://www.biomerieux-diagnostics.com/sites/clinic/files/2018\_eccmid\_kerr\_clinical\_evaluation\_of\_fa\_pneumonia\_panel\_plus.pdf. Accessed March 26, 2019.

27. Lim WS, Baudouin S V, George RC, et al. BTS guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax*. 2009;64(Suppl 3):iii1 LP-iii55. doi:10.1136/thx.2009.121434

28. National Clinical Guidelines Centre. *Diagnosis and Management of Community- and Hospital-Acquired Pneumonia in Adults. NICE Clinical Guideline 191*. NICE; 2014. https://www.nice.org.uk/guidance/cg191. Accessed March 4, 2019.

29. Musher DM, Mandell LA, Niederman MS, et al. Infectious Diseases Society of America/American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults. *Clin Infect Dis*. 2007;44(Supplement\_2):S27-S72. doi:10.1086/511159

30. Yamana H, Matsui H, Tagami T, Hirashima J, Fushimi K, Yasunaga H. De-escalation versus continuation of empirical antimicrobial therapy in community-acquired pneumonia. *J Infect*. 2016;73(4):314-325. doi:10.1016/J.JINF.2016.07.001

31. Jones RN. Microbial Etiologies of Hospital-Acquired Bacterial Pneumonia and Ventilator-Associated Bacterial Pneumonia. *Clin Infect Dis*. 2010;51(S1):81-87. doi:10.1086/653053

32. Kalil AC, Metersky ML, Klompas M, et al. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis*. 2016;63(5):e61-e111. doi:10.1093/cid/ciw353

33. Falguera M, Ruiz-González A, Schoenenberger JA, et al. Prospective, randomised study to compare empirical treatment versus targeted treatment on the basis of the urine antigen results in hospitalised patients with community-acquired pneumonia. *Thorax*. 2010;65(2):101-106. doi:10.1136/thx.2009.118588

34. Kim JW, Chung J, Choi S-H, et al. Early use of imipenem/cilastatin and vancomycin followed by de-escalation versus conventional antimicrobials without de-escalation for patients with hospital-acquired pneumonia in a medical ICU: a randomized clinical trial. *Crit Care*. 2012;16(1):R28. doi:10.1186/cc11197

35. Leone M, Bechis C, Baumstarck K, et al. De-escalation versus continuation of empirical antimicrobial treatment in severe sepsis: a multicenter non-blinded randomized noninferiority trial. *Intensive Care Med*. 2014;40(10):1399-1408. doi:10.1007/s00134-014-3411-8

36. Van Der Eerden MM, Vlaspolder F, De Graaff CS, et al. Comparison between pathogen directed antibiotic treatment and empirical broad spectrum antibiotic treatment in patients with community acquired pneumonia: a prospective randomised study. *Thorax*. 2005;60:672-678. doi:10.1136/thx.2004.030411

37. Public Health England. Laboratory surveillance of Staphylococcus aureus bacteraemia in England, Wales and Northern Ireland: 2017 Health Protection Report Volume 12 Number 30. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\_data/file/734747/hpr3018\_staph-aureus.pdf. Published 2018. Accessed March 4, 2019.

38. Self WH, Wunderink RG, Williams DJ, et al. *Staphylococcus aureus* Community-acquired Pneumonia: Prevalence, Clinical Characteristics, and Outcomes. *Clin Infect Dis*. 2016;63(3):300-309. doi:10.1093/cid/ciw300

39. Sader HS, Castanheira M, Arends SJR, Goossens H, Flamm RK. Geographical and temporal variation in the frequency and antimicrobial susceptibility of bacteria isolated from patients hospitalized with bacterial pneumonia: results from 20 years of the SENTRY Antimicrobial Surveillance Program (1997–2016). *J Antimicrob Chemother*. March 2019. doi:10.1093/jac/dkz074

40. Intensive Care National Audit and Research Centre. Key statistics from the case mix program 2017-1018. https://www.icnarc.org/Our-Audit/Audits/Cmp/Reports/Summary-Statistics. Accessed March 4, 2019.

41. Cowley MC, Ritchie DJ, Hampton N, Kollef MH, Micek ST. Outcomes Associated With De-escalating Therapy for Methicillin-Resistant Staphylococcus aureus in Culture-Negative Nosocomial Pneumonia. *Chest*. 2019;155(1):53-59. doi:10.1016/J.CHEST.2018.10.014

42. Public Health England. English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) - Report 2018. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\_data/file/759975/ESPAUR\_2018\_report.pdf. Published 2018. Accessed March 4, 2019.

43. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine Leukocidin--Producing Staphylococcus aureus in Primary Skin Infections and Pneumonia. *Clin Infect Dis*. 1999;29(5):1128-1132. doi:10.1086/313461

44. Haney Carr J, Hageman J. Guidance on the diagnosis and management of PVL-associated Staphylococcus aureus infections (PVL-SA) in England, 2nd Edition. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\_data/file/322857/Guidance\_on\_the\_diagnosis\_and\_management\_of\_PVL\_associated\_SA\_infections\_in\_England\_2\_Ed.pdf. Published 2008. Accessed March 4, 2019.

45. Public Health England. Monthly Legionella Report December 2018 National Surveillance Scheme for Legionnaires’ disease in Residents of England and Wales. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\_data/file/776445/Monthly\_LD\_Report\_December\_18.pdf. Published 2019. Accessed March 4, 2019.

46. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. *Lancet*. 2011;377(9773):1264-1275. doi:10.1016/S0140-6736(10)61459-6

47. Jennings LC, Anderson TP, Beynon KA, et al. Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax*. 2008;63(1):42-48. doi:10.1136/thx.2006.075077

48. Choi S-H, Hong S-B, Ko G-B, et al. Viral Infection in Patients with Severe Pneumonia Requiring Intensive Care Unit Admission. *Am J Respir Crit Care Med*. 2012;186(4):325-332. doi:10.1164/rccm.201112-2240OC

49. Mauskopf J, Klesse M, Lee S, Herrera-Taracena G. The burden of influenza complications in different high-risk groups: a targeted literature review. *J Med Econ*. 2013;16(2):264-277. doi:10.3111/13696998.2012.752376

50. Li G, Yilmaz M, Kojicic M, et al. Outcome of critically ill patients with influenza virus infection. *J Clin Virol*. 2009;46(3):275-278. doi:10.1016/j.jcv.2009.07.015

51. Reed C, Chaves SS, Perez A, et al. Complications Among Adults Hospitalized With Influenza: A Comparison of Seasonal Influenza and the 2009 H1N1 Pandemic. *Clin Infect Dis*. 2014;59(2):166-174. doi:10.1093/cid/ciu285

52. Taylor G, Abdesselam K, Pelude L, et al. Epidemiological features of influenza in Canadian adult intensive care unit patients. *Epidemiol Infect*. 2016;144(4):741-750. doi:10.1017/S0950268815002113

53. Madhi SA, Klugman KP, Vaccine Trialist Group. A role for Streptococcus pneumoniae in virus-associated pneumonia. *Nat Med*. 2004;10(8):811-813. doi:10.1038/nm1077

54. George SN, Garcha DS, Mackay AJ, et al. Human rhinovirus infection during naturally occurring COPD exacerbations. *Eur Respir J*. 2014;44(1):87-96. doi:10.1183/09031936.00223113

55. McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. *Nat Rev Microbiol*. 2014;12(4):252-262. doi:10.1038/nrmicro3231

56. Irwin DE, Weatherby LB, Huang WY, Rosenberg DM, Cook SF, Walker AM. Impact of patient characteristics on the risk of influenza/ILI-related complications. *BMC Health Serv Res*. 2001;1:8. http://www.ncbi.nlm.nih.gov/pubmed/11580874. Accessed March 4, 2019.

57. Muthuri SG, Venkatesan S, Myles PR, et al. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. *Lancet Respir Med*. 2014;2(5):395-404. doi:10.1016/S2213-2600(14)70041-4

58. Tackling drug-resistant infections globally: final report and recommendations chaired by Jim O’Neill. https://amr-review.org/sites/default/files/160525\_Final paper\_with cover.pdf. Published 2016. Accessed February 12, 2019.

59. Public Health England. *UK Standards for Microbiology Investigations Investigation of Bronchoalveolar Lavage, Sputum and Associated Specimens*.; 2018. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\_data/file/684559/B\_57i3.4.pdf. Accessed March 12, 2019.

60. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia. *Am J Respir Crit Care Med*. 2005;171(4):388-416. doi:10.1164/rccm.200405-644ST

61. Trupka T, Fisher K, Micek ST, Juang P, Kollef MH. Enhanced antimicrobial de-escalation for pneumonia in mechanically ventilated patients: a cross-over study. *Crit Care*. 2017;21(1):180. doi:10.1186/s13054-017-1772-4

62. Faron, M.L., Mahmutoglu, D, Huang, A., Balada-Llasat, J.M., Relich, R.F., Humphries, R., Miller, S., Harrington, A., Murphy, C., Leber, A., Dien Bard, J., Zimmerman, C., Kerr, S., Graue, Ledeboer, C.N.A., Buchan BW. Clinical Evaluation of a Semi-Quantitative Multiplex Molecular Assay for the Identification of Bacteria, Viruses and Fungi in Lower Respiratory Specimens. In: *CVS*. ; 2017. https://wa3k33tsiyh3dl4v410mijmu-wpengine.netdna-ssl.com/wp-content/uploads/2018/08/Faron-M-et-al.-CVS.-2017..pdf. Accessed March 12, 2019.

63. Brendish NJ, Malachira AK, Armstrong L, et al. Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial. *Lancet Respir Med*. 2017;5(5):401-411. doi:10.1016/S2213-2600(17)30120-0

64. Morris AC. Management of pneumonia in intensive care. *J Emerg Crit Care Med*. 2018;2:101-101. doi:10.21037/jeccm.2018.11.06

65. Armstrong RA, Kane AD, Cook TM. Outcomes from intensive care in patients with COVID‐19: a systematic review and meta‐analysis of observational studies. *Anaesthesia*. 2020;75(10):1340-1349. doi:10.1111/anae.15201