University of Southampton

Faculty of Medicine

Clinical Experimental Sciences

The Clinical Impact of Rapid Molecular Diagnostics in Pneumonia

Volume 1 of 1

by

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Thesis for the degree of Doctor of Philosophy

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Abstract

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Pneumonia is a leading cause of mortality and morbidity worldwide. Novel syndromic molecular platforms have been developed which test for many different pneumonia causing organisms. These platforms have been shown to deliver reliable microbiological results in fraction of the time of conventional diagnostics with much greater sensitivity. Such investigations could revolutionise the care of patients with respiratory illness by influencing antibiotic prescribing and infection control decisions in near real-time. This thesis describes two clinical impact trials of molecular point-of-care testing (mPOCT) for respiratory pathogens in pneumonia.

The Severe Acute Respiratory Illness Point-Of-Care (SARIPOC) trial was a randomised controlled trial which recruited adults with pneumonia in critical care. 200 patients were randomised to either standard clinical care or a molecular stewardship investigation including molecular testing with the Filmarray pneumonia panel, serum procalcitonin, and clinical infection advice. A contributory pathogen was identified in a 71% of mPOCT patients, compared to 51% of standard care patients. The median time to result was 1.7 hours for mPOCT and 66.7 hours for standard care. As a result of these increased, rapid detections, we demonstrated that 51% more patients in the mPOCT arm went onto optimal therapy for a microbiological result, with no observed difference in safety outcomes.

The CoV-19 POC study was a non-randomised interventional trial, which recruited 500 adults presenting to secondary care with suspected SARS-CoV-2 infection. Patients were tested with the QIAstat-Dx respiratory SARS-CoV-2 panel within the first 24 hours of admission and compared to 555 contemporaneously identified control patients, who were admitted via the same admission pathways but were tested by standard laboratory PCR. Median time to results was quicker in the mPOCT tested group (by 19.6 hours), and as a result, patients spent almost 20.8 fewer hours in cohort assessment areas, where they could potentially be exposed to SARS-CoV-2 infection, before arriving in their definitive clinical area.

These two trials demonstrate that mPOCT for respiratory pathogens are associated with reduced time to results compared to conventional diagnostics and with improvement in clinical care, including timely infection control decisions and in the appropriate use of antibiotics. Routine use of mPOCT for SARS-CoV-2 is now widespread in the UK. Further confirmatory trials are needed to before mPOCT for pneumonia can become standard practice in UK hospitals.

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Prizes and awards arising from this work

1. Academy of Medical Sciences Publication prize 2021 at the University of Southampton Faculty of Medicine Conference: £250 for the CoV-19 POC study

2. Hospital Superhero 2021 “Clinical innovation of the year”, Winner. University Hospitals Southampton NHS Trust (team award).

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Research Thesis: Declaration of Authorship

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I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

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Other papers published during this PhD candidature

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2. Livingstone R, Lin H, Brendish N J, **Poole S,** et al. Routine molecular point-of-care testing for SARS-CoV-2 reduces hospital-acquired COVID-19. *Journal of Infection* 2022. In press

3. Mansbridge C, Tanner A, Beard K.. **Poole S,** et al. FebriDx host response point-of-care testing improves patient triage for COVID-19 in the emergency department. *Infection Control and Hospital Epidemiology* 2022. In press.

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Definitions and Abbreviations

16S: A rRNA gene that is highly conserved between different species of bacteria

(NIHR) WTCRF: NIHR Wellcome Trust Clinical Research Facility

AE: Adverse Event

AMR: Antimicrobial resistance

APACHE score: Acute Physiology and Chronic Health Evaluation score

ARDS: Acute respiratory distress syndrome

ATS: American Thoracic Society

BAL(F): Bronchoalveolar Lavage (fluid)

BCYE: Buffered charcoal yeast extract: a culture medium for Legionella.

bla: Beta-lactamase

BRC: NIHR Southampton Biomedical Research Centre

BTS: British Thoracic Society

CAP: Community Acquired Pneumonia

CE marking: Indicates conformity with health and safety standards for products to be sold within the European Economic area.

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

CF: Cystic Fibrosis

CI: Chief Investigator

CIRU: Clinical Informatics Research Unit

CL2: Biosafety Containment laboratory level 2

COP: Cryptogenic organising pneumonia

CRE: Carbapenem Resistant Enterobacteriaceae

CRP: C-reactive protein

CRF: Case Report Form or Clinical Research Facility

CTIMP: Clinical trial of an investigational medicinal product

DNA: Deoxyribonucleic acid

ECCMID: European Congress on Clinical Microbiology and Infectious Diseases

ELFA: Enzyme-linked fluorescence assay

ERGO: Ethics and research governance online

ESβL: Extended spectrum beta-lactamase

ET(A): Endotracheal tube (aspirate)

FDA: United States Food and Drug Administration – responsible for oversight of diagnostics and drugs in the USA.

FTD: Fast-track diagnostics

GAS: Group A Streptococcus (*Streptococcus pyogenes*)

GCS: Glasgow Coma Scale

GDPR: General data protection regulation

GICU: General Intensive Care Unit

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

HDU: High Dependency Unit

Hib: Haemophilus influenzae type B

HRA: Health Research Agency

ICU: Intensive Care Unit

IDSA: Infectious Diseases Society of America

ILD: Interstitial Lung Disease

LRT(I): Lower respiratory tract (infection)

MERS CoV: Middle-Eastern Respiratory syndrome Coronavirus.

Definitions and Abbreviations

mNGS: metagenomic Next-generation sequencing

MREJ: *mec* Right extremity junction

MRSA: Methicillin Resistant *Staphylococcus aureus*

MSSA: Methicillin sensitive *Staphylococcus aureus*

MSC: Medical safety cabinet

NAAT: Nucleic acid amplification tests

NAI: Neuraminidase inhibitor

NDM: New Delhi Metallobetalactamase

NICE: National Institute for Clinical Excellence

NICU: Neurological Intensive Care Unit

NIHR: National institute for health research

NTHi: Non-typeable *Haemophilus influenzae*

PCT: Procalcitonin

PCR: Polymerase chain reaction

PI: Principal Investigator

POC(T): Point-of-care (test)

PSI: Pneumonia severity index

R&D: University Hospitals Southampton Research and Design department

REC: Regional Ethics Committee

RHDU: Respiratory High Dependency Unit

RNA: Ribonucleic acid

RSV: Respiratory syncytial virus

SAE: Serious Adverse Event

SARIPOC: Severe acute respiratory illness point-of-care

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

SCBR: Southampton Centre for Biomedical Research

SMI: Standards for microbiological investigation – National standards defined for UK laboratories for specific investigations.

SOFA: Sepsis related organ failure assessment score

Tm: Melt-temperature of amplicons of PCR: the specific temperature that an amplicon will melt, releasing LCGreen dye and reducing fluorescence as detected by the Filmarray instrument.

UAT: *Streptococcus pneumoniae* urinary antigen testing

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 document, refers to RHDU, GICU and NICU at Southampton, and to prevent confusion, critical care is the preferred term.

Chapter 1 Introduction

1.1 Overview

Pneumonia is a lower respiratory tract condition characterised by alveolar inflammation which can be caused by both infectious and non-infectious aetiologies. Most commonly, it arises as a result of bacterial infection, however viruses, fungi and other infectious and non-infectious mechanisms also cause to disease. Lower respiratory tract infections were accountable for an estimated 2.7 million deaths in 2015, making them the third most common cause of death worldwide[1].

Large amounts of empirical 'broad spectrum' antibiotics are used to treat pneumonia which inadvertently promote antimicrobial resistance (AMR): a problem identified by the World Health Organisation as one of the leading threats to global health today. The O'Neill report, commissioned by the UK government in 2014, has highlighted the need for developed nations to take a lead in tackling AMR. As part of this report a specific recommendation was made that all antibiotic prescriptions should be supported by diagnostic tests where available for that condition by 2020 with a view to more judicious and targeted use of antibiotics[2].

Timely administration of appropriate antibiotics is a central tenant of care for patients with pneumonia[3,4] and yet the gold-standard for microbiological diagnosis remains culture based methods which take several days to return results. These take greater than 24 hours to identify an organism and often greater than 72 hours to provide phenotypic antibiotic sensitivity data. Culture is also insensitive, only detecting a pathogen in 23-40% of patients with clinically diagnosed pneumonia[5–8] and an even smaller proportion when samples are obtained after the initiation of antibiotics.

In recent years several rapid syndromic molecular tests for pneumonia have been developed. These offer the potential to revolutionise treatment of pneumonia by providing information to clinicians in near 'real-time' on the pathogens present and their likely antibiotic sensitivity by also detecting genotypic markers of resistance. Multiple studies have demonstrated both the high concordance between these tests and culture, and the significantly increased yield of pathogens detected[8–11].

The main aim of this body of work was to determine whether these diagnostic benefits can be translated into clear, tangible benefits for patients who are critically unwell with pneumonia. To achieve this I have designed, setup and run the SARIPOC (severe acute respiratory illness point-of-

care testing) trial: a pragmatic randomised controlled trial in critically unwell patients with pneumonia. The study also allowed me to consider wider issues relating to the diagnosis and treatment of pneumonia in these settings with novel insights provided by multiplex syndromic molecular testing.

The emergence of a novel pneumonia-causing pathogen during my studentship, SARS-CoV-2, has also provided me with a unique opportunity to apply the molecular technologies I have been working with for the benefit of patients. To this end, I also present work from the CoV-19 POC trial, a study evaluating the clinical impact of near-patient molecular testing for SARS-CoV-2, for which I am joint first author.

1.2 History of pneumonia

1.2.1 Clinical characterisation

Pneumonia has been affecting humans for thousands of years. Hippocrates (c460-360 BCE) provided the first recorded description of pneumonia in Ancient Greece[12]:

> "Peripneumonia, and pleuricic affections, are to be thus observed: If the fever be acute, and if there be pains on either side, or in both, and if expiration be if cough be present, and the sputa expectorated be of a blond or livid color, or likewise thin, frothy, and florid, or having any other character different from the common."

He also described many of the characteristic features[12]:

> "When pneumonia is at its height, the case is beyond remedy if he be not purged and it is bad if he has dyspnoea, and the urine is thin and acrid and if sweat comes out about the neck and head, for such sweats are bad as proceeding from the suffocation, rales, and the violence of the disease which is obtaining the upper hand, unless there be a copious evacuation of thick urine, and the sputa be concocted [sputa are concocted when they resemble pus]; when either of these come on spontaneously, that will carry off the disease."

1.2.2 Appreciation for microbiological causes

It was not until 1875, some 2,000 years later in Europe, that the German-Swiss physician Edwin Klebs (1834-1913) first observed bacteria in the bronchial secretions of patients with pneumonia using microscopy[13], although their role in disease was not initially understood.

The first isolation of *Streptococcus pneumoniae*, the major cause of pneumonia at the time, was in 1881 by Louis Pasteur (1822-1895)[14] and George Sternberg (1838-1915)[15], a US Army surgeon. They recovered the bacterium from rabbits injected with human saliva (an interesting parallel to the rabbit antibodies for *S. pneumoniae* that I used in the SARIPOC study (2.10.3). Whilst they linked the bacterium with disease, it was not specifically linked to pneumonia.

Carl Friedländer (1847-1887), the German microbiologist, was the first person to demonstrate that bacteria were a consistent feature in pulmonary tissue of patients dying from pneumonia in 1882, and propose that they were causative[16]. He described cultivating two organisms from patients with pneumonia that had differing colonial growth. One resembled a round headed nail and the other a flat headed nail with a central depression: a morphological characteristic of pneumococcus. It is believed the former of these was *Klebsiella pneumoniae* (named after Edwin Klebs) which became known as Friedländer's bacillus.

The birth of modern microbial diagnostics for pneumonia arguably began two years later in 1884. Hans Christian Gram (1853-1938), a Danish microbiologist who worked in the same lab as Friedländer, developed the Gram's stain which allowed rapid differentiation between two major classes of bacteria based upon the properties of their cell wall. This technique was published in 1884[17], a year after he completed medical school, and is still used around the world today.

1.2.3 Towards effective treatment

Prior to the emergence of antibiotics, pneumonia was always a serious condition and frequently fatal. William Osler (1849-1919), famously referred to it as the 'Captain of the men of death' and 'the old man's friend'. A series of 465,000 cases from different studies which was compiled in 1939 had mortality rates between 20-40%[18].

Treatments before 1850 included frequent bleeding and purging (inducing vomiting). These fell out of practice and were replaced with more supportive therapy. General management advised by physicians included:

> "a light airy room with ample ventilation is desirable, as fresh air in abundance is required. The room should be quiet and an open fireplace is advantageous for purposes of ventilation. The patient should be screened from drafts. The exposure of patients to

> very cold air, a procedure widely used several years ago, failed to produce beneficial results"
>
> "Delay in hospitalization has doubtless cost the lives of many patients. The patient should be carried to hospital on a stretcher and preferably by ambulance and should not be dressed or permitted to sit up during the trip."
>
> **Roderick Heffron MD, Pneumonia with special reference to pneumococcus lobar pneumonia, 1939**

S. pneumoniae was the most frequently identified causative organism of pneumonia[18]. As a result, organism specific serum therapy was used in the early part of the 20th Century[19]. This involved inoculating horses with known pneumococcal strains, and then transplanting their serum after recovery into patients who were suffering with pneumonia (see Figure 2). Allergic reactions occurred frequently[20]. After the success of Edward Jenner in combating smallpox by vaccination a century earlier, attempts were also made to create vaccines against pneumococcus, beginning with Almroth Wright (1861-1947) in South Africa in 1911[21]. These did not come to fruition until after the widespread availability of penicillin.

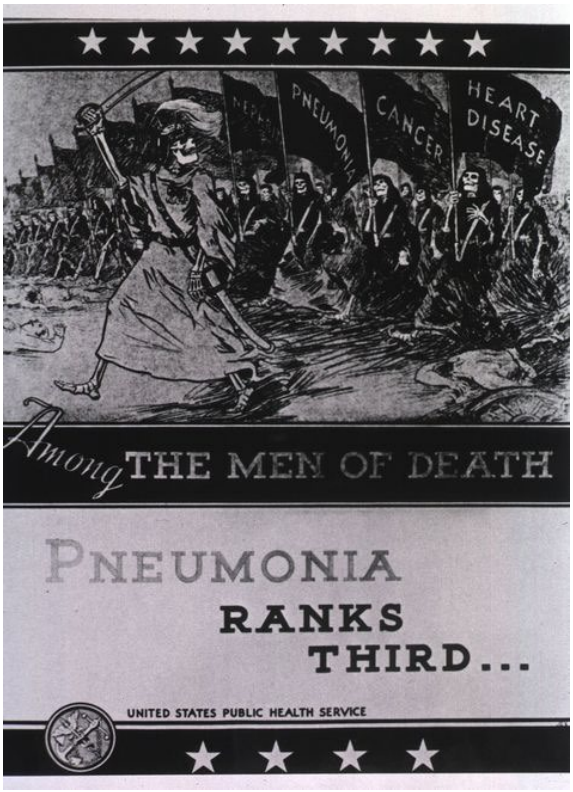


Figure 1: US public health service poster on pneumonia (1930s)

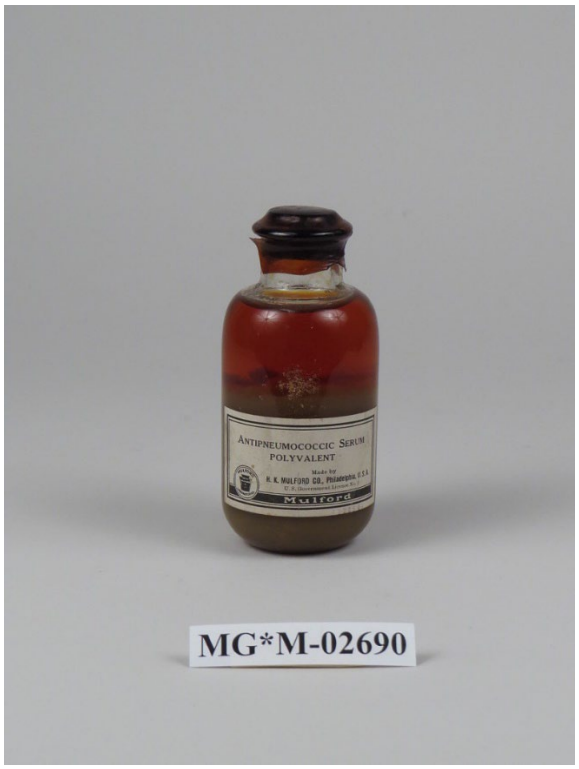


Figure 2: Antipneumococcic Serum, The Smithsonian, circa 1929[22]

1.2.4 Antibiotics

Antibiotics are chemical compounds which have a negative impact on bacteria and are used as medical therapy to treat disease. They are likely to have been used in different forms for thousands of years. Tetracycline has been identified by mass spectrometry chelated into the mineralised bone of Sudanese Nubians dating from 350-550AD, suggesting ingestion of the antibiotic in their diets[23].

Paul Ehrlich (1854-1915) was credited with the discovery of the first modern antibiotic in 1909, using a compound derived from dyes to treat syphilis. Arsphenamine (see Figure 3) was introduced in 1910 and widely used until the discovery of penicillin[24] although it did not have an application for pneumonia.

Chemotherapy treatments, as they were known at the time, were not widely available and often limited by toxicity. Optichin is one such example which was found to have activity against pneumococcus in 1915[25]. It is still used in the laboratory to differentiate pneumococcus from other α-haemolytic streptococci (see 2.9.6).

It was not until 1928, that Alexander Fleming (1881-1955, Figure 4) famously stumbled upon the discovery of penicillin. He returned from a holiday to find that bacterial plates which he had left uncovered had become contaminated by the fungus *Penicillium notatum*. Around the colonies of

fungus were clear zones where the bacterium was unable to grow[26,27]. The work of Howard Florey (1898-1968) and Ernst Chain (1906-1979) in refining penicillin from the other substances produced by *Penicillium notatum* allowed mass production in the early 1940s and led to an age of readily available treatment for many common infections.

(a) (b)

Figure 3: (a) Drug label for Arsphenamine (Salvarsan) and (b) a post-card from Paris, 1910, referring to Arsphenamine: 606 refers to it being the 6th compound in the 600th series that Ehrlich and team tested for efficacy, and the bullet referring to the phrase "magic bullet" (Zauberkugel) which Ehrlich popularised.

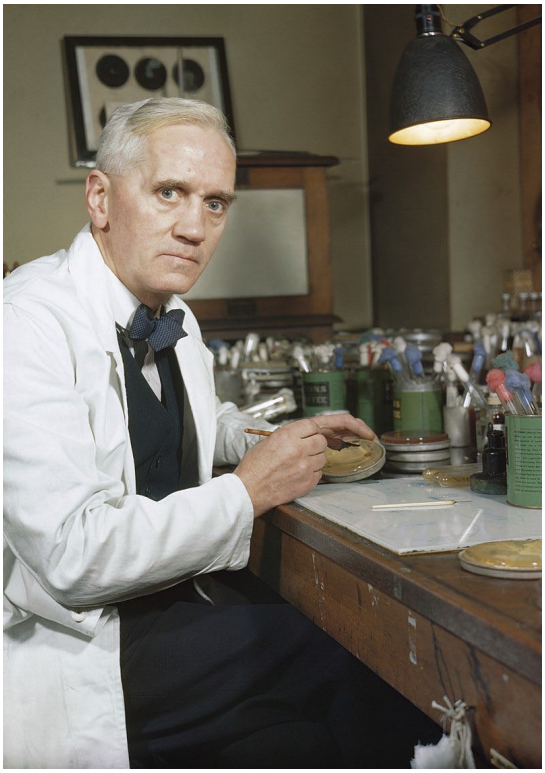


Figure 4: Alexander Fleming at St Marys Hospital, London, 1947. Image released by the Imperial War Museum on the IWM Non-commercial Licence[28].

1.2.5 An evolving threat

> "The time may come when penicillin can be bought by anyone in the shops. Then there is a danger that the ignorant man may easily underdose himself and by exposing the microbes to non-lethal quantities of the drug make them resistant."
>
> **Alexander Fleming, Nobel Lecture, 1945**

Researchers developing early antibiotics, including Fleming, were aware of the potential for bacteria to become resistant to treatment. Wide scale, often over-the-counter availability of antibiotics after the war led to huge, unrestricted consumption. This was unsurprisingly followed rapidly by increasing resistance: one paper in the Lancet in 1948 reported 59% of staphylococcal isolates were resistant to penicillin, compared to "few such strains" found before 1944[29].

Scientists discovered new classes and adapted existing antibiotics to try and overcome new resistance mechanisms occurring as a result of evolutionary pressure. Invariability, whenever a new agent was used, bacterial resistance followed (Figure 5). Methicillin resistant *S. aureus* (MRSA) was the first resistant organism to reach widespread public attention in the 1990s.

By 2014, the pipeline of new antibiotic classes was looking sparse. the World Health Organisation (WHO) released a report on global antibiotic resistance, highlighting high levels of resistance around the world and the prospect of a post-antibiotic era where simple infections can kill again.

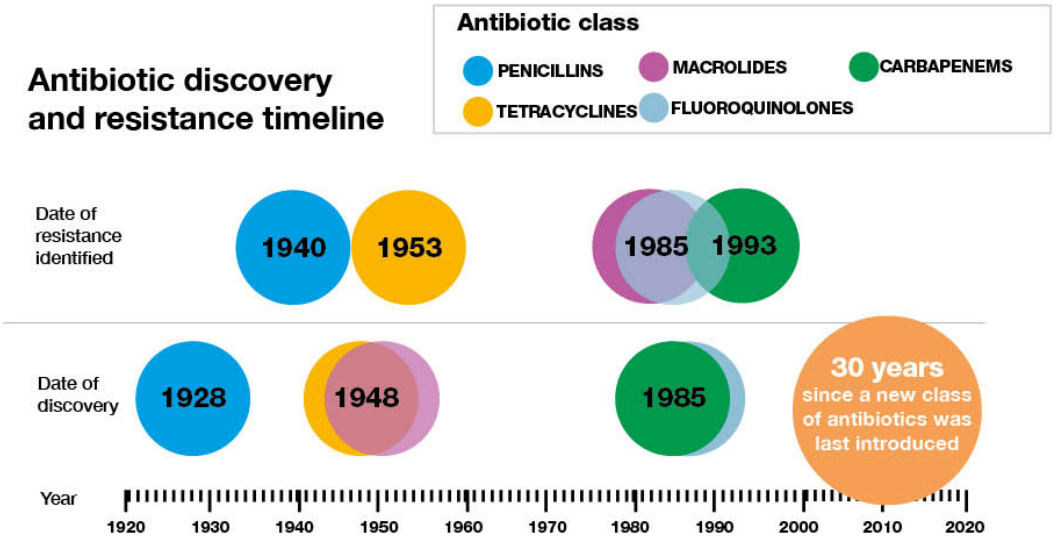


Figure 5: Antibiotic discovery timeline, from Public Health England Health matters[30]

1.3 Pathophysiology of pneumonia

Pneumonia occurs when pathogenic organism(s) infect the lower respiratory tract. These organisms typically gain entry by inhalation from the upper aerodigestive tract. Infection occurs when the virulence or volume of the infecting agent evades or overwhelms the host immune response. An impaired immune response can reduce the threshold at which infection occurs[31].

Studies using orally administered radiolabelled dyes have demonstrated that aspiration of oropharyngeal contents frequently occurs in small volumes as part of normal physiology, potentially creating a port of entry for pathogenic organisms (micro-aspiration)[32]. It has long been believed to be a major pathogenic mechanism in many pnuemonias[33] because of the frequent isolation of oropharyngeal flora in CAP and HAP, and the greatly increased risk of pneumonia for those patients with an impaired swallow. However, the theory that aspiration is a major mechanism for pneumonia is clearly not satisfactory in all cases. Dolphins have evolved entirely divided digestive and respiratory tracts and still frequently suffer from pneumonia[34].

Until recently, the lower respiratory tract was believed to be sterile. The advent of bacterial 16s rRNA sequencing technology, and metagenomic next generation sequencing (mNGS) have challenged our understanding. The respiratory tract is not sterile and is colonised by a diverse community of micro-organisms. The first paper that sequenced the lower respiratory tract was published as recently as 2010[35] and found an abundance of different phyla. The constitution of these communities in the healthy lung are similar to the oropharynx but at much lower concentrations[36] and the make-up changes during disease states[37]. Exactly how or why these changes occur, and whether they are cause or result of infection, remain unclear.

A novel emerging theory is that micro-aspiration events may even be protective against pneumonia. Wu et al modelled aspiration in mice by direct inoculation using a mixture of human oral commensal bacteria and found reduced susceptibility to disease caused by *S. pneumoniae* for 2 weeks[38].

1.4 Standard clinical definitions and microbiology of pneumonia

Many syndromes of pneumonia have been described with a broad range of different characteristics. For the purposes of this work, the diagnosis of pneumonia requires the combination of a compatible clinical illness (typically fever and cough), radiographic evidence of pulmonary infiltrates, and evidence of an inflammatory response (for example fevers, or elevated blood white cell count).

The most widely adopted definitions of pneumonia are sub-divided by where the infection originated. These definitions are practically useful as they predict common bacterial agents present, and therefore which antibiotics are likely to be effective. These definitions and the common organisms are considered below.

The microbiology of pneumonia worldwide is heterogenous and varies by geographical location, time of year and microbiological sampling methods used. New evolutionary pressures, such as vaccination for *S. pneumoniae*[39] impact the microbial aetiology. The arrival of new pathogens, such as SARS-CoV-2, can dramatically shift the landscape of pneumonia-causing agents.

1.4.1 Community acquired pneumonia

Community acquired pneumonia (CAP) is defined as pneumonia occurring prior to hospital admission, or within the first 48 hours of hospital admission. It is estimated that 25 per 10,000 adults are hospitalised with CAP each year[5] and it causes nearly 30,000 deaths annually in England and Wales[40]. Studies have estimate the financial cost in Europe is around €10 billion annually[41].

Frequently identified pathogens in CAP are presented in Table 1. Many patients with CAP do not have any pathogen identified[7,8]. Organisms and clinical syndromes associated with 'atypical' bacterial and viral infection are considered in 1.5.2 and 1.5.3 respectively. The management of CAP is discussed in 1.7.1.

The most frequently identified bacterial pathogen in CAP is *S. pneumoniae*[5,42,43]. *S. pneumoniae* is an aerobic, Gram positive diplococcus which is present in the oropharynx of about 5% of healthy adults[44]. It is a major cause of bacteraemia and meningitis, which are forms of invasive pneumococcal disease. Pneumococcal pneumonia was classically described as a syndrome of acute onset fever, cough, pleuritic chest pain and rusty coloured sputum[18]. It differs from other bacterial pneumonia pathogens in that it is associated with bacteraemia in a greater proportion of cases[45]. Pneumococcal disease peaks in the winter months and is less common in summer. The frequency of pneumococcal pneumonia has been declining, before 1945 it was identified in >90% of patients with CAP, and typically it is now reported in <50% of cases[42]. The reason for this decline is not fully explained. There are 90 known serotypes, classified by differences in polysaccharide capsule. Certain serotypes are related to more severe clinical course[46]. Vaccines introduced in the last 30 years have led to shifts in the prevailing serotypes[39,47]. Invasive pneumococcal disease (i.e. grown from a sterile site) is a notifiable illness in the UK as outbreaks can occur, particularly in care facilities[48].

Haemophilus influenzae is a small aerobic Gram-negative coccobacillus frequently found in the respiratory tract. Its only known reservoir is humans. As with pneumococcus it is frequently grown as a commensal, present in around 20% of healthy adults[49], and 30-40% of patients with stable COPD[50,51]. There are six typeable strains of *H. influenzae* (a to f) which are classified based on surface polysaccharide antigens. Type b used to be the most frequent cause of invasive disease, especially in children, however widespread Hib vaccination has dramatically reduced the incidence. Non-typeable strains (NTHi) also cause disease, however they are heterogenous and vary widely in their pathogenicity. It is these strains that are most commonly detected in patients with pneumonia[51].

Moraxella catarrhalis is another common coloniser of the upper aerodigestive tract which may rarely cause pneumonia. The organism is a Gram-negative diplococcus. Like *H. influenzae*, it is particularly seen in patients with underlying COPD[52]. There are no features that make it clinically distinguishable from other causes of CAP.

Staphylococcus aureus is a Gram-positive coccus which colonises more than 20% of healthy adults. Carriers have greater chance of infection[53]. It typically causes severe disease: Jacobs et al reported a mortality rate between 5 and 10% in 104,562 hospitalised cases of CAP due to *S. aureus* in the USA[54]. The organism is frequently associated with endocarditis[55]. As discussed in 1.2.5, MRSA emerged as a public health threat in the 1990s but rates have been steadily declining worldwide, and in the UK have reduced by 80% since the mid-2000s[56] so that MRSA CAP is now rare. *S. aureus* pneumonia is more common during influenza season, and the presence of these viruses upregulates bacterial virulence factors[57].

Streptococcus pyogenes (Group A Streptococcus/ GAS) is a beta-haemolytic Gram-positive coccus which colonises the oropharynx of 2-3% of adults. It causes a wide range of infections, most commonly pharyngitis but also rarely causes pneumonia. GAS can cause a severe, invasive, toxic shock syndrome associated with a high mortality. Invasive GAS infections are notifiable to PHE as outbreaks can occur.

Table 1: Frequency of identification of common Infectious agents in patients presenting to hospital with CAP

Organism	Proportion of total patients positive for organism, %		
	United States, Culture[5] **(n=2259)**	**Europe, Culture**[43] **(n=3524)**	**Europe, Molecular**[8] **(n=323)**
Typical' bacteria			
Streptococcus pneumoniae	5.1	17.4	35.6
Haemophilus influenzae	0.6	2	40.2
Moraxella catarrhalis	0	0.1	13.6
Staphylococcus aureus	1.6	0.7	10.2
Enterobacteriaecae	1.4	0.8	11.5
Pseudomonas aeruginosa	0.3	1.4	2.8
'Atypical' bacteria			
Legionella species	1.4	3.3	1.8
Mycoplasma pneumoniae	1.9*	1.8†	1.9
Chlamydia pneumoniae	0.4*	1.4†	0
Viruses			
Influenza viruses (A and B)	5.8	NR	7.1
SARS-CoV-2	NT	NT	NT
Respiratory syncytial virus	3	NR	1.2
Parainfluenza viruses	3	NR	3.4
Human metapneumoviruses	1.6	NT	0.9
Adenovirus	1.4	NR	2.2
Human coronaviruses	2.3	NT	0.9
Human rhinovirus	8.6	NT	12.7
No organism detected	62	58	13

NT, not tested. NR, not reported. *Diagnosed using PCR on nasopharyngeal and oral swabs. †Diagnosed using serology

1.4.2 Hospital acquired pneumonia

Hospital acquired pneumonia (HAP) is defined as occurring >48 hours after admission to a healthcare facility. Non-ventilator associated HAP affects 0.5-1% of adult hospital admissions in the UK[58]. In a US national dataset, adult admissions complicated by HAP costed ~$36,500 more than for severity adjusted controls[59].

HAP is caused by a different spectrum of organisms and associated with more antibiotic resistant bacterial pathogens than those occurring in the community (Table 2). These are typically enterobacteriaeceae, but also include the same agents seen in community acquired illness. These are heterogeneous between different locations and seasonalities. The organisms responsible for HAP vary between departments within the same institution[60]. There are few studies that specifically consider non-ventilator associated HAP, most studies of nosocomial pneumonia include majority VAP patients.

Alterations in the colonising flora of the aerodigestive tract happen rapidly after admission to hospital. One study reported that colonisation (as measured with MC&S with a cut off of $>2^{10}$ CFUs from serial oropharyngeal swabs) occurred in 51% of patients admitted to ICU within a median of 7 days[61]. The majority of these organisms were *S. aureus*, enterobacteriaceae and *Pseudomonas aeruginosa*. The acquisition of these organisms as commensals are theorised to cause pneumonia, however the presence of them in the upper respiratory tract was not predictive of those who would go on to develop pneumonia in the study. The treatment of HAP is discussed in 1.7.2.

Table 2: Frequency of identification of common Infectious agents in patients with HAP

Organism	Proportion of total patient samples positive for organism, %	
	United States[62], culture n=174	**Europe[63], culture n=898**
Bacteria		
Escherichia coli	2.3	9.2
Klebsiella species	1.7	8.4
Enterobacter species	1.1	4.3
Staphylococcus aureus	9.8	14.7
Acinetobacter baumanii	0	1.3
Pseudomonas aeruginosa	5.1	9.6
Viruses		
SARS-CoV-2	NT	NT
Influenza A and B	4	NT
Respiratory syncytial virus	2.2	NT
Parainfluenza viruses	3.4	NT
Human metapneumoviruses	2.2	NT
No pathogen identified	56	48.9

NT, not tested.

1.4.3 Ventilator associated pneumonia

Ventilator associated pneumonia (VAP) is a subgroup of HAP, defined as occurring >48 hours after intubation for invasive artificial ventilation. The incidence of VAP in intubated patients is about 10%[64] and the development of VAP is associated with an excess mortality of around 10%[65]. A retrospective matched cohort study by Kollef et al[66] 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 to be $40,000 per patient. Pneumonia (HAP and VAP) is the most common nosocomial infection in the developed world[67], complicating around 2% of hospital admissions[59].

Tracheal colonisation in mechanically ventilated patients occurs very frequently, in as much as 90% of patients, in one study where patients were assessed by serial culture[68], however only a relatively small number of patients go on to develop VAP. The link between the presence of bacteria and disease is poorly understood. Sequencing studies have shown conflicting results as to whether changes in relative abundance predict disease[69].

The common causative organisms of VAP are similar to HAP, except with a higher predominance of *S. aureus* and *P. aeruginosa*, and higher rates of resistance[70]. Esperatti et al compared aetiology of ventilator associated pneumonia to non-ventilator associated hospital acquired pneumonia within a single institution. 117 (71%) of 164 patients with VAP had an infecting organism identified, comparted to 64 (42%) of 151 of patients with HAP[71]. The common microbiological aetiology observed in VAP are presented in Table 3. Data for the proportion of VAP caused by respiratory viruses are lacking, although outbreaks are reported in ventilated patients[72]. Respiratory virus prevalence in the ICU is considered separately in section 1.5.3. Antimicrobial therapy for VAP is discussed in 1.7.2.

Table 3: Frequency of identification of common Infectious agents in patients with VAP

Organism	**Proportion of total patient sample positive for organism, %**	
	United States, culture[73] n=8133	**Europe, culture[71] n=164**
Escherichia coli	5.9	7
Klebsiella species	11	4
Enterobacter species	8.9	6
Staphylococcus aureus	27	23
Acinetobacter baumanii	7.1	2
Pseudomonas aeruginosa	18	24
Stenotrophomonas maltophilia	NR	4
No bacteria identified	15	29

1.5 Other pneumonia syndromes

1.5.1 Aspiration pneumonia

Aspiration of gastric contents can cause a spectrum of different respiratory diseases including pneumonia. The clinical diagnosis of aspiration pneumonia specifically refers to pneumonia following suspected aspiration of a large volume of gastrointestinal secretions (macro-aspiration). Aspiration pneumonia is not a clearly defined clinical entity and definitions in the literature are varied. As a result, there are limited data on incidence. There are no studies that have assessed frequency of aspiration in HAP or VAP. Studies in CAP suggest 5-15% of cases are aspiration pneumonias[74]. The mortality from aspiration pneumonia is greater than non-aspiration pneumonia after accounting for co-morbidity[75].

A prospective observational study of ventilated patients following a macro-aspiration event with radiological changes by Lascarrou et al[76] identified pathogenic bacteria from BAL fluid in 46.7%. Studies in the 1970s consistently found anaerobes in the majority of cases of aspiration pneumonia, especially in community acquired cases, so these were frequently believed to be causative[77]. This has not been the case in later studies where they are very infrequently isolated[78]. Community acquired cases of aspiration are more frequently typical CAP organisms, including *S. pneumoniae* and *H. influenzae*, whilst hospitalised patients more frequently have enteric Gram negative organisms and *S. aureus*[78,79]. As such, aspiration pneumonia can be seen as overlapping the spectrum of CAP/HAP.

Classically, the chest radiograph of a patient with aspiration pneumonia shows involvement of the lower lobes, and especially the right lower lobe (or posterior segments of upper lobes, and superior segments of lower lobes if the patient is bed bound): see Figure 6.

The key differential diagnosis following a macro-aspiration event for pneumonia is aspiration pneumonitis. The syndrome is typically characterised by profound, rapid hypoxia which corrects within 48 hours (in contrast to aspiration pneumonia which is sub-acute and does not resolve)[80]. In contrast to aspiration pneumonia, chest radiographs show bilateral patchy infiltrates. In rabbits, a pH of less than 2.4 is required to cause vigorous pulmonary inflammation[81]. Based on animal experiments with differing volumes of gastric contents, DiBardino et al estimated that a 70kg person would need to aspirate at least 120ml of gastric contents (assuming a pH of 1) to cause pneumonitis[80].

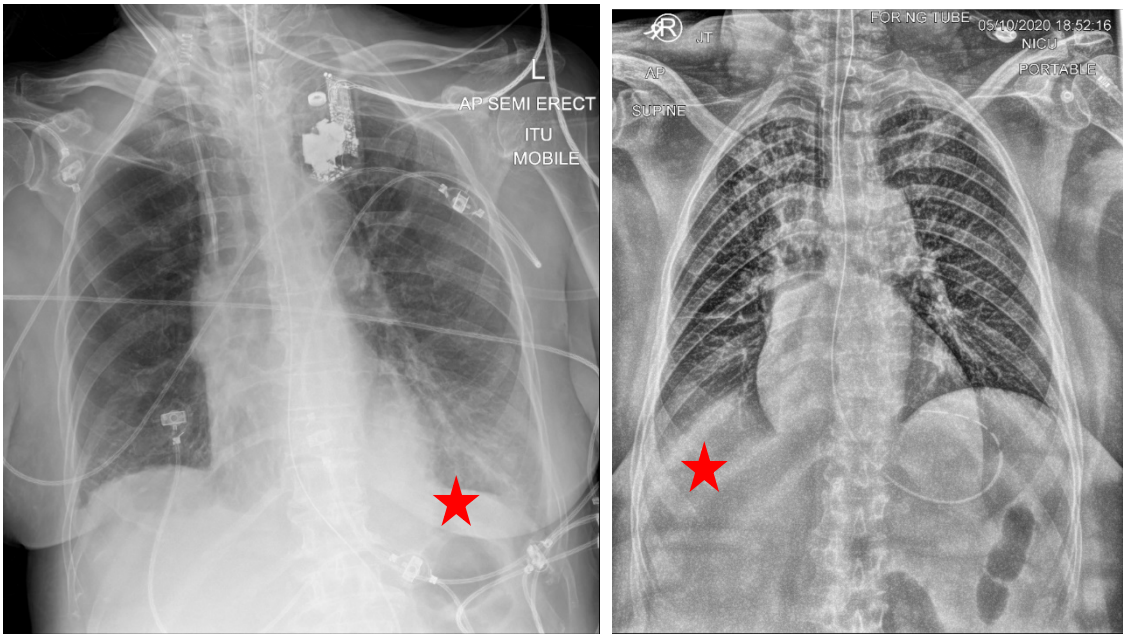


Figure 6: Two examples of chest radiographs from patients recruited to the SARIPOC trial following macro-aspiration events with pneumonia: red stars highlight dependent pneumonic changes

1.5.2 Atypical pneumonia

Atypical pneumonia is a very commonly used phrase in clinical practice. It is poorly defined but is generally used to describe pneumonia caused by a group of 'atypical' pathogens. These micro-organisms differ from common organisms like *S. pneumoniae* or *H. influenzae* in their clinical manifestations, lack of susceptibility to first-line antibiotics, and difficulty demonstrating them in the diagnostic laboratory. The most common atypical bacterial causes of CAP are *Legionella* spp., *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*.

1.5.2.1 Legionnaire's disease

Legionella pneumophilia serotype 1 makes up around 80% of Legionellosis infections[82]. The remaining ~20% include the other (>15) serotypes and other *Legionella* species including *L. longbeachae*, *L. bozemanii* and *L. micdadei*. Legionnaire's disease is the severe end of the spectrum of illness caused by *Legionella* species, which can also cause a mild, non-pneumonic illness called Pontiac fever. There are typically 400-500 cases of Legionellosis reported annually in England and Wales, of which >90% were Legionnaire's disease[83]. The mortality rate of Legionnaire's in patients admitted to ICU is up to 30%[84].

Legionella is a common environmental Gram-negative aerobe which is found in warm water sources. Outbreaks can occur associated with these, classically in air conditioning units. Patients frequently have severe pneumonia and experience more non-pulmonary symptoms including headaches, and diarrhoea, than those with 'typical' bacterial pneumonia. Radiologically Legionnaires can be indistinguishable from 'typical' lobar pneumonia, although it commonly presents with multifocal bilateral infiltrates as it did for a patient recruited to the SARIPOC trial who was found to have Legionnaires (see Figure 7). The organism is difficult to culture (see 1.6.3.2). Nosocomial cases occur, but these are very rare in the UK[83].

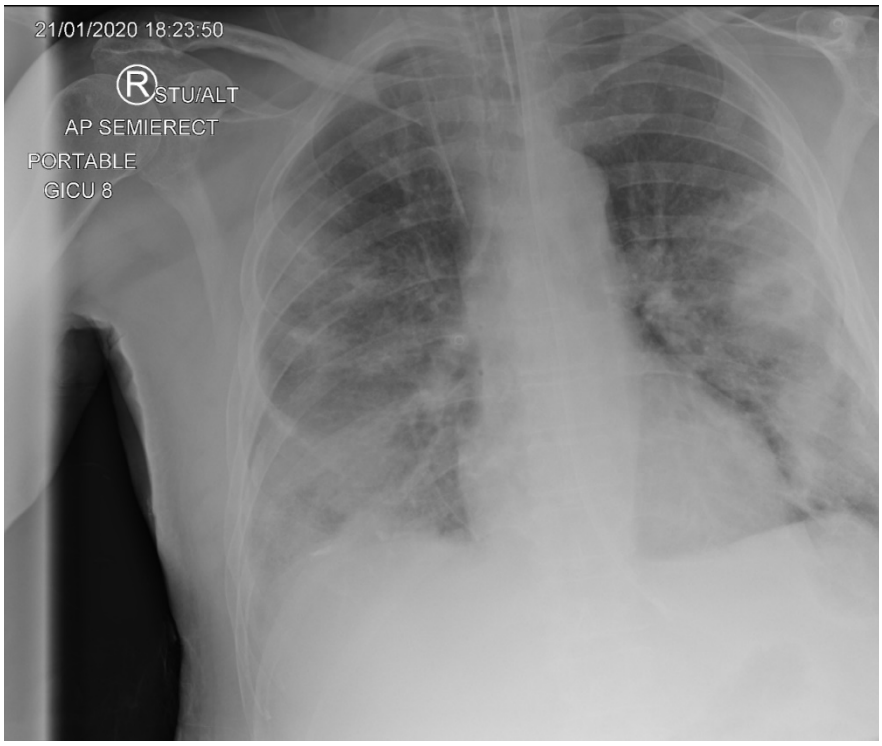


Figure 7: Chest radiograph of a patient with community acquired *Legionella* pneumonia in the SARIPOC trial

1.5.2.2 *Mycoplasma pneumoniae*

Mycoplasma pneumoniae is a fastidious aerobe which causes pneumonia. It is one of the smallest known free-living bacteria. The organism does not have a cell wall, so will not be visible with Gram's stain. It is believed to be spread by droplet transmission from an infected host. Infections peak in Autumn, although epidemics within shared facilities can occur at any time of year. It causes around 2% of hospitalised cases of pneumonia[5]. Pneumonia caused by *M. pneumoniae* is typically milder than other pathogens and has a longer, more insidious onset. The mortality in one study of hospitalised adults with CAP was 0.7%[85].

Extra-pulmonary features are much more common than with typical pathogens, including skin rashes, haemolysis (as a result of cold agglutinins) and cardiac involvement. Radiographically it can appear like any other pneumonia, although patchy reticular infiltrates are most common. It is extremely difficult to isolate *M. pneumoniae* in the laboratory (see 1.6.4). It is not part of normal human oropharyngeal flora.

1.5.2.3 *Chlamydia pneumoniae*

Chlamydia pneumoniae is an obligate intracellular Gram-negative bacterium which is rarely found to be the cause of CAP. The limitations of conventional diagnostics (see 1.6.4.2) mean that the extent of disease caused by *C. pneumoniae* is largely unknown: at least 80% of adults have IgG in

seroprevalence studies[86]. Only 0.4% of adults hospitalised with CAP had evidence of *C. pneumoniae* infection[5].

As with *Legionella* species, outbreaks occur within communal shared areas that are poorly ventilated. Clinically, *C. pneumoniae* pneumonia is mild, with an insidious onset (>2 weeks) and prominent upper respiratory tract symptoms, like laryngitis. Radiographically, there are patchy infiltrates.

1.5.3 Viral pneumonia

Before the start of the SARS-CoV-2 pandemic, respiratory viruses were detected in ~30% of adult patients presenting to hospital with CAP[5,87,88]. The breakdown of different respiratory viruses detected by nucleic acid amplification tests (NAAT) in CAP are presented in Table 1 and in HAP in Table 2. 30-40% of patients admitted to intensive care units with pneumonia have a respiratory virus detected, with a broad range of viruses present[89,90].

The role which viruses play in pneumonia is not well understood but they are clearly important: a meta-analysis of 31 studies of CAP demonstrated that patients with viral/ bacterial co-infections had a higher risk of death (OR 2.1, 95% CI 1.32-3.31)[88]. Several respiratory viruses clearly have the potential to cause a severe primary pneumonia in the immunocompetent host (see Table **4**) whilst others are frequently detected, but their significance and role in disease is unclear.

There is good evidence that viruses interact with bacteria, for example the upregulation of *S. aureus* virulence factors discussed in 1.4.1. There is also growing evidence that viruses interact with one another (see 1.5.3.2).

Table 4: Respiratory viruses commonly detected in pneumonia

Strong evidence for causality in pneumonia	**Less evidence for causality in pneumonia**
SARS-CoV-2	Human Rhinoviruses
Influenza A and B	Human Coronavirus*
RSV	
Human metapneumovirus	
Adenovirus	
Parainfluenza viruses	

*includes OC43, HKU1, 229E, NL63

1.5.3.1 COVID-19

SARS-CoV-2 emerged as a novel pneumonia pathogen in December 2019 in Wuhan, China[91], causing the disease COVID-19. Since that time, it has spread around the world causing many millions of deaths worldwide. It is a betacoronavirus which causes a severe illness after a short (median 4 day) incubation period. The most specific symptoms are persistent cough, fever and anosmia[92]. Radiographs have a typical appearance of bilateral, multifocal patchy consolidation (see Figure 8).

Patients with COVID-19 requiring hospitalisation have a severe course, with up to 17% requiring critical care admission during the first wave in the UK, and at least 26% not surviving to discharge[93]. Our contribution towards improving diagnosis and mitigation of COVID-19 in hospital are presented in Chapter 5.

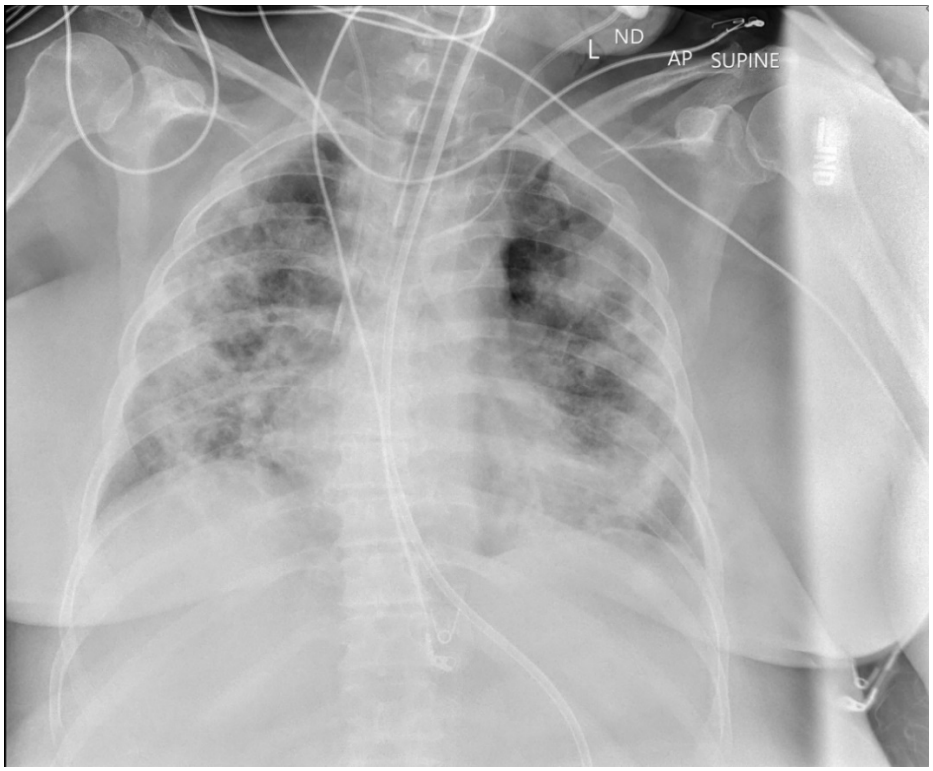


Figure 8: A typical chest radiograph of a patient with Covid-19 from the SARIPOC trial

1.5.3.2 Influenza

Prior to the emergence of SARS-CoV-2, Influenza viruses were the major causes of seasonal respiratory virus epidemics and pandemics. The emergence of SARS-CoV-2 has, so far, dramatically reduced the prevalence of influenza viruses (see Figure 9) due to social distancing measures and travel restrictions. There are four subtypes of influenza, A-D. Of these, A and are B are the main cause of disease in humans. The two main envelope glycoproteins of influenza A,

haemagglutinin and neuraminidase, each have several described subtypes (H1, H2 and H3, and N1 and N2 respectively). The main reason for the pandemic potential of Influenza A is how readily these glycoprotein antigens can change.

Influenza can cause primary viral pneumonia but can also often be complicated by secondary bacterial pneumonia. A study reviewing lung histology and microbiology from the 1918-1919 Spanish flu pandemic (prior to the availability of antibiotics) found pneumonia pathogens in > 90% of samples, with histology consistent with bacterial pneumonia in almost all samples[94]. It has been theorised that the spread of previous epidemic influenza viruses have been slowed by interaction with existing viral infections[95], and there is a growing body of epidemiological evidence to support this phenomenon in other respiratory viruses[96].

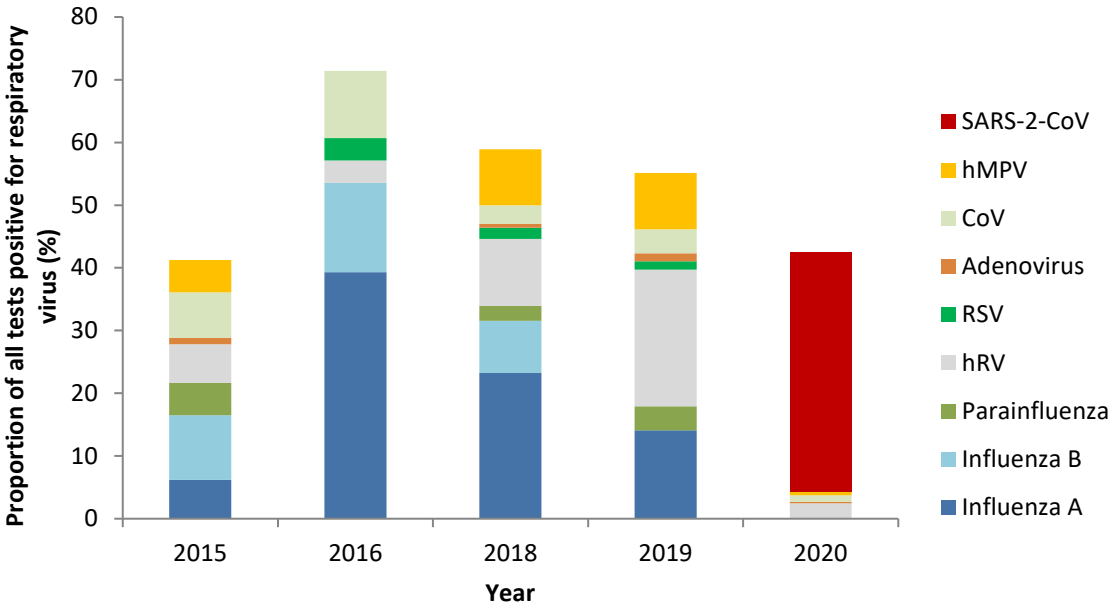


Figure 9: Proportion of tests positive for different respiratory viruses at presentation to secondary care[97]

1.5.3.3 RSV

RSV is a single-stranded RNA virus which is a frequent cause of seasonal respiratory illness, particularly affecting children and the elderly in the winter months. The severity of these infections can vary from asymptomatic to severe pneumonia. There are two major subtypes, A and B, which are typically both present in outbreaks. Subtype A causes more severe disease[98]. One study in adults found that 26% of healthy adults with RSV infection developed clinical features of a lower respiratory tract infection[99].

1.5.4 Uncommon causes of pneumonia

There are an enormous variety of micro-organisms which have been associated with pneumonia, and to review these is beyond the scope of this work. The microbiology of pneumonia is particularly diverse in the context of the immunosuppressed host, where more unusual agents cause disease. There are also a very wide variety of non-infectious mimics which can fulfil the criteria for pneumonia, but which do not respond to antibiotic therapy. A non-exhaustive list of non-infectious mimics and unusual infectious causes are presented in Table 5.

Table 5: Unusual pneumonia causes and non-infectious mimics

Infectious causes	**Non-infectious mimics**
Mycobacterium tuberculosis	Alveolar haemorrhage
Non-tuberculous mycobacteria	Pulmonary vasculitidies (granulomatosis with polyangiitis)
Pneumocystis jirovecii	Cryptogenic organising pneumonia (COP)
Aspergillus species	Eosinophilic pneumonias
Coxiella burnetii	Pulmonary embolus
	Pulmonary oedema
Cytomegalovirus	Acute interstitial pneumonia

1.6 Current standard diagnostic testing for pneumonia

Current widely used tests for pneumonia are considered in the following section. Novel molecular diagnostics, and the emerging evidence base for their accuracy and impact are reviewed in 1.10.

1.6.1 Microbiological culture and sensitivity (MC&S)

The bedrock of modern microbiological testing remains culture of sputum and blood on solid media. These tests are very rarely used in mild disease, and not recommended in expert guidelines in this context due to the relatively high cost and low yield. They are both advised in all cases of severe pneumonia, or pneumonia where this a high likelihood of organisms which will be resistant to empirical therapies[100,101]. There is little direct evidence that culture based investigations improve outcomes[102]. Culture typically takes 48-72 hours for full organism identification and phenotypic sensitivity.

1.6.1.1 Sputum

Lower respiratory tract samples may be expectorated by the patient (either by their own accord or using a cough assist device). Not every patient with pneumonia is able to expectorate sputum so invasive techniques can be employed when sampling is deemed necessary. Invasive methods include bronchoalveolar lavage (BAL) and endotracheal aspiration (ETA). BAL is direct sampling from the lung using a bronchoscope inserted via the mouth. A small amount of fluid is injected into the area to be sampled (as a lavage/ wash) and then extracted by suction. In clinical practice it is considered the 'gold standard' diagnostic test as it allows visualisation of the parenchyma and direct sampling. ETAs are obtained by inserting a suction catheter into the lower respiratory tract via an endotracheal tube. As such, they are not under direct visualisation.

Protected specimen brush (PSB) samples are another technique for obtaining samples that are not routinely used in clinical practice. A brush is introduced via bronchoscope to the area of interest but is covered during its passage in and out of the upper aerodigestive tract to avoid contamination. The standard method for laboratory processing of sputum for bacterial culture is described in 2.9.6.1.

1.6.1.2 Blood

Blood cultures yield a positive organism in less than 10% of patients presenting to hospitals with CAP[103], and only a small proportion of these lead to a meaningful change in antibiotics. A prospective observational cohort study by Campbell et at found that 1.97% (15/760) patients who were tested had a meaningful change of antibiotic therapy based on a positive result[103].

Blood samples are obtained by venesection into anaerobic and aerobic culture media (BACTEC™). These are incubated at 36°C for 5-7 days within an automated device. If respiring organisms are present at sufficient concentration, there is a change in the fluorescence through the bottle which is detected automatically and flagged to the operator. At this stage, the medium is subbed onto solid media for growth. The standard method for laboratory processing of blood for bacterial culture is described in 2.9.6.2.

1.6.2 Respiratory virus polymerase chain reaction (PCR) and antigen testing

Respiratory virus PCR of upper respiratory tract was recommended during periods of high community prevalence of influenza viruses. After the emergence of SARS-CoV-2, this extended to all admissions to critical care. PCR testing can be either uniplex real-time PCR (RT-qPCR) or multiplex PCR (such as with the Filmarray, discussed in 1.10.1.1). Viral PCR in any format is typically performed on nasopharyngeal swabs suspended in viral transfer medium and is highly sensitive and specific.

Highly conserved regions of different viral RNA are reverse-transcribed and amplified creating a detectable fluorescent signal. Lower respiratory tract samples may have a greater sensitivity for viral infection than nasopharyngeal sampling in patients with lower respiratory tract infection[104].

The advent of the COVID-19 pandemic has driven the requirement for readily available testing for respiratory viruses in hospitalised adults. This is discussed in Chapter 5.

1.6.3 Urinary antigen testing

Pneumococcal and legionella urinary antigen testing are recommended by both the BTS and IDSA/ATS in severe CAP[100,101]. Both guidelines also recommend urinary antigen testing for Legionella in the context of recent travel, patient specific risk factors or during suspected outbreaks. Expert bodies do not make recommendations for use in HAP and VAP, and antigen testing is rarely used in this context due to them being infrequent pathogens in hospitalised patients.

1.6.3.1 *Streptococcus pneumoniae*

Urinary antigen testing for pneumococcus is appealing as it is a frequent cause of CAP and the test modality is culture-independent, so less likely to be affected by antibiotic administration. Tests can remain positive for several weeks after infection[105].

There are several urinary antigen test kits for *S. pneumoniae* which are commercially available. The Abbott (formerly Alere) BinaxNow! kit is by far the most frequently used and investigated (for specific details about how the test works and is performed, see 2.10.3). It is validated for use on any urine sample and CSF, up to 24 hours after starting antibiotics. The C-polysaccharide which is targeted is present in all pneumococcal serotypes[106]. The package insert reports a sensitivity of 90% (95% CI 60-98%) and specificity of 71% (59-80%) when compared to the reference standard of blood culture. A systematic review and meta-analysis of 11 studies using positive sputum Gram stain or blood culture reported a sensitivity of 60.3% (46.4-74.4%) and a specificity of 89.2% (82.5-94.4%)[107].

The use of urinary antigen testing is still debated[108], and guideline recommendations are weak, and based on limited evidence. None of the RCTs that have been performed have been suitably powered to look at clinical outcomes. Falguera et al randomised 177 patients 1:1 to either receive empirical treatment or urinary antigen testing for Legionella and pneumococcus guided therapy[109]. There was no significant difference in outcomes for those patients randomised to the targeted treatment arm, however only 25/88 in the intervention arm had a positive antigen, and the study only randomised patients who were clinically stable. Of note, 3/25 patients with a

positive result relapsed on targeted therapy, compared to 3/152 who were empirically treated (12% vs 3%, p=0.04).

1.6.3.2 *Legionella pneumophilia*

Legionella species infection can cause life-threatening disease and identification of the causative organism is extremely difficult. It takes at least 3 days to grow on special media (buffered charcoal yeast extract: BCYE). As with *S. pneumoniae*, therefore, a culture independent method of identification is highly desirable.

In comparison to *S. pneumoniae*, there is a greater variety of kits used. One of the forerunners was again the BinaxNow! kit which works by the same method as the pneumococcal urinary antigen cards. All of the widely used kits target the serogroup 1 antigen. The package insert for the BinaxNow! kit reports a sensitivity of 95% (88.7-98.4%) and specificity of 95% (91-97.6%) for detecting *L. pneumophilia* serogroup 1 from retrospectively tested samples.

1.6.4 Serological testing

The clinical utility of serological testing in pneumonia is very limited. This largely owes to the fact seroconversion takes several weeks, by which point the patient may have recovered and treatment is unlikely to be of benefit[110].

1.6.4.1 *Mycoplasma pneumoniae*

Mycoplasma culture is technically challenging and is not available in most diagnostic laboratories. Culture takes 2-3 weeks on specialised media and frequently fails as a result of contaminating bacterial overgrowth. Acute and convalescent serology have historically represented the gold standard diagnostic test for *M. pneumoniae* but only provide retrospective evidence of infection, so are rarely performed. PCR of respiratory tract samples is increasingly being used for diagnosis, although it is still not widely available in the UK.

1.6.4.2 *Chlamydia pneumoniae*

As an obligate intracellular bacterium, *C. pneumoniae* can only be cultured in cell culture, like viruses. Acute and convalescent serology are most widely used in diagnosis, but in practice these are very rarely performed.

1.7 Antimicrobial therapy

The bedrock of therapy for pneumonia is antimicrobials: compounds which specifically target an infectious organism. No trial has specifically compared treated and untreated patients. Pertel et al presented data from two phase-3 RCTs for Daptomycin treatment of CAP during the drug development, comparing it to Ceftriaxone for the treatment of hospitalised adults[111]. Unbeknownst to the study group, Daptomycin is avidly bound to pulmonary surfactant so does not reach the pulmonary parenchyma, so the trial has been viewed as effectively one of beta-lactam vs placebo. Reassuringly, cure rates were significantly lower in the Daptomycin arm, however a clinical cure rate of 70.9% was observed, compared to 77.4% in the Ceftriaxone arm (95% CI of difference -12.4% to -0.6%).

Large retrospective datasets from hospitalised adult patients with CAP in the US have shown strong associations between time to first dose and mortality, especially when administered less than 4 hours after presentation[112]. These findings have not been consistent in other studies[113].

Antimicrobial choice is informed by local resistance patterns, the likely responsible organism, and the availability/ cost of therapies. As such, there is no universal choice for pneumonia. Local prescribing guidelines are presented in Table 6.

1.7.1 Community acquired pneumonia

The British Thoracic Society recommend empirical treatment of mild CAP (as determined by CURB score, see 1.9.1) with amoxicillin, owing to the likelihood of *S. pneumoniae* being the responsible organism and penicillin resistance rates in the UK being low. For moderate CAP, amoxicillin with a macrolide is the recommendation, on the basis that atypical pathogens account for approximately 20% of infections. For severe CAP, co-amoxiclav with a macrolide is the recommendation, based on a concern that *Legionella* spp. and enteric bacteria can cause more severe disease[100].

In the IDSA/ATS CAP guideline, the recommendation in choice of antimicrobials is the same for severe CAP, however the rationale for the use of macrolide therapy is different[101]. Macrolides have a broad range of immunomodulatory effects, including decreasing the number of neutrophils and reducing cytokine concentrations, which some have believed to be beneficial in pneumonia[114]. There are no RCTs comparing beta-lactam monotherapy with a macrolide combination therapy in severe pneumonia. A systematic review and meta-analysis including 9850 adult patients with severe pneumonia observed a small but significant mortality benefit between patients with combination therapy of any antibiotic when comparted to those without (RR 0.84,

95%CI 0.71-1.00, p=0.05), however this study did not include background aetiology (including Legionella infection), and included patients on non-beta-lactam antibiotics[115].

Current expert guidance is that treatment duration for CAP should be at least 5 days, based on multiple RCTs which demonstrated non-inferiority to longer courses in mild CAP[116]. NICE and BTS guidelines recommend a 7-10 day course for severe CAP[100,117]. Interestingly, a recently published RCT has demonstrated non-inferiority of 3 days of antibiotics in patients hospitalised with CAP, providing they met pre-defined clinical stability criteria[118].

1.7.1.1 Legionnaire's disease

Once identified, atypical organisms require individualised treatment as they are resistant to beta-lactams. International guidelines do not make specific recommendations on therapy for atypical organisms, with the exception of the BTS CAP guidelines in confirmed *Legionella* infection.

Legionella species are intrinsically resistant to beta-lactams. The BTS guidelines recommend a respiratory quinolone such as levofloxacin or moxifloxacin. Azithromycin is also highly active in vitro against *Legionella* species however observational evidence suggests quinolones lead to faster symptomatic cure than macrolides in Legionnaires disease[119,120]. No RCT has compared different therapies.

1.7.2 Hospital acquired pneumonia and ventilator associated pneumonia

The NICE and IDSA/ATS guidelines recommend that empirical antibiotic treatment for HAP and VAP should be based on local antibiotic resistance data, as the level of resistance varies dramatically in different locations and changes through time[121,122]. As a result, there is no universal guidance on empirical antimicrobial agents. Empirical options depending on local susceptibility suggested in the NICE guidelines are piperacillin-tazobactam, ceftazidime, ceftriaxone, cefuroxime, meropenem, ceftazidime-avibactam or levofloxacin[122].

The optimal duration of treatment for HAP has not been studied. In the context of VAP, two systematic reviews found no difference in mortality or recurrence when comparing patients treated with a 7 or 8 day course of antibiotics when compared to a longer course (>8 days)[123,124] A subgroup in one of these meta-analyses of patients with VAP due to *P. aeruginosa* and *Acinetobacter* species found short courses of antibiotics were associated with recurrent infection (OR 2.18)[123]. This was not replicated in the other study, and a meta-analysis of all of the RCTs from both reviews did not support this[121]. The 2016 IDSA guidance recommends 7 days therapy for VAP on the basis of this, and extrapolates it to HAP as well[121]. The NICE HAP guidance recommends a review for the need for therapy after 5 days, but does not comment on VAP[122].

Table 6: Local trust antimicrobial guidance in ICU during the study period

	CAP	**HAP**		**VAP**	
		<5 days after admission	**≥5 days after admission**	**<5 days after admission**	**≥5 days after admission**
1st Line	Cefuroxime or co-amoxiclav AND Azithromycin	Ceftriaxone	Piperacillin-tazobactam	Co-amoxiclav	Piperacillin-tazobactam
2nd line	Levofloxacin	Chloramphenicol	Ceftazidime	Levofloxacin	Meropenem
2nd line		Vancomycin and ciprofloxacin	Vancomycin and ciprofloxacin		

1.7.3 Viral infection

Antiviral treatment options for respiratory viruses are limited. Of the common respiratory viruses, only Influenza and SARS-CoV-2 have approved antiviral therapies, although antiviral treatments for other respiratory viruses including respiratory syncytial virus (RSV) are in development. The most widely used antiviral treatment for influenza are neuraminidase inhibitors (NAIs). NAIs are recommended by national guidelines[125] for all hospitalised patients with influenza and their use is associated with improved outcome including reduced mortality, especially when used early in the disease course[126]. There are two approved anti-viral agents for SARS-CoV-2 at the time of writing, remdesivir[127] and molnupiravir[128] with others in late-stage clinical trials.

1.7.4 Aspiration pneumonia

Antibiotic treatment for aspiration pneumonia should follow the same guidelines as for CAP/HAP/VAP (see section 1.7). Occasional clinical practice is to give metronidazole as an adjunct to a beta-lactam/ beta lactamase combination for suspected aspiration pneumonia. No trials have considered this directly. Several interventional trials have compared different treatment options in hospitalised patients (including ampicillin-sulbactam rather than co-amoxiclav, moxifloxacin and carbapenems) and have broadly found equivalence of these agents[129].

The evidence for antibiotic therapy are unclear in suspected aspiration pneumonitis due to difficulty differentiating it from pneumonia and acute respiratory distress syndrome (ARDS). Expert opinion is that empirical antibiotics should be discouraged in suspected aspiration pneumonitis after macro-aspiration unless the patient has small bowel obstruction, has severe disease, or is taking gastric acid suppressing medication[33]. This should be re-considered if the patient has not responded within 48 hours. One potential exception to this is in patients who are

cooled and mechanically ventilated following a shockable out of hospital cardiac arrest. A recent RCT by François et al of 198 patients in this cohort had lower rates of early VAP when given 2 days of co-amoxiclav compared to controls[130].

1.7.5 De-escalation

Antibiotic de-escalation is the alteration or discontinuation of antimicrobial therapy from broad spectrum empirical therapy to a more targeted, narrow spectrum antimicrobial. Antibiotics are considered broad-spectrum when they have activity against many different species of bacteria. De-escalation is a key component of antibiotic stewardship and widely accepted as good practice[121,122,131]. It is theorised to reduce harms related to antibiotic use, including drug toxicity, *Clostridioides difficile* infection, and reducing antimicrobial resistance.

Trials evaluating the efficacy and safety of antimicrobial de-isolation based on culture results are sparse. The vast majority of published studies are observational and difficult to compare directly due to differences in the patient populations (HAP, CAP, VAP, ICU/ non-ICU, severe sepsis etc) No interventional studies have evaluated the safety or efficacy of antimicrobial de-escalation based on the results of rapid multiplexed PCR for LRT pathogens of pneumonia. Studies to date have made their de-escalation intervention after at least 48 hours when the patient has stabilised, and culture results are available. 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 therapy[121,132].

The IDSA and the American Thoracic Society advise antibiotic 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'[121]. There a small number of interventional studies looking at antibiotic de-escalation based upon microbiological culture results in HAP/VAP which have suggested this practice is safe[133,134]. High quality data for outcomes, including length of intensive care stay and antibiotic savings, are lacking and conflicting. A meta-analysis by Khan et al[135] of observational studies reviewing antibiotic de-escalation in pneumonia in ICU (HAP and VAP only) found no difference in mortality between those who were de-escalated according to culture result and those that weren't.

In the context of CAP, both the IDSA[116] and NICE/BTS[132] guidelines recommend organism directed therapy when a pathogen has been identified by culture. High quality data is lacking but observational data and limited interventional data suggests this is safe[109,136,137]. A systematic review by Paul et al[138] included studies with CAP, HAP, VAP and Blood stream infection. The

reviewers found no association between de-escalation and survival with pneumonia (OR 0.97, 95% CI 0.45–2.12).

1.8 Biomarkers and their utility in pneumonia

A biomarker is any molecule, gene or characteristic which identifies a pathological process. A large number of proteins have been investigated as potential biomarkers in pneumonia, mainly designed with the goal of predicting mortality for stratification of treatment[139], or guiding decisions to start antibiotics. In this section I will consider the most widely used and researched blood biomarkers in pneumonia, C-reactive protein and Procalcitonin. I will also discuss sputum specific biomarkers briefly.

1.8.1 Serum C-reactive protein

C-reactive protein is an 'acute-phase' (defined as an increase of at least 25% in inflammatory disorders) protein produced in the liver. As such, it is a non-specific marker of inflammation. CRP was initially discovered in patients with pneumonia. It is so-named because it reacts with the pneumococcal C-polysaccharide in the plasma of patients during the acute phase of pneumococcal pneumonia[140]: the same protein detected by pneumococcal urinary antigen testing in 1.6.3.1. It has been most thoroughly investigated as a tool to reduce unnecessary antibiotic prescribing in acute respiratory illness is primary care, although it has not been widely adopted in the UK for this purpose. A previous NICE guideline [CG191] recommending point-of-care testing with CRP (with a cut off for withholding antibiotics of <30mg/L) in primary care has been retracted since the start of the SARS-CoV-2 pandemic.

There are no studies directly comparing patient outcomes for patients who have antibiotics started based on CRP levels for patients hospitalised with pneumonia. The use of CRP in initiating antibiotics is contentious in HAP and VAP[141]. Limited observational trial data showed CRP levels were similar in patients with and without VAP[142]. Whilst there is little evidence to support the use of CRP in withholding antibiotics, it has been shown to identify response to treatment when falling adequately by day 3[143].

1.8.2 Serum Procalcitonin

Procalcitonin (PCT) is a protein precursor to calcitonin. It is increasingly used as a biomarker to aid antibiotic prescribing in the context of lower respiratory tract infections. In normal physiology, it is only produced in thyroid parafollicular cells and not released into the circulation. In systemic

inflammatory states however, it is produced in almost all tissues and readily released into the blood stream within 4-12 hours of provoking inflammation[144].

PCT measurements are used in a variety of different ways by clinicians in CAP, HAP and VAP. The strongest evidence for its use is in early discontinuation of antibiotics in the context of CAP, however others have used it to decide on initiation of therapy. The IDSA/ATS CAP guidelines do not currently advocate the use of PCT as an adjunct to decide on initiation of antimicrobials[101], citing a high variability in the sensitivity of PCT for determining the presence of a bacterial pathogen from studies ranging between 38-91%[145]. The guidelines do not make a recommendation on its use in shortening courses of therapy[101]. The BTS guideline makes no recommendations[100]. A Cochrane review of 26 RCTs studying acute respiratory tract illness in 6708 adults in a hospitalised and non-hospitalised patients, found lower mortality and reduced antibiotic exposure in PCT guided (i.e. both discontinuation and initiation of therapy) therapy arms[146]. These trials used similar cut offs for their prescribing algorithms and included a variety of interventions including both withholding antibiotic initiation in patients with a low PCT (typically <0.25ng/ml or <0.1ng/ml) or stopping antibiotics courses once PCT had reduced by a proportion of peak levels (typically >80% or below a threshold). Studies enrolled patients from critical care (37%), the emergency department (49%) and primary care (15%). 1468 (43.5%) patients had CAP. Small proportions of patients (5-8% of patients respectively) had a diagnosis of VAP or HAP.

Use of PCT to withhold antibiotics in critical care is contentious, and the 2016 IDSA/ATS HAP/VAP guidelines do not recommend PCT as an adjunct to decide on initiating therapy[121]. A meta-analysis performed as part of the guideline showed an area under the curve of the summary receiver operating characteristic curve of 0.76 (95% CI 0.72-0.79) for diagnosing pneumonia, which was deemed inadequate for widespread use. The guidelines do however recommend PCT use to guide discontinuation of therapy and there is increasing agreement for its use. A large multicentre European study in critical care units (SAPS)[147] enrolled 1575 critically ill patients with any suspected infection and advised ICU clinicians to stop antibiotics when PCT fell below <0.5ng/ml or levels decreased by 80% from peak. They reported significantly reduced antibiotic exposure and 28-day mortality compared to standard care. Adherence to the algorithm was 53% at 48 hours.

There is limited data on the use of PCT in VAP. Only one small interventional study has used PCT for guiding early cessation of antibiotics and reported a median reduction in the duration of antibiotics of 4.5 days. This trial did not observe any differences in safety outcomes (albeit without being adequately powered to look for this)[148].

1.8.3 Sputum-specific biomarkers

There are no sputum-specific biomarkers in routine clinical use, however a recent multicentre study in the UK trialled one. Hellyer et al examined host-response proteins in BAL fluid from patients hospitalised with VAP and compared them to microbiological culture. They reported that a combination of interleukin-1β (IL-1β) and interleukin-8 (IL-8) excluded VAP with a sensitivity of 100%[149]. After validation, they took this forward to a large multicentre RCT with the aim of reducing the amount of antibiotic used. Unfortunately, they did not see a significant reduction in the number of antibiotic free days following the investigation. The investigators put this lack of change down to entrenched prescribing behaviour[150].

1.9 Severity scoring

Multiple severity scores for CAP have been validated to help inform need for hospitalisation and to guide empirical antimicrobial treatment. The most widely used of these in the UK is the CURB-65 score[151]. The pneumonia severity index (PSI)[152] is another severity score which is used more widely in the USA for the same purpose. It is more complex but has been more extensively investigated.

Predicting mortality in HAP and VAP is difficult as it is often largely determined by the underlying reason for hospital admission and critical care illness. As such, specific predictive scores have not been validated for these entities.

Scoring systems have been developed to predict mortality in non-specific critical illness, and of these the Acute Physiology and Chronic Health Evaluation score (APACHE) II[153] and Sepsis related organ failure assessment (SOFA)[154] scores are the most widely used and reported. Separate scoring systems have also been validated for determining the requirement for ICU level care in severe CAP, most notably the IDSA/ ATS 2007 criteria[116].

1.9.1 CURB-65

In the UK, CURB-65 is the most widely adopted severity score for CAP. It is a 6-point scale which has been validated to predict 30 day mortality in adults presenting with CAP (see Figure 10) and features in the BTS and NICE guidelines for directing empirical antibiotic prescribing[100] (discussed in 1.7.1). Patients are stratified into low, intermediate, and high-risk groups based on their score. Those in the low-risk group are recommended to be managed at home with narrower spectrum antimicrobials. Of note, the validation study excluded patients in whom pneumonia was not the primary cause of admission and also patients who had been in hospital in the past 14 days[151].

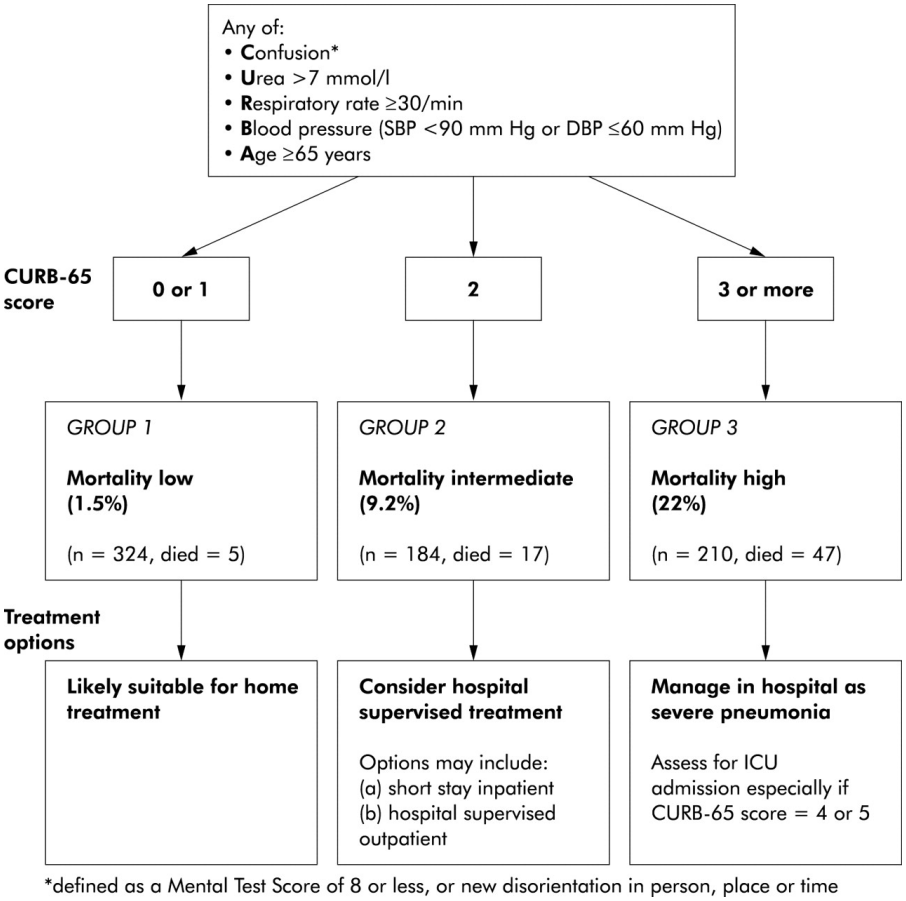


Figure 10: CURB-65 Score from Lim et al (with permission)[151]

1.9.2 Pneumonia Severity Index (PSI)

The PSI[152] is another CAP prognostication tool that is considerably more complex than CURB-65. It does not feature in UK clinical practice guidelines: unlike CURB, it was validated in a US cohort (as opposed to the British, Dutch and antipodean healthcare settings used for CURB-65). It is recommended in preference to CURB-65 in the IDSA/ ATS guidelines[116] with the authors citing greater evidence from clinical trials and identification of more low risk patients.

The validation studies for PSI only excluded patients who had been hospitalised within the last 10 days and those known to be HIV positive (i.e. patients were required to have symptoms and a radiographic diagnosis of pneumonia, but it was not required to be the main presenting complaint). Reported 30 day mortality was 0.1% for class I, 0.6% for class II, 0.9% for class III, 9.3% for class IV and 27% for class V[152]. Two large North American cluster randomised trials demonstrated that PSI is safe as a triage tool and led to fewer admissions of low-risk patients[155,156]. A European RCT was not adequately powered to report on mortality but did show improved patient satisfaction scores as a result of increased outpatient management[157].

1.9.3 2007 IDSA/ATS Criteria for severe CAP

The 2007 IDSA/ ATS CAP guidelines[116] recommended using specific criteria to assist in decision making for patients with CAP who may require ICU level care. The latest IDSA/ ATS guideline recommends that the presence of either one major, or three minor criteria listed on Table 7, in combination with clinical judgement, should be used to determine requirement for higher levels of care. This scoring system was found to be the best predictive score for ICU requirement in a meta-analysis with a sensitivity of 84% and specificity of 78%[158].

Table 7: 2007 IDSA/ATS criteria for defining severe CAP[116]

Minor Criteria	**Major Criteria**
Respiratory rate ≥30 breaths/min	Respiratory failure requiring mechanical ventilation
PaO_2/FiO_2 ratio ≤250	
Multilobar infiltrates	
Confusion	Septic shock with vasopressor requirement
Uraemia (Blood urea level>20mg/dl)	
White cell count <4,000 cells/µl due to infection	
Platelet count <100,000/µl	
Temperature <36°C	
Hypotension requiring aggressive fluid resuscitation	

1.9.4 APACHE-II

APACHE was first used in 1981 to predict mortality in critical illness. It is not specific for pneumonia. APACHE II was released in 1985[153] and is still the most commonly used prognostic score, despite the release of APACHE III and IV, as their statistical methods are still under copywrite.

APACHE II generates a score from 12 clinical and biochemical indices. The score for each increases the further the observed result is from normal physiology. Each of the indices are the worst recorded for any given parameter within the first 24 hours of admission. APACHE II is only validated for general ICU patients at admission. Table 8 shows how predicted mortality varies with score.

Table 8: Estimated mortality by APACHE II score[153]

APACHE II Score	Post-operative mortality	Non-operative mortality
0-4	4%	1%
5-9	8%	3%
10-14	15%	7%
15-19	25%	12%
20-24	40%	30%
25-29	55%	35%
30-34	73%	73%
>34	85%	88%

1.9.5 SOFA

The SOFA score was initially developed as a predictor of sepsis in critical care[154]. It is a simpler score than APACHE II, and easier to calculate. Scores (0-4) are given for to represent levels of dysfunction of six organ systems: respiratory (PaO_2/FiO_2), haematological (platelet count), neurological (GCS), cardiovascular (hypotension/ ionotropic support), renal (urine output/ creatinine) and hepatic (bilirubin). This creates a total score between 0-24. Unlike APACHE II, SOFA is performed on admission (rather than the worst measurement of the first 24 hours). How SOFA relates to mortality is presented in Table 9.

Table 9: Predicted mortality by SOFA score[154]

SOFA score	In-hospital mortality
0-1	0%
2-3	6.4%
4-5	20.2%
6-7	21.5%
8-9	33.3%
10-11	50%
12-14	95.2%
>14	95.2%

1.10 Syndromic Molecular Tests

Syndromic molecular testing is the process of using one test to simultaneously target multiple pathogens with overlapping signs and symptoms.

At the time of writing there are 2 FDA approved, CE marked syndromic molecular panels for pneumonia which are commercially available: the Filmarray (Biofire diagnostics LLC, Salt Lake City, Utah, US) Pneumonia panel and the Unyvero (Curetis GmbH, Holzgerlingen, Germany) Hospitalised Pneumonia (HPN) panel. Fast Track Diagnostics respiratory panel 33 (Fast Track Diagnostics SARL, Luxembourg) is another available platform with a large number of targets, but contains insufficient bacterial targets for it to be considered a true pneumonia panel and so this will only be considered in brief.

In the following sections, the first two panels will be considered in detail, including their diagnostic performance and use in interventional trials. The commercially available platforms are summarised in Table 10.

There are a multitude of other 'respiratory pathogen' multiplex panels which have targets only for respiratory viruses, atypical bacterial targets or a very small range of typical bacteria. These are considered to be beyond the scope of this review and we have only included assays with targets for a wide range of typical pathogens for pneumonia.

Table 10: Syndromic molecular tests for pneumonia

Panel	**Turn-around time (Hands on)**	**Targets**	**Comments**	**Refs**
BioFire Filmarray Pneumonia panel[159]	75 minutes (5 minutes)	15 Bacterial (Semi-quantitative) 3 Atypical bacteria 8 Resistance genes 8 Viruses	CE marked, FDA approved Potentially deployable as point-of-care test Semi-quantification (Genome copies)	160–163
Curetis Unyvero Hospitalised Pneumonia panel (HPN)[164]	5 hours (5 minutes)	17 Bacterial 3 Atypical bacterial 1 Fungal (*Pneumocystis jirovecii*) 19 Resistance genes	CE marked, FDA approved equivalent LRT panel (latter only validated on ET aspirates) Very extensive range of resistance genes No viral targets	9,165–170
FTD Respiratory Pathogens 33[171]	Platform dependent* >6 hours	8 Bacterial 4 Atypical bacterial 20 Viruses 1 Fungal (*Pneumocystis jirovecii*)	CE marked Laboratory based Insufficient bacterial targets for true pneumonia panel: lacking critical Gram negative targets Not automated No resistance targets Qualitative only	10

*Validated on Applied Biosystems® 7500 and NucliSENS® easyMag®, other platforms are compatible.

Further panels are in development from Mobidiag, Bruker, Accelerate and Axo Science[172] but published data unavailable. There are also several research groups who have developed their own syndromic molecular pneumonia tests, most notably Gadsby et al[8].

1.10.1 The BioFire Filmarray

This is an FDA approved and CE marked test 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 *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, *S. pyogenes*, *Streptococcus agalactiae*, *P. aeruginosa*, *E. coli*, *Enterobacter cloacae* complex (including *E. cloacae, E. asburiae*, *E. hormaechei*, *E.kobei* and *E. ludwigii*) *Acinetobacter calcoaceticus-baumanii* complex (including *A. baumanii, A calcoaceticus, A. pittii, A. seifertii* and *A. nosocomialis*), *Klebsiella aerogenes*, *K. oxytoca, K. pneumoniae* group (including *K. pneumoniae, K. quasipneumoniae* and *K. variicola)*, *Proteus* species and *S. marcescens*. The qualitative bacterial targets are *C. pneumoniae*, *L. pneumophilia* and *M. pneumoniae*. Resistance gene targets are 5 carbapenemases (bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}*)*, one ESβL (bla_{CTX-M}*)* and two MRSA genes (*mecA/C* and *MREJ)*. The viral targets are Influenza A, Influenza B, Rhinovirus/Enterovirus, Adenovirus, RSV, non-SARS Human Coronavirus (HKU-1, NL63, OC43, 229E), human Metapneumovirus, and Parainfluenza viruses (types 1, 2, 3 and 4).

1.10.1.1 How the device works

The test kits include individually packaged, sterile, pre-mixed hydration vials and sample buffer vials. The hydration buffer is injected directly into the Filmarray pouch, and the sample buffer added to an injection cartridge whilst preparing the patient sample. LRT samples are obtained in a sterile universal container, sputum trap or sputum pot. A sterile swab (individually packaged and contained within the Filmarray kit) is inserted into the sample and stirred for 10 seconds. This is inserted and broken off into the injection cartridge, which already contains the sample buffer, and the mixture inverted three times. This mixture is then injected into the sample port of the Filmarray test cartridge. The cartridge is then inserted into the analyser port.

After the short initial setup, the remainder of the test run is fully automated within the analyser, including nucleic acid extraction, purification, reverse transcription, DNA amplification by PCR and detection of PCR product on the microarray card.

The pouch is heat sealed within the Filmarray device, the sample is mixed with freeze-dried positive control material (as discussed 2.10.1) and moved into the large cell lysis area (see Figure 11). Lysis is then performed in this area by the mechanical action of ceramic beads. Nucleic acids are then isolated by passing the lysate over silica-magnetic beads: these are washed three times. A retractable magnet captures and releases the beads during washes. After this, reverse transcription of RNA targets occur during three minutes at 54°C before first stage PCR.

First stage PCR consists of 26 cycles. Each cycle begins with heating the mixture to 94°C for four seconds causing double strand DNA (dsDNA) denaturation. This is followed by cooling to 60°C for 19 seconds to allow primer annealing and then extension, using Taq DNA polymerase.

Prior to second stage PCR the nucleic acid mixture is diluted 225-fold. There are 30 cycles of 94°C for four seconds and 63° for 19 seconds in the second stage PCR using different primers. “Outer primers” are used in the first stage of PCR to amplify out sequences of interest from background nucleic acid and different “inner primers” are used in the second stage which are made up of sequences “nested” within the products of the first stage. The benefit of this adaptation to PCR is that it helps improve sensitivity by removing highly prevalent background nucleic acids (for example from other colonising organisms or human cells).

The final stage of the process is a melt-curve analysis. The DNA-binding dye LCGreen® plus is included within the mastermix for the PCR reaction within the Filmarray pneumonia pouches, so incorporates into the copies of DNA that are produced by each cycle. The dye fluoresces when bound to DNA, and this is detected by the Filmarray instrument. After the final cycle of PCR 2, the temperature is gradually increased from 60°C to 94°C. Different amplicon lengths and sequences determine the temperature at which they denature and the fluorescence drops, so are specific for different targets. These are known as the melting temperature (Tm) and are detected in a melt curve. Reactions occur in triplicate, and at least 2/3 positive melt curves are required for a positive result (displayed in Figure 12).

The relative abundance of organism for the 15 bacterial targets is estimated based on real-time PCR relative to control material of known quantity and is grouped for reporting into bins. These represent approximately 10^4, 10^5, 10^6 and $>10^7$ genomic copies of bacterial nucleic acid per millilitre of specimen respectively. In diagnostic accuracy studies, concordance with reference molecular testing is very high[173] but as expected the overall concordance between FA bin and reference sputum culture (CFU/ml) concentration was lower at around 40%[159] and was highly variable between organisms. As such the manufacturer advises clinical correlation in interpretation of semi-quantitative results.

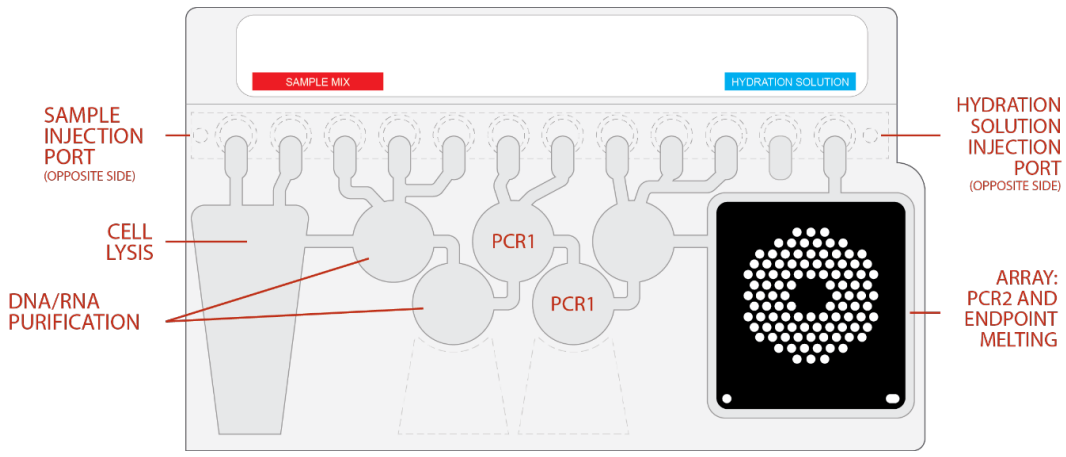


Figure 11: Filmarray Test pouch

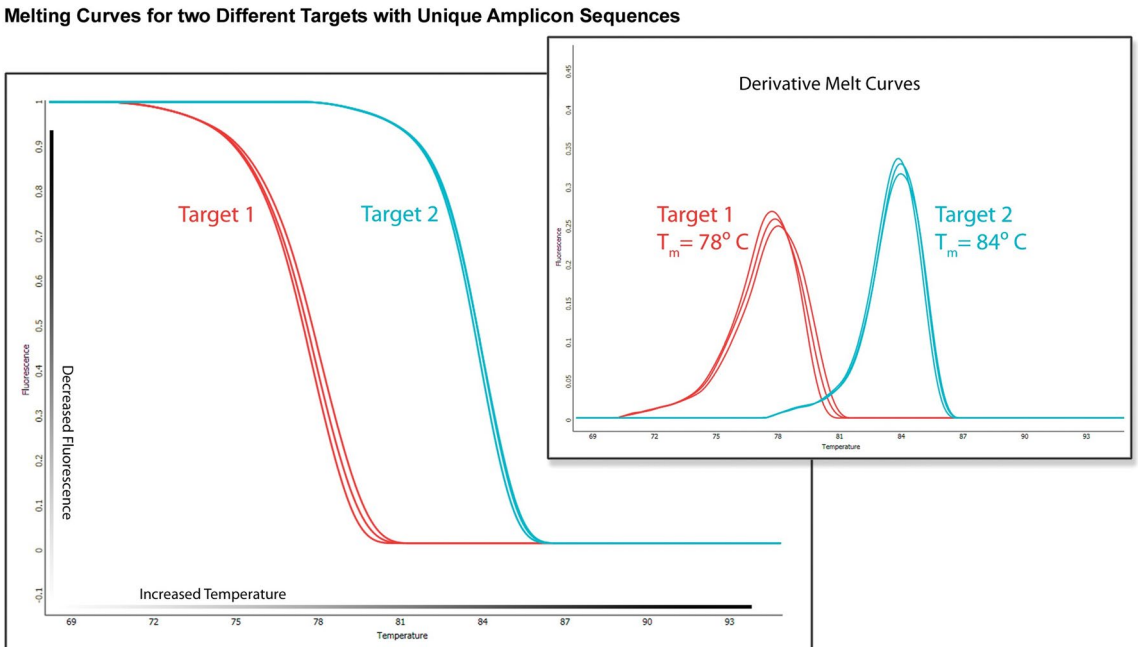


Figure 12: Filmarray Melt Curves

1.10.1.2 Technical details

The assay is validated on several sample types; sputum (including expressed sputum), bronchoalveolar lavage fluid and endotracheal aspirates. Sample preparation takes 5 minutes and the test has a run time of around an hour and 15 minutes. A Pneumonia *plus* panel is also available which has an additional Middle-Eastern Respiratory Syndrome Coronavirus (MERS CoV) target.

The negative percent agreement (NPA) is the specificity of a test when compared to a non-reference standard. Some authors use this when reporting results *in lieu* of specificity as a result of the imperfect nature of current diagnostics. The NPA of bacterial detection between culture-based methods and the FA pneumonia panel varies between different organisms but is consistently very high. In the manufacturers dataset only two organisms on the panel had an NPA below 95%: *H. influenzae* (91.4% [95% CI 89.3-93.1%]) and *S. aureus* (91.2% [95% CI 89.1-93.0%])[160,162,163] Furthermore, the pneumonia panel detected pathogens in a much higher proportion of samples than culture. Buchan et al[160] reported that the Filmarray detected a bacterial target in 71% more specimens than routine culture, equating to over 100% increase in total bacterial detections.

To date there have been no published prospective interventional studies evaluating the clinical impacts of using the pneumonia panel in patients with pneumonia. 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[160,161].

1.10.2 Curetis Unyvero Hospitalised Pneumonia (HPN) panel[164]

The HPN panel is CE marked and runs on the Unyvero platform which includes the Unyvero Lysator, the Unyvero Cockpit and the Unyvero Analyzer. Amplicons generated by 8 parallel multiplex PCR reactions are qualitatively detected by hybridisation on arrays in a single use cartridge. It has a wide range of bacterial and resistance gene targets including 29 pathogens and 19 resistance genes. The bacterial targets are *S. pneumoniae*, *S. aureus, Citrobacter freundii, E. coli*, *E. cloacae complex, E. aerogenes, Proteus species, K. pneumoniae, K. oxytoca, K. variicola, S. marcescens, Morganella morganii, M. catarrhalis, P. aeruginosa, A. baumanii complex, S. maltophilia, L. pneumophilia, H. influenzae*, *C. pneumoniae*, and *M. pneumoniae*. Resistance gene targets are: *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, *bla*$_{OXA-48}$, *bla*$_{OXA-58}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *sul1, ermB,* GyrA83 *and* GyrA87 *for E. coli and P. aeruginosa, mecA/C*. There is one fungal target (*Pneumocystis jirovecii*).

The assay is validated for use on sputum (including expectorated sputum, BAL and ET aspirate). The Unyvero is automated like the Filmarray system, but requires an additional extraction step, so requires slightly longer manual preparation (5 minutes of low skill hands-on time). The total turnaround time is 4-5 hours. An equivalent test, the lower respiratory tract panel (LRT) has FDA approval in the US but is only validated for use on tracheal aspirates.

Manufacturer reported diagnostic PPA for bacterial detection (when compared to reference culture and molecular detection in cases of discrepancy) is between 80-100% with the majority of targets >90%: the exceptions are *A. baumanii complex* (88.9%), *K. pneumoniae* (80%) and *S. marcescens* (90%). Reported NPA is 98.3%-100%. Enne et al tested 608 surplus ICU samples and reported PPA of bacterial targets of between 50-100%: with the majority of targets >90%[174]. The most notable exceptions were *E. aerogenes* (50% [95% CI, 12-88%]) and *S. marcescens* (77.8% [95% CI, 40-97%]).

In the diagnostic performance data presented by the manufacturer, resistance marker detection aligned poorly with organism antibiogram: for example, matching in only 4/11 *mecA* detections or 9/13 quinolone resistance markers in *E. coli*. This issue was noted by Gadbsy et al[168] for the P55 assay where the sensitivity for antibiotic resistance detection was 18%.

To date there are no published randomised controlled trials evaluating the clinical impact of the Unyvero HPN system in patients with pneumonia. Jamal et al[170] performed a non-randomised

interventional study using the P50 assay where antibiotics were adjusted based on the results and pathogens detected were compared to culture. The turnaround time for result was very quick (~4 hours) compared to culture (48-96 hours) and a large proportion of patients had antibiotics changed based on the P50 results, however the small number of patients studied and the lack of a comparator group make definitive conclusions impossible. Gadsby et al retrospectively tested BAL samples with the P55 and reviewed patient notes. They reported that 53.6% of patients who had positive standard of care microbiology could potentially have had a change in antibiotics earlier based on P55 results[168]. Conversely, they reported a false negative P55 result in ~20% of those with a positive culture which could have caused harm if acted upon.

1.10.3 Fast Track Diagnostics (FTD) Respiratory pathogens 33[171]

The Respiratory pathogens 33 panel differs from the first two tests discussed in that it is exclusively a laboratory centred assay. The CE marked Respiratory pathogens 33 kit can be used on several standard laboratory PCR cyclers. As such there is no reported standard turnaround time although it is greater than 6 hours. Positive signals are detected from eight multiplex real-time PCR reactions. It is not an automated process so will have a considerably longer hands-on time requiring skilled extraction and setup. The panel has 12 bacterial targets, 20 viral targets and 1 fungal target (*P. jirovecii*). The bacterial targets are: *H. influenzae (with additional specific HiB target), Bordatella* species *(excluding B. parapertussis), M. catarrhalis, Salmonella* species*, L. pneumophilia/longbeachiae, K. pneumoniae, S. aureus, S. pneumoniae, C. pneumoniae and M. pneumoniae.* The viral targets are*:* Influenza (A, A(H1N1), B, C), Rhinovirus, Coronaviruses (NL63, 229E, OC43, HKU1), Parainfluenza (1-4), Metapneumoviruses A/B, Bocavirus, RSV, Adenovirus, Enterovirus and Parechovirus.

1.10.4 Comparing systems

There is very little published data comparing different syndromic molecular pneumonia tests. Enne et al and the INHALE group presented data at ECCMID 2019 where they compared the Unyvero and the Filmarray on 654 surplus intensive care respiratory tract samples[174]. The Filmarray had slightly greater sensitivity for common pathogens, fewer major discordances (defined as routine culture finding 1 or more undetected organisms) and fewer machine failures. The Unyvero had slightly higher specificity and overall concordance with reference culture.

1.11 Potential therapeutic benefits of Syndromic molecular tests for pneumonia

1.11.1 Directed antibiotic use

The greatest potential clinical benefit of a rapid syndromic test for pneumonia is facilitating better utilisation of antibiotics. The superior diagnostic yield of multiplex PCR means that a pathogen is detected rapidly in a much greater proportion of patients[8–11], so therapy can be quickly tailored to the responsible organism. In some situations, this will allow narrowing of antimicrobial therapy: for example, identification of *S. pneumoniae* facilitating a change of antibiotics to penicillin, in geographical areas with a low prevalence of penicillin resistant *S. pneumoniae*. In other cases, it may facilitate a change or escalation of antimicrobial therapy: for example, the identification of MRSA which would not be covered by empirical regimens in many areas. The absence of detection is also helpful: the sensitivity when compared to culture of molecular assays is very high[175,176] so can reassure clinicians that organisms are not present and so support decisions to stop unnecessary antibiotics or to deescalate antibiotics that were used empirically to cover organisms subsequently not detected.

The impact of this improved use of antibiotics are twofold: firstly, earlier appropriate antibiotics should improve clinical outcomes including reducing time to recovery and preventing avoidable deaths. Secondly, it may prevent unnecessary broad-spectrum antibiotic use, which facilitates antibiotic stewardship, reduces antibiotic related adverse events and subsequent development of antimicrobial resistance.

S. pneumoniae, H. influenzae, *S. aureus*, *M. catarrhalis* are all cultured from the sputum of patients with CAP. Many of these organisms have predictable resistance patterns when interpreted with local epidemiological data. Gadsby et al developed and internally validated their own syndromic molecular assays for pneumonia. They used this to test sputum samples of 323 adults admitted to hospital with CAP[8]. Their assay detected a pathogen in 87% of patients (as opposed to 39% of patients using only routine culture). As a result, they proposed that the vast majority (77%) of antibiotic prescriptions in CAP could have been de-escalated based on results from multiplex PCR testing. The majority of these potential interventions involved stopping clarithromycin when atypical organisms were not detected or ‘narrowing’ antibiotics when a likely sensitive pathogen had been detected.

In HAP and VAP, empirical regimens are broad spectrum and large numbers of antibiotics are consumed. The absence of detection of certain organisms (for example *P. aeruginosa*) may

facilitate a narrowing of the antimicrobial spectrum with the subsequent effect of reducing antibiotic related adverse effects and improving stewardship. Furthermore, common Gram negative isolates are increasingly resistant in pneumonia surveillance studies[177]. Rapid molecular detection of these resistance genes might facilitate earlier initiation of effective antibiotics leading to better patient outcomes.

1.11.2 Improving treatment and identification of other infective agents

Detection of certain viruses such as influenza and RSV which are known to cause pneumonia, coupled with the absence of detection of bacteria and low levels of serum biomarkers such as procalcitonin (which is elevated in patients with bacterial infection), could support decisions to stop or use an abbreviated course of antibiotics. The ResPOC trial was a pragmatic randomised controlled trial that tested patients with community acquired acute respiratory illness using the BioFire Respiratory Panel (which tests comprehensively for respiratory viruses and atypical bacteria) at the point-of-care. It found that patients who were tested with the Filmarray were significantly more likely to receive a single dose or shorter course of antibiotics[178] than those who were not. It also found a significant reduction in length of hospital stay in the intervention group along with improved use of neuraminidase inhibitors (NAI) in patients with influenza.

There are currently no approved specific antiviral agents for respiratory viruses other than SARS-CoV-2 and influenza. The benefit from NAI treatment in patients with influenza is greatest when they are started within 48 hours of symptom onset although there is evidence in adults to suggest benefit when started beyond this time[126] and also that treatment started earlier in the course of hospital admission improves outcome, irrespective of overall duration of illness[179]. As such, timely identification and treatment is critical.

1.11.3 Infection control and public heath

Since the 1990s infection control methods including patient source isolation and deep cleaning with targeted decolonisation, have been highly successful at reducing the spread of MRSA. Enhanced infection control practices are recommended for a number of pathogens that may be present in patients with pneumonia. Early identification of these should reduce the spread of these organisms, especially in hospitalised patients. Some examples of these which are found on commercially available molecular tests are extended spectrum beta lactamases (ESBLs), carbapenemase producing enterobacteriaceae (CPEs), MRSA, Influenza and RSV.

In the UK there is a mandatory requirement to report certain infectious diseases to Public Health England, so they can be investigated. *L. pneumophilia* is associated with outbreaks from devices

that aerosolize water. Earlier sensitive detection of these would allow outbreak investigation to occur sooner and potentially stop further cases occurring.

Chapter 2 The SARIPOC trial methods

2.1 Overview

The SARIPOC trial was a single centred, pragmatic randomised controlled trial of rapid molecular diagnostics versus routine clinical care in critically ill adults with pneumonia.

2.2 Aims and objectives

The main aim of the study was to assess the clinical impact of rapid, multiplex molecular testing for respiratory pathogens in critically unwell patients with pneumonia. The primary outcome was the proportion of patients who were on pathogen directed antimicrobial treatment as a measure of improved antimicrobial stewardship.

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 [8,11,161].

As an outcome measure, results directed antimicrobial therapy is desirable as it encompasses both pathogen directed therapy where antimicrobial agents 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 (i.e. a negative result). This strategy aims to improve clinical outcomes, reduces antibiotic associated adverse events, decrease costs of care and facilitates 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[2].

2.3 Trial design

This was a single centre, randomised controlled, study. Participants were randomised 1:1 to the intervention and control (standard clinical care) arms. Participants randomised to the intervention arm had multiplex testing for pneumonia pathogens, serum procalcitonin measurement and urinary pneumococcal antigen testing with accompanying specialist infection prescribing advice.

2.4 Study setting

Patients were recruited from intensive Care Units (ICUs) including General ICU (GICU), Neurological ICU (NICU), and Respiratory High Dependency Unit (RHDU) within Southampton General Hospital, which is part of University Hospital Southampton (UHS) NHS Foundation Trust**.**

Southampton General is a large, tertiary referral centre in Hampshire, UK. GICU and NICU are part of a major trauma centre, taking patients from across the South of England, the Isle of Wight and Channel Isles. Tertiary neurosurgical services are provided on NICU.

2.5 Trial approvals

Regional ethics committee and human research authority approvals were obtained prior to starting the study. The REC South Central-Berkshire Committee initially approved the study in March 2017 (Reference 17/SC/0110) when it was designed exclusively as a pilot study of respiratory virus testing in critical care. Fewer than 20 patients were recruited. The technology available for multiplex testing for bacteria was not licensed at this stage and when it became available in 2019, a major amendment of the protocol was submitted and approved on 23rd May 2019 to repurpose the study for broader scientific value. This changed the definition of severe acute respiratory illness to specifically mean pneumonia and changed the outcome measures to reflect this change.

A second amendment was approved in July 2019 to remove the inclusion criteria of duration of respiratory illness <72 hours.

Local Southampton University Ethical Approval (ERGO) was also obtained prior to starting the study.

2.6 Study sponsor

The study sponsor is University Hospitals Southampton Foundation Trust. The trial was overseen by the research and development (R & D) department at Southampton General hospital. The internal study code is RHM MED 1387.

2.7 Trial registration

The trial was prospectively registered with the ISRCTN registry ISRCTN65693049. All amendments were communicated to and updated on the registry.

2.8 Participant eligibility criteria

2.8.1 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.

- Duration of respiratory illness <72 hours[†]

*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'[100]. 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'[180]. 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'[180]

[†]Inclusion criterium removed 2 months after opening recruitment to main trial

2.8.2 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 was at the CI's discretion.

2.9 Trial process

2.9.1 Screening

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

2.9.2 Consent

The study team obtained informed consent for those with capacity, or assent via consultee for those without capacity, as per the dedicated study forms. 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 did not apply.

Discussion of the study was provided to patients, or their consultee for those lacking capacity, by study staff. This included supply of a participant information sheet for the participant or witnesses to read and retain.

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

Each patient was assumed to have capacity unless it was established that they lacked capacity. For patients unable to consent for themselves, the study complied with the Mental Capacity Act 2005 and in such cases, the patient's family member, carer or friend may have been 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) was asked if they would provide assent. Both the person taking assent and the consultee were required to personally sign and date the relevant form.

The personal / nominated consultee was 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 always took precedence.

In the event of the patient recovering capacity following enrolment by consultee, they were asked to read the patient information sheet and provide consent for themselves. In this circumstance the patient had the option to 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 were retained).

Although the study procedures were very brief, if a patient lost capacity after enrolment but before the study procedures were completed, consultee consent was sought to continue with any study procedures.

Patient information sheets and consent forms, for those with and without consent are included in the supplementary appendices (participant and consultee study stationary).

2.9.3 Randomisation and allocation

Participants were enrolled and assigned a participant identification number consecutively. Once a patient had been screened and found eligible, and consent had been obtained, a study team member used the in-built CRF ALEA randomisation software to obtain a randomisation code for the patient who was then allocated to either the intervention or control group in a 1:1 ratio. The software used random permuted blocks of varying sizes to ensure total unpredictability of allocation and allowed for audit-linked and reliable randomisation.

2.9.4 Procedures for all participants at enrolment

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

A blood sample (maximum 15mls) was obtained from all participants, processed in the closely located NIHR Southampton Clinical Research Facility laboratory and included a serum tube (5mls), PAXgene RNA tube (5mls) and EDTA whole blood (5mls).

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

All respiratory samples left-over from Filmarray testing were frozen and stored for further study. Participant consent for this was included in the consent form. Patients could be approached for additional samples to be collected and stored for further study.

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

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

2.9.5 Procedures for participants randomised to the intervention group

The LRT sample obtained as described above was taken as soon as possible after recruitment and 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. An extended explanation about how this was done, including a detailed explanation of how the Filmarray works are available in chapter 1.10.1.

At least one Filmarray machine was located in a critical care area for this purpose. Test results were generated in about 1 hour. In the event of a run failure, the analysis run was repeated using the same sample; if there was insufficient sample left, further samples were taken. The LRT sample was also be tested by bacterial 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, was taken as soon as possible after recruitment and tested promptly at the point-of-care using VIDAS (bioMerieux) for biomarkers including procalcitonin (PCT). The use of this machine, including scientific principles, are discussed in 2.9.8.

The urine sample obtained as described above, was taken as soon as possible after recruitment, and tested at the point-of-care for Pneumococcal antigens using the Abbott (Formerly Alere) *Streptococcus pneumoniae* (formerly Binax NOW!) antigen testing kit.

The results of the investigations were documented in the patient's case notes and specialist infection advice immediately given on appropriate/optimal antimicrobial therapy. Antibiotic advice was given on a case by case basis taking into account clinical, biochemical and radiographic

features of disease. Additionally, ongoing advice was provided during the following days. The participant was informed of the result where appropriate. The infection prevention and control team were informed of any reportable pathogen in real time (according to local hospital infection guidelines).

2.9.6 Procedures for participants randomised to routine clinical care

The lower respiratory tract sample obtained as described above, was stored at -80°C and subsequently tested by the Filmarray pneumonia panel at least 30 days after collection. This allowed 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 did not influence participant care. Data submitted by the manufacturer as part of the FDA approval process demonstrated no change in the performance of the pneumonia panel from freezing and thawing samples[181].

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

The blood samples obtained as described above, were stored at -80°C and subsequently tested using VIDAS® (bioMerieux, Marcy l'Étoile, France) for biomarkers including procalcitonin, at least 30 days after collection, and did not influence participant care.

Standard microbiological investigation for patients randomised to the control arm, including culture of respiratory tract secretions and viral PCR, was at the discretion of the clinical team and was reported in the usual way by the electronic results system. In UHS there was ongoing regular Microbiology liaison on ICU and NICU from a consultant microbiologist and this did not change during the period of the study. This liaison service discussed patients on a case-by-case patients in an afternoon ward round setting, rather than on release of results.

The standard locally advised investigation panel for CAP and HAP included blood cultures and a sputum sample for microscopy and culture. Additionally, urine testing for pneumococcal urinary antigens (Using Alere BinaxNOW®) was advised in all patients, and legionella urinary antigen testing (Using Tinity Biotech Uni-Gold™ Legionella Urinary Antigen PLUS) when clinically suspected. Respiratory virus PCR was recommended in patients where there was a clinical suspicion of influenza. For patients with VAP the recommendation was the same with the exception of performing a BAL at time of diagnosis instead of sputum sample collection.

2.9.6.1 Sputum culture procedures

The standard operating procedure for sputum culture processing conformed to UK SMI B57[182]. Sputum samples (including BALs and ETT) were mucolysed to homogenise the sample prior to culture. BAL samples were additionally centrifuged at 1200rpm for 10 minutes prior to culture and a Gram stain prepared from the centrifuged deposit. The presence (or absence) of organisms, white blood cells and epithelial cells was reported electronically in-hours. All samples were inoculated on chocolate (with bacitracin disc), blood (with optichin disc), CLED and Sabouraud agar plates. Other specific media were added at the discretion of the biomedical scientist. The sample was not diluted prior to inoculation. Plates were read at 24 and 48 hours and relative quantification was provided (+, ++ or +++) in the final report. Organism identification was performed by MALDI-TOF mass spectrometry. Disc antimicrobial sensitivity testing was performed according to EUCAST standard methods and breakpoints.

2.9.6.2 Blood culture procedures

The standard operating procedure for blood culture processing conformed to UK SMI B37[183]. When BACTEC bottles flagged positive in automated incubator, a Gram stain was performed, and blood was subbed onto different plates. These depended on the Gram stain and clinical details, and included: Blood agar, fastidious anaerobe agar, chocolate agar and others according to UK SMI B37[183] depending on clinical history. Organism growth was identified using MALDI-TOF mass spectrometry. EUCAST standard methods and sensitivity plates were used for phenotypic sensitivity.

2.9.7 Additional procedures after enrolment for all participants

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

2.9.8 The mini-VIDAS PCT assay

The PCT assay on the mini-VIDAS (Figure 14) is a sandwich enzyme linked fluorescence assay (ELFA): the principles of which are summarised in Figure 13. It uses two single-use devices for

each test: the solid phase receptacle (SPR) which serves both as the solid phase for the reaction and as a pipette, and the PCT test strips which contain the reagents required for the reaction.

Collected patient serum samples are left to clot in the SST tube for a minimum of 30 minutes. They are then centrifuged at 20G for 10 minutes and the serum removed from the supernatant. 200µl of serum is pipetted into the end of the test strips which are inserted into the mini-VIDAS device. SPRs are put in a separate section of the machine.

The sample is transferred into wells containing anti-procalcitonin antibodies, labelled with alkaline phosphatase, creating an antibody/ antigen mixture. This mixture is cycled in and out of the SPR several times, where the procalcitonin antigen binds to immunoglobulins which are pre-spotted on the SPR creating a sandwiched PCT antigen/antibody complex which is labelled with alkaline phosphatase. Unbound products are removed during a washing phase.

4-Methyl-umbelliferyl phosphate is then cycled in and out of the SPR (as the reaction substrate). The alkaline phosphatase catalyses the reaction of this to 4-Methyl-umberlliferone which fluoresces. This is measured at a wavelength of 450nm. A pre-entered calibration curve allows the machine to calculate the relative concentration of PCT from the intensity of the fluorescence.

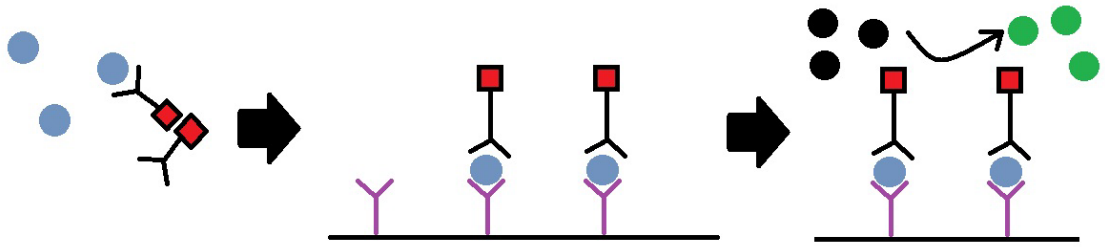


Figure 13: Principles of the ELFA technique used in the PCT assay*

*Blue circles represent PCT molecules, red squares alkaline phosphatase linked to anti-PCT antibodies, purple lines the antibodies present on the solid state (SPR) and the black and green circles 4-Methyl-umbelliferyl phosphate and 4-Methyl-umberlliferone respectively.

Figure 14: the mini-VIDAS

2.9.9 The Abbot Streptococcus pneumoniae antigen card

The *S. pneumoniae* antigen card is an immunochromatographic membrane assay which detects the presence of the C-polysaccharide antigen of the cell wall. To perform the test, a swab is dipped into the urine specimen until the swab head is completely covered, and then inserted into the base of the test card (indicated by two arrows in Figure 15). Three drops of reagent are added to the lower hole: this is a citrate buffer solution. The test card is closed bringing the sample solution suspended in buffer into contact with the test strip.

The test strip begins with an inert fibrous support (not visible externally) which contains rabbit anti-*S. pneumoniae* antibodies (sample) and goat anti-rabbit antibodies (control) that are both conjugated to visualising particles. The sample moves along this region by capillary flow: any present pneumococcal antigen binds to the anti- *S. pneumoniae*/conjugate antibody present on the test strip. The resulting complexes move through the proceeding nitrocellulose membrane and are captured by an adsorbed stripe of immobilised anti-*S. pneumoniae* antibodies at the

sample line (indicated by 'sample' on Figure 15). The aggregation of visualising particles indicates a positive result.

The conjugated goat anti-rabbit antibodies from the fibrous support are captured by an adsorbed stripe of immobilised control antibody, again causing an aggregation of visible conjugate particles which indicates a valid result. The result lines are read at 15 minutes and a result is disregarded if the internal control fails.

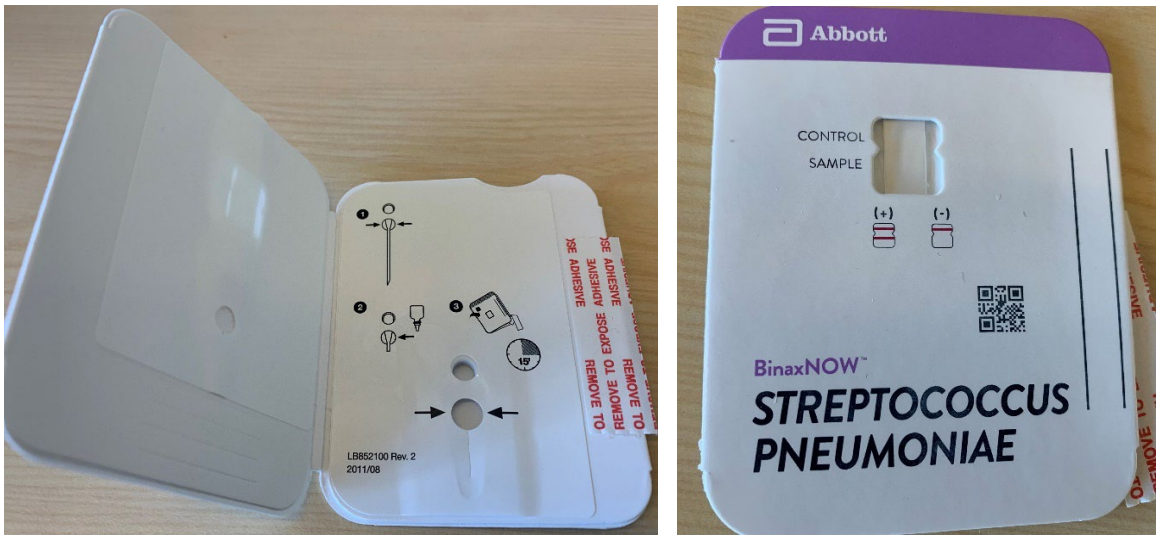


Figure 15: The *S. pneumoniae* antigen card

2.10 Assay validation and quality control

2.10.1 Biofire Filmarray Pneumonia*plus* panel

The Filmarray pneumonia platform was validated prior to use according to the manufacturer's instructions. All of the targets on the Filmarray pneumonia panel were tested using a synthetic control matrix, containing a combination of synthetic RNA and DNA (Maine Molecular Quality Controls, Maine, USA). Further validation for quantification was performed using pooled samples of inactivated organisms at varying concentrations (Zeptometrix Pneumonia panel-Quantifiable bacteria, New York, USA). Controls were repeated every two months or every new lot number, using the Maine Molecular synthetic positive and negative control matrices. Additionally, culture results were obtained and recorded in real time, when available.

Each pouch of the Filmarray pneumonia panel has two internal process controls. An RNA process control targets a yeast RNA transcript and is carried through all the stages of the test, indicating that these steps have completed successfully. The Quantified standard media (QSM) assay detects

a predetermined quantity of synthetic nucleic acid for to ensure quantification of bacterial analytes are occurring correctly. Failure of either control leads to a run failure and invalid result.

2.10.2 Mini-VIDAS Procalcitonin assay

The mini-VIDAS (Biomerieux) for Procalcitonin was calibrated every 30 days or new Master lot entry in accordance with manufacturer's instructions. Calibration curves were checked weekly using the QCV (Quality control Vidas) materials supplied by the manufacturer.

2.10.3 Abbott BinaxNOW Streptococcus pneumoniae urinary antigen tests

The urinary antigen cards had positive and negative controls performed at the beginning of each new lot number.

2.11 Laboratory infection control precautions

All samples were handled in a designated containment level 2 (CL2) laboratory in the Clinical Research Facility. Sputum samples were handled in a clean class II medical safety cabinet (MSC). After the emergence of SARS-CoV-2, a further risk assessment was performed with the laboratory management. All patients testing positive for SARS-CoV-2 or with suspected COVID-19 had all samples, including blood and urine, handled in the MSC in CL2 prior to inactivation with Filmarray Sample buffer in line with updated PHE guidance[184].

2.12 Outcome measures

2.12.1 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).

2.12.2 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 al[185]

Table 11: Antimicrobial stewardship ranking

Rank	**Antimicrobial agent**
5	Carbapenems, Polymixins, Novel cephalosporin/ beta-lactamse inhibitor combinations, Monobactams, Tigecycline
4	Piperacillin-tazobactam, Respiratory Quinolones (Moxifloxacin, Levofloxacin), Chloramphenicol
3	Ceftriaxone, Ceftazidime, Ciprofloxacin
2	Co-amoxiclav, Co-trimoxazole
1	Amoxicillin, Doxycycline, Clarithromycin and other Macrolides, Vancomycin, Teicoplanin, Linezolid

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.

Clinical/ 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.

2.12.3 Exploratory Outcomes

Association between pathogen quantification and clinical outcome measures

Utility of serum procalcitonin in differentiating between bacterial infection from non-bacterial infection and prediction of clinical outcome

Pathogen detection in pneumonia occurring following macro-aspiration

Pathogen detection in patients with SARS-CoV-2 infection

Pathogen detection in relation to preceding duration of antibiotic therapy

2.12.4 Filmarray Pneumonia panel performance (not pre-specified in protocol)

Diagnostic yield of the Filmarray for typical bacterial detection, irrespective of quantification (Diagnostic yield defined as concordance of pathogens detected, and positive and negative percent agreement, between a single Filmarray test and all LRT cultures performed from 48 hours before the start of antibiotics, to 72 hours after)

Diagnostic accuracy of Filmarray for typical bacterial detection, irrespective of quantification (Diagnostic accuracy defined as concordance of pathogens detected, and positive and negative percent agreement, between Filmarray testing and culture performed on the same samples)

Frequency of detection by culture of 'off panel' bacteria (i.e. pathogens grown which are not targets on the Filmarray panel)

Comparison of Filmarray Semi-quantitation with culture relative quantification

Number of Filmarray pathogen detections depending on sample type (BAL-like or non-BAL like)

Frequency of Filmarray resistance gene detection, relationship to phenotypic sensitivity and clinical implications

Diagnostic accuracy of the Filmarray for atypical bacterial detection and virus detection compared to standard of care diagnostics

Additional identification of respiratory viruses as a result of syndromic testing

2.12.5 Additional post hoc analyses

Impact of the SARS-CoV-2 pandemic on viral detections in critical care

Association between *S. pneumoniae* growth in blood and sputum, urinary antigen positivity, and molecular detection of *S. pneumoniae*.

2.13 Sample size calculation

The study initially aimed to recruit a total of 300 patient-participants: about 100 per year for three years. With 1:1 allocation to groups, this was 150 patients per group. This was 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 panel[186]), using a 5% significance level a sample size of 150 per group will have an 80%

power to detect this difference. This difference was considered to be clinically relevant in terms of antibiotic use and patient outcome. Based on the authors previous studies the drop-out rate was expected to be very low (<1%)[178]. This was revised to 200 patients following an unplanned interim review which is discussed in section 2.21.

2.14 Data collection

Demographic and clinical data were 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 additionally had the duration of invasive ventilation recorded. Data were recorded on a standardised Case Report Form and transferred to a secure electronic database. Once patients had been discharged or after 30 days (whichever is soonest), clinical data were 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 were also collected on the turnaround time of respiratory pathogen test results in each group.

For those allocated to the intervention group, the Filmarray pneumonia panel was run on enrolment as soon as a sample was available. For those allocated to standard clinical care, samples were taken on enrolment, frozen and stored, and run on the Filmarray pneumonia panel at least 30 days after collection. This did not influence patient management but allowed retrospective comparison of pathogen detection.

2.15 Data Management

The subjects' anonymity was maintained. The study team kept a confidential log of each subject's name, hospital ID number, date of birth, and unique participant trial number. The participant details were recorded on the secure NHS Edge system in a similar manner, including NHS number. This participant trial number was used on documents after screening to maintain confidentiality. Documents that were not anonymous (e.g. signed informed consent forms) are maintained

separately, in strict confidence. The study staff were responsible for entering study data in the CRF.

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

2.15.1 General Data Protection Regulation (GDPR)

The patient information sheet given to participants provided 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 would be retained with the minimal patient identifiable information possible and no further data collected.

2.15.2 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 took part in 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.

2.15.3 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 was planned. A trial management committee oversaw the trial.

2.16 Statistical Analysis

Analysis was by intention to treat (ITT) and the framework was superiority. Statistical analysis was performed by a dedicated medical statistician from University Hospital Southampton NHS

Foundation Trust (HM) who was independent from the study team. The use of multiple imputation was planned should missing data exceed 5% for the primary outcome or for key secondary outcomes but was not needed as missingness was below this threshold (<1% for the primary outcome and <5% overall).

We used difference in proportions and difference in medians to compare the groups. The primary outcome was compared between groups using logistical regression. We used an adjusted logistic regression model to assess the effect of the following variables on the primary outcome; age, sex, pneumonia diagnosis (CAP, HAP or VAP), recruitment location (rHDU, GICU or NICU), SARS-CoV-2 infection, time on antimicrobials at recruitment, initial PCT value, and SOFA score.

Secondary outcome measures were measured in all patients. Difference in proportions between the groups was assessed using quantile regression (for median values) for continuous data. Competing-risks regression with in-hospital death specified as a competing risk was used to model time to results-directed therapy and to create a plot of the estimated cumulative incidence of the event in the presence of the competing risk, with associated sub-hazard ratio. Competing-risks regression was also used for time to discharge outcomes, with in-hospital death specified as a competing risk. This method was also used for mortality outcomes, with the competing risk being discharge from hospital within 30 or 60 days. Patients still in hospital at 30 or 60 days were censored. Analysis was carried out using Prism version 7.0 (GraphPad Software Inc; La Jolla, California), and Stata version 17 (StataCorp, College Station, Texas).

2.17 Rationale, methods and statistical analysis for exploratory outcomes

2.17.1 Association between pathogen quantification and clinical outcome measures

An association between Filmarray relative pathogen quantification and clinical outcomes is not established. Rand et al have suggested there may be a link: but only found significant association between higher relative abundances and sputum white cell count, and highest temperature on the day of recruitment[187].

The clinical indices of all patients testing positive for any semi-quantifiable bacteria on the molecular test (regardless of group allocation) were compared by relative abundance/ bin value (copies/ml) of organism detected using standard descriptive and comparative statistical methods using Prism (GraphPad Software Inc; La Jolla, California) and SPSS (SPSS, Inc; Chicago, Illinois). Summaries of all baseline characteristics were presented using means and standard deviations, medians and interquartile ranges, or frequencies and percentages, as appropriate. Continuous

variables (Apache II score, CRP, FiO2, PCT and time to ICU discharge) were analysed by multiple independent Kruskal-Wallis 1-way analysis of variance tests for association with Filmarray bin value. Categorical variables (single target detection, co-detections, diagnosis of CAP, HAP and VAP, 30-day mortality, invasive and non-invasive ventilation use) were analysed with the chi-square linear-by-linear test.

2.17.2 Utility of serum procalcitonin in differentiating between bacterial infection from non-bacterial infection and prediction of clinical outcome

We compared patients with any molecular detection of a bacterial pathogen, including atypical bacteria, with patients who had either no pathogen detected, or an exclusively viral aetiology. We constructed a receiver operating characteristic (ROC) curve, using SPSS version 26 (SPSS, Inc; Chicago, Illinois), to evaluate the utility of PCT in distinguishing bacterial infection from non-bacterial infection, using the Filmarray Pneumonia panel as a molecular reference standard. These were calculated for all patients with pneumonia on critical care, and for CAP specifically, as the majority of the existing evidence base is in CAP. We additionally compared PCT to a higher threshold (>10^5 copies/mL) of molecular detection, as others have demonstrated much stronger concordance with culture at 10^6 copies/ml and above[176,188,189].

We calculated the sensitivity and specificity for molecular organism detection at PCT cut-offs of 0.1ng/ml, 0.25ng/ml and 0.5ng/ml as those commonly used in clinical practice. As a reference we also presented the ROC curve data for PCT compared to conventional diagnostic testing.

We stratified patients into PCT groups (<0.1ng/ml, 0.1 to <0.25ng/ml, 0.25 to <0.5ng/ml and ≥0.5ng/ml) in order to examine association with clinical outcome measures. Continuous variables were analysed by multiple independent Kruskal-Wallis 1-way analysis of variance and categorical variables were analysed with the chi-square linear-by-linear test. We constructed multiple receiver operator characteristic curves to examine the utility of PCT in predicting all cause 30-day mortality in all patients at recruitment, Day 3 (recruitment + 48 hours) and day 6 (recruitment + 5 days). We then applied the same methodology to exclusively patients who had tested positive for a bacterial pathogen on the Filmarray pneumonia panel.

2.17.3 Pathogen detection in pneumonia occurring following macro-aspiration

To date, no published literature has considered bacterial molecular detection following macro-aspiration events. We identified all recruited patients within the trial who had a macro-aspiration event in the run up to the development of their pneumonia. The definition of a macro-aspiration

event was directly witnessed large airway soiling or radiographic evidence strongly suggestive of aspiration in the context of a likely aspiration event.

We compared the rate of molecular positivity in patients with a recent history of macro-aspiration, to the same rate in patients who had not recently aspirated, using Fishers exact test. We then compared the relative frequency of pathogen detection between cases of aspiration occurring in the community, and those occurring in hospital, using Fishers exact test.

Lastly, we compared the rate of previous regular proton pump inhibitor therapy between patients with aspiration and non-aspiration pneumonias using Fishers exact test. These patients are theorised to be at greater risk of pneumonia, as PPIs increase the gastric pH which is believed to protect against bacterial growth in the upper GI tract. All comparative statistics for this outcome were performed using Prism (GraphPad Software Inc; La Jolla, California).

2.17.4 Pathogen detection in patients with SARS-CoV-2 infection

The extent of bacterial co-infection in cases of COVID-19 is unknown. The fear of under-treating potential bacterial secondary infection has led to huge consumption of antibiotics worldwide for which more accurate, rapid diagnostics are urgently needed[190]. We presented data on the frequency and type of bacterial co-detection in cases of SARS-CoV-2 who were tested with the Filmarray pneumonia panel. We compared the relative frequency of molecular pathogen detection in patients co-infected with SARS-CoV-2, with non-SARS-CoV-2 pneumonia, for those patients with community acquired illness and with secondary hospital acquired pneumonia, using Fishers exact test using Prism (GraphPad Software Inc; La Jolla, California).

2.17.5 Pathogen detection in relation to preceding duration of antibiotic therapy

One of the theoretical benefits of molecular diagnostics for pneumonia are that they do not require culturable, viable organism to give a valid result. Since the advent of the surviving sepsis campaign, there is a huge emphasis on timely antibiotics in possible cases[3]. As a result, diagnostic tests are not always performed before the start of treatment and patients receive antibiotics before sampling, which reduce the yield. A prospective cohort study by Scheer et al found that blood culture positivity almost halved (50.6% to 27.7%) when patients with sepsis had received at least one dose of antibiotics before having blood cultures obtained[191]. Musher et al assessed the sputum culture positivity of 58 patients with bacteraemic pneumococcal pneumonia in relation to preceding antibiotic duration, stratified intro groups of no antibiotics, <6 hours, 6-24 hours and >24 hours. Sputum culture was positive in 80% of those who had not received antibiotics , which

fell to only 29% in those who had received in excess of 24 hours therapy[192]. The extent to which molecular results decline over time following antibiotic administration is not known.

We grouped any LRT samples according to the preceding duration of antibiotics before being obtained into: no antibiotics, under 12 hours, 12-24 hours and over 24 hours. We included all Filmarray pneumonia results and all sputum culture results taken from 48 hours prior to starting antibiotics, to 72 hours afterwards. Molecular detections of S. agalactiae, L. pneumophilia, C. pneumoniae and M. pneumoniae were excluded as organisms that would be either unlikely to grow in routine culture, or unlikely to be specifically identified. Sputum cultures of yeasts or oral flora were considered negative. We tested trends within groups using the chi-squared analysis for significance of the trend. We compared the rates of positivity (i.e. of bacterial pathogen detection/growth) in different time groups between both testing methods using Fishers exact test. As a sub-group, we selected only patients with a semi-quantitative molecular detection which was also tested by culture, and presented the proportional rate of growth of every positive target on culture, according to the duration of preceding antibiotics. We tested trend using the chi-squared analysis for significance of the trend.

2.18 Methods and statistical analysis for Filmarray Pneumonia panel diagnostic performance

The SARIPOC study was not primarily designed as a diagnostic accuracy study, and these analyses were not pre-specified in the study protocol.

2.18.1 Diagnostic accuracy and yield of Filmarray for typical bacterial detection, irrespective of quantification

It was not mandated in the methods for all samples to be tested by both culture and Filmarray. In some patients, samples were completely expended in testing, and different samples may have been tested by culture than were tested on the Filmarray. These results are still of interest, as they highlight the reproducibility of Filmarray results and how they may ultimately be utilised in routine clinical practice, where sputum samples are not unlimited. For this reason, qualitative 'diagnostic accuracy' of the Filmarray was compared only to paired samples, whereas 'diagnostic yield' considered all samples (including taken at different times) from the same patients, although it is not diagnostic accuracy in the truest sense of the phrase.

The concordance between Filmarray testing and sputum culture was categorised into fully concordant (i.e. exactly the same on-panel pathogens identified), concordant but Filmarray

detected additional bacterial target(s), and discordant (i.e. molecular testing failed to identify an organism on the Filmarray panel which grew). Additional organisms detected by the Filmarray were presented.

The positive and negative percent agreement of Filmarray bacterial detection compared to culture as the reference standard was calculated for each analyte with 95% confidence intervals, and then for all the targets pooled. A true positive (TP) was defined as a Filmarray result which was culture positive. A false negative (FN) was defined as a negative Filmarray result which was culture positive. The positive percent agreement was calculated by dividing TP by TP + FN.

A true negative (TN) was defined as a negative FA result which was culture negative. A false positive (FP) was defined as a positive FA with a negative culture result. The negative percent agreement was calculated by dividing TN by TN + FP.

2.18.2 Frequency of detection by culture of 'off panel' bacteria

'Off panel' organisms are those which are not targeted by the Filmarray Pneumonia panel but are still pneumonia pathogens. All such occurrences in the trial were presented and any clinical impact as a result of their lack of detection were considered.

2.18.3 Comparison of Filmarray Semi-quantitation with culture relative quantification

The Filmarray semi-quantification was compared to the relative abundance grown in culture in all samples that were tested directly by both methods. Concordance between the two was calculated, with scanty growth (+) considered equivalent to a molecular abundance of 10^4 and 10^5 copies/ml, moderate growth (++) considered equivalent to 10^5 and 10^6 copies/ml and heavy growth (+++) considered equivalent to $\geq 10^6$ copies/ml.

2.18.4 Number of Filmarray pathogen detections depending on sample type (BAL-like or non-BAL like)

Murphy et al noted that sputum like samples (ETA and expectorated sputa) typically are positive for multiple bacterial targets more often than BAL-like samples[175]. We compared the number of bacterial targets identified per sample from sputum like samples (including ETA and expectorated sputa) and BAL-like samples (including BALs and undirected/ mini-BALs) using the Mann-Whitney-U test. We then directly compared the proportion of all tests that were positive for a pathogen from BAL-like samples with sputum like samples using Fishers exact tests. Lastly, we compared the proportion of positive results that had greater than one pathogen detected between sampling

methods using Fishers exact test. Statistics were performed using Prism (GraphPad Software Inc; La Jolla, California).

2.18.5 Frequency of Filmarray resistance gene detection, relationship to phenotypic sensitivity and clinical implications

We retrospectively examined the case records, prescribing history, extended microbiological history and specialist microbiology notes of all patients who had a resistance gene detected on the Filmarray pneumonia panel.

2.18.6 Diagnostic accuracy of the Filmarray for atypical bacterial detection compared to standard of care diagnostics

All cases of *L. pneumophilia, C. pneumoniae* and *M. pneumoniae* were identified from culture, serology, urinary antigen testing and Filmarray testing in the trial. The relative performance of different diagnostic modalities was considered.

2.18.7 Additional identification of respiratory viruses as a result of syndromic testing

No parallel samples were taken for testing by laboratory viral PCR at the time of recruitment, therefore we were unable to present diagnostic accuracy data on viral detections. We identified all positive respiratory virus detections by conventional laboratory PCR or Filmarray testing. We compared retrospective Filmarray testing of control arm samples, to standard of care viral PCR results to identify the number of respiratory viruses 'missed'.

2.19 Rationale, methods and statistical analysis for additional post-hoc analyses

2.19.1 Impact of the SARS-CoV-2 pandemic on viral detections in critical care

In other work, we identified profound impact on the frequency of detection of existing respiratory viruses in hospitalised adults following the emergence of SARS-CoV-2 and subsequent social distancing measures[97,193]. We identified all patients testing positive for a non-SARS-CoV-2 respiratory virus in critical care and compared the proportional rate of positivity for human rhinovirus/enterovirus, seasonal coronaviruses, parainfluenza viruses, human metapneumovirus and RSV (no influenza viruses were detected in the study) before and after March 2020, using chi-square tests.

2.19.2 Association between *S. pneumoniae* growth in blood and sputum, urinary antigen positivity, and molecular detection.

We identified all patients with a positive blood culture for *S. pneumoniae*, all patients with a positive pneumococcal urinary antigen test, an all patients with *S. pneumoniae* present in LRT sample by culture and/or molecular testing. We compared positivity between tests.

2.20 Safety

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

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

SAEs were reported via the sponsor's standard forms and processes within 24 hours of the study team learning of the occurrence. In the event of a SAE, the PI was involved in deciding whether this was a study-related event.

As this trial involved participants who were 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 were not recorded as a life-threatening SAE. Transfer either way between ICU and RHDU did not count as a SAE. Transfer to another critical care facility (including repatriation to a local hospital) did not count as a SAE except when it was for a level of care that could not be provided at UHS. Similarly, complications or sequelae of conditions that they had at the time of recruitment were not counted as a SAE of persistent or significant disability, or incapacity, or an SAE related to prolongation of existing hospitalisation.

Hospitalisation, life-threatening event or death more than 30 days after the original presentation did not count as SAEs because of the time lapsed in relation to the event.

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 were developed to streamline the reporting process, promote efficiency and maximise safety.

2.21 Protocol amendments

The initial trial protocol was approved in 2016 using the BioFire Respiratory panel to diagnose viral infections in critical care. Version 2.0 was submitted in March 2019 and approved May 2019 where the inclusion criteria were narrowed from all acute respiratory illness to exclusively CAP, HAP and VAP. The main test used was changed to the BioFire Pneumonia panel (which only got CE marking in 2018). PCT and urinary antigen measurements were added in, and the primary outcome/ secondary outcomes re-written to reflect the use of this new technology. A new size calculation was performed.

The third substantial amendment was approved August 2nd 2019 which removed the inclusion criteria for 'duration of respiratory illness <72 hours'. The reason for this being included initially was to try and limit the duration of preceding antibiotics which patients had received prior to recruitment. In practice, many patients had been unwell for several weeks prior to starting therapy which precluded their participation in the trial, and made recruitment of adequate numbers almost impossible. We recorded the preceding duration of antimicrobials for recruited patients and demonstrated that patients had only been on a median of 24 hours therapy prior to recruitment.

The fourth substantial amendment was approved in March 2021, this reduced the sample size to 200 patients after an interim review, clarified definitions of time points for secondary outcome measures, and removed the mandated reporting of all deaths as SAEs (given the expected risk of death in this cohort would be ~30% regardless of study).

2.22 Contributions of myself and others

I co-designed all aspects of the study with the study CI and my PhD supervisor Dr Tristan Clark. The study protocol was initially written in 2017 for the original iteration of the trial (when initially designed as an exclusively respiratory viral study). I rewrote the protocol for the substantial

amendment discussed above. I re-wrote all of the study documents, including the patient information sheet, patient consent form, consultee information sheets and consultee consent forms. I performed a literature review whilst re-designing the study to inform the process which was later published[194].

I submitted both REC amendments and altered the integrated research application system (IRAS) form accordingly. I obtained University ethics and research governance online (ERGO) approval. I presented the study to the Biomedical Research Centre (BRC) clinical studies forum (CSF) to gain approval for the study to be adopted by the BRC.

I designed, road tested and oversaw the use of the electronic case report form (CRF) which was produced by Dr Nicole Vaughan-Spickers and colleagues, clinical research analyst programmers at the Southampton clinical informatics research unit (CIRU).

I carried out the trial procedures as described above. Overall, I recruited over 90% of the study participants. I was fortunate to be supported in obtaining samples and with trial administrative procedures by many of the BRC research nurses and clinical trials assistants from both the Critical care and respiratory teams. The people involved with the study changed during its course, as a result of moving priorities within the BRC due to the COVID-19 pandemic. I am particularly thankful for the help from Rachel Burnish (critical care), Clare Bolger (critical care), June Law (respiratory), Jithin John (respiratory) and Oli Jones (critical care).

Other research fellows contributed to running the trial. Dr Alex Tanner and Dr Vasanth Naidu participated in running the study and recruited patients. Dr Nathan Brendish helped advise Dr Tanner and Dr Naidu in my absence. Sample processing was supported by the Southampton Clinical Research Facility (CRF) laboratory staff led by Gavin Babbage. The CRF team received samples from the ward, centrifuged serum samples then catalogued samples in the freezer. Within that team I am particularly grateful to Phaedra Marius and Sabeha Sabeha who performed these duties for most of the study. Testing of samples was performed by myself and the other research fellows (AT and VN).

I collected follow up retrospective data with Hang Phan and Florina Borca: University and BRC R&D data analysts, who collected data from UHS electronic patient systems and collated it on the BC genomes platform.

Helen Moyses (Biomedical Research Centre Medical statistician) performed statistical analysis separately on the primary and secondary outcomes, and performed both the logistic regression model, and the competing risks regression analysis. I performed the statistical analysis separately using comparative statistics on primary and secondary outcomes. I performed all of the statistical

analysis described for exploratory outcomes, pneumonia panel performance and additional *post hoc* analyses.

Chapter 3 The SARIPOC trial results

3.1 Recruitment

Between July 10, 2019, and May 5, 2021, 296 patients were assessed for eligibility in the study and 200 were randomly assigned to either mPOCT (n=100) or standard clinical care (n=100). Patient flow through the study is shown in Figure 16. 96 patients who were approached were ineligible. No patients were withdrawn. One patient was transferred to another hospital before the results of their microbiological testing were available (in the control group), and so did not have data available for the primary endpoint. All patients were analysed in the intention to treat analysis.

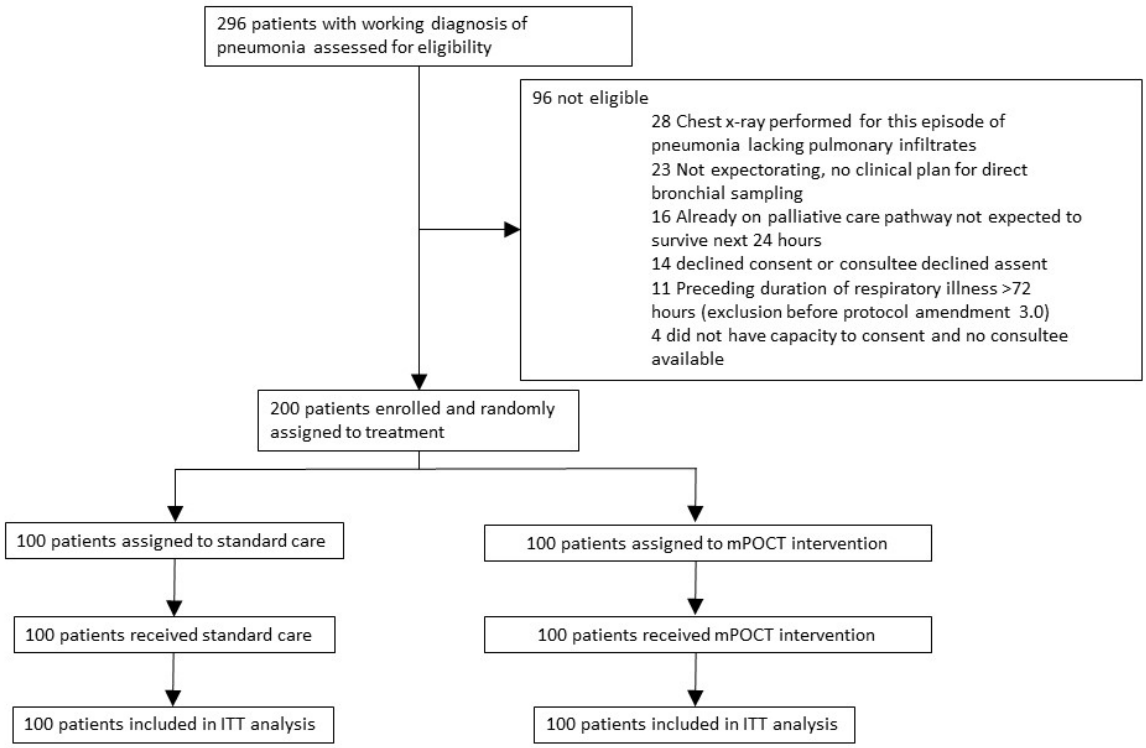


Figure 16: Recruitment diagram for SARIPOC trial

3.2 Baseline characteristics

Baseline characteristics for all patients are shown in Table 12 and were well matched between groups. The number of patients with a diagnosis of CAP was similar in each group, although there were more patients with VAP in the intervention group (28 versus 18), and more patients with

HAP (39 versus 30) in the control group. 15 (15%) of 100 in the mPOCT group and 12 (12%) of 100 in the control group were SARS-CoV-2 PCR positive prior to enrolment.

Table 12: Baseline characteristics

	mPOCT group, n=100	**Control group, n=100**
Age, years	63 (52-72)	63 (54-73)
Sex		
Female	31 (31)	29 (29)
Male	69 (69)	71 (71)
Ethnicity		
White British	87 (87)	87 (88)
White other	5 (5)	2 (2)
Black African	3 (3)	3 (3)
Afro-Caribbean	2 (2)	1 (1)
Indian, Pakistani or Bangladeshi	3 (3)	5 (5)
Chinese	0 (0)	1 (1)
Unknown	0 (0)	1 (1)
Smoking status		
Current smoker	25 (25)	19 (19)
Ex-smoker	37 (27)	28 (28)
Never smoker	26 (26)	37 (37)
Unknown	12 (12)	16 (16)
Pregnant		
Yes	1 (1)	0 (0)
No	99 (99)	100 (100)
Co-morbidities		
Respiratory disease (any)	35 (35)	25 (25)
COPD	14 (14)	9 (9)
Asthma	15 (15)	9 (9)
Chronic kidney disease	7 (7)	7 (7)
Chronic liver disease	8 (8)	8 (8)
Diabetes mellitus	19 (19)	22 (22)
Immunosuppression	7 (7)	8 (8)
Charlson co-morbidity score	5.5 (0-11)	4 (0-11)

	mPOCT group, n=100	Control group, n=100
Observations		
Heart rate, beats per min	91 (80-108)	85 (71-104)
Respiratory rate, breaths per min	22 (17-26)	21 (18-26)
Oxygen saturation, %	96 (93-98)	96 (94-99)
Blood pressure, mmHg		
Systolic	133 (114-152)	128 (113-145)
Diastolic	65 (56-77)	65 (57-76)
Fraction of inspired oxygen, %	50 (40-70)	50 (40-60)
Temperature, °C	36.9 (36.2-37.6)	37.0 (36.3-37.6)
Glasgow coma scale score	10.0 (3.0-15.0)	9.0 (3.0-15.0)
Respiratory support		
Invasive ventilation	55 (55)	53 (53)
Non-invasive ventilation	16 (16)	20 (20)
Severity		
APACHE II score	16.0 (11.5-21.0)	17.5 (13.0-23.0)
SOFA score	6 (4-9)	5 (4-9)
Recruitment diagnosis		
Community acquired pneumonia	42 (42)	43 (43)
Hospital acquired pneumonia	30 (30)	39 (39)
Ventilator associated pneumonia	28 (28)	18 (18)
Known COVID-19 infection	15 (15)	12 (12)
Laboratory indices		
C-reactive protein, mg/L	120 (70-193)	144 (80-212)
White blood cell count, 10^9/L	13.1 (9.4-18.2)	12.1 (9.6-17.0)
Neutrophils, 10^9/L	10.9 (7.7-15.2)	10.3 (7.8-14.9)
Lymphocytes, 10^9/L	0.9 (0.6-1.3)	1.0 (0.5-1.3)
Duration of antimicrobials prior to recruitment, hours	24.1 (16.7-41.2)	23.7 (14.3-39.1)
Duration of critical care admission prior to recruitment, days	2.2 (1.0-4.3)	2.2 (0.9-4.1)

Data are n (%) or median (IQR). ITT, intention to treat. mPOCT, molecular point-of-care testing. COPD, chronic obstructive pulmonary disease, APACHE II, Acute physiology and chronic health evaluation. SOFA, sequential organ failure assessment.

3.3 Outcomes

The median (IQR) time to results was 1.7 (1.6 to 1.9) hours in the mPOCT group and 66.7 (56.7 to 88.5) hours in the control group (difference of -65.0 hours, 95% CI -68.0 to -62.0; p<0.0001), shown in Figure 17**.** Seventy one (71%) of 100 patients in the mPOCT group had a credible pathogen identified, compared to 51 (51%) of 100 in the control group (difference of 20%, 95% CI 7 to 33; p=0.004, Table 13). The details of the pathogens detected are shown according to pneumonia type (Table 14).

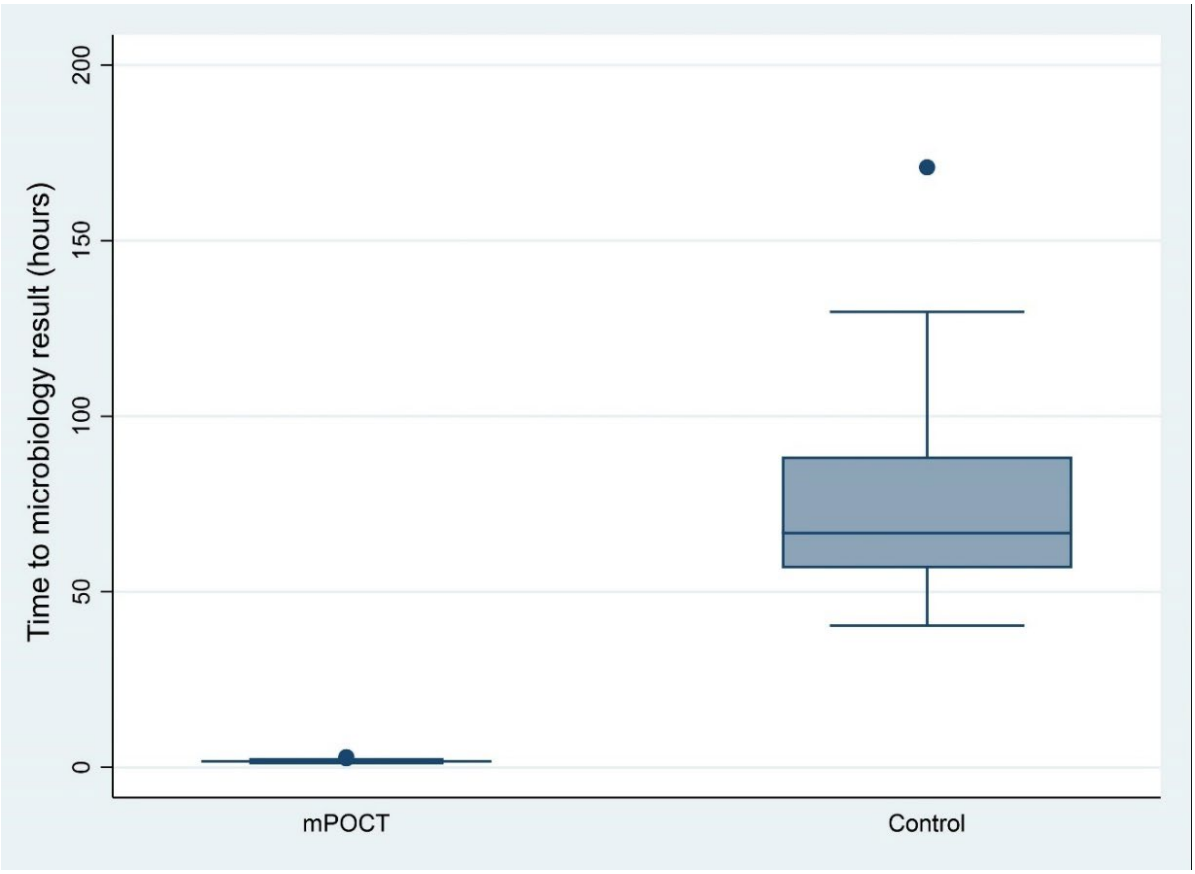


Figure 17: Time to microbiology result

Table 13: Microbiological testing outcome measures

Outcome	**mPOCT group n=100**	**Control group n=100**	**Absolute difference (95% CI)**	**p value**
Time to microbiology result from request, hours	1.7 (1.6-1.9)	66.7 (56.7-88.5)	-65.0 (-68.0 to 62.0)	<0.0001
Patients with credible pathogen identified	71 (71)	51 (51)	20 (7 to 33)	0.004
Procalcitonin, ng/ml*	0.38 (0.15-2.61)	0.51 (0.22-3.73)	-0.13 (-0.66 to 0.40)	0.63
Procalcitonin value <0.5ng/ml*	50 (53)	47 (49)	4 (-11 to 18)	0.61
Positive pneumococcal urinary antigen†	1 (1)	1 (1)	0 (-3 to 3)	0.99

All data are presented as n (%) and median (IQR). mPOCT, molecular point-of-care testing. CI, 95% confidence interval. CAP, Community acquired pneumonia. HAP, hospital acquired pneumonia. VAP, ventilator associated pneumonia. *assessed in 94 patients in the mPOCT group and 95 in the control group †assessed in 96 patients in the mPOCT group and 95 patients in the control group.

Table 14: Causative organisms of pneumonia identified

	CAP		HAP		VAP	
	mPOCT	**Control**	**mPOCT**	**Control**	**mPOCT**	**Control**
Any pathogen, n/n (%)	25/42 (60)	20/43 (47)	20/30 (67)	18/39 (46)	26/28 (93)	13/18 (72)
Single pathogen, n/n (%)	15/25 (60)	17/20 (85)	8/20 (40)	15/18 (83)	15/26 (58)	8/13 (62)
Multiple pathogens, n/n (%)	10/25 (40)	3/20 (15)	12/20 (60)	3/18 (17)	11/26 (42)	5/13* (38)
Frequency of identification of individual pathogens						
S. pneumoniae	1	0	2	2	1	1
S. aureus	6	6	7	0	2	3
H. influenzae	5	0	3	2	2	1
M. catarrhalis	0	0	1	0	0	0
H. parainfluenzae	0	1	0	0	0	1
L. pneumophilia	0	1	0	0	0	0
K. pneumoniae group	1	2	2	3	2	3
E. coli	3	1	6	3	6	1
S. marcescens	2	0	3	2	2	1
E. cloacae complex	1	0	1	2	1	0
K. aerogenes	1	1	2	0	0	0
K. oxytoca	2	0	1	1	5	1
S. maltophilia	0	0	0	1	0	0
P. mirabilis	1	0	0	1	3	1
Citrobacter spp	1	1	0	0	1	0
P. aeruginosa	5	1	3	3	15	3
Rhinovirus/enterovirus	3	2	1	0	0	0
Respiratory syncytial virus	1	0	0	1	0	0
Parainfluenza virus	1	0	0	0	0	0
Seasonal human coronavirus[†]	0	0	2	0	0	0
SARS-CoV-2[‡]	6	7	0	0	0	0

CAP, Community acquired pneumonia. HAP, Hospital acquired pneumonia. VAP, ventilator associated pneumonia. Seasonal human coronavirus includes HKU1, 229E, NL63 and OC43. SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2. *one culture reported as mixed coliforms. ‡only with diagnosis at start of this episode of pneumonia (i.e. HAP/VAP developing later in a primary COVID-19 illness not included, but noted in baseline characteristics)

80 (80%) of 100 patients in the intervention arm received results-directed therapy, compared to 29 (29%) of 99 in the control arm (difference of 51%, 95% CI 39 to 63, P<0.0001). The median (IQR) time to results-directed therapy was 2.3 (1.8-7.2) hours in the mPOCT group and 46.1 (23.0-

51.5) hours in the control group (difference of -43.8 hours, 95% CI -48.9 to -38.6; p<0.0001). 56 (56%) of 100 patients in the intervention arm received specific pathogen-directed therapy, compared with 18 (18%) of 99 in the control arm (difference 38%, 95% CI 25-50; p<0.0001), (Table 36, supplementary appendix A). The impact of multiple clinical variables on the association between mPOCT and results-directed therapy was explored using a logistical regression model (Table 37, supplementary appendix A) which did not demonstrate any significant impact of any variable, including initial PCT level at recruitment. A time-to-event curve showing result-directed therapy in the groups is shown in Figure 18.

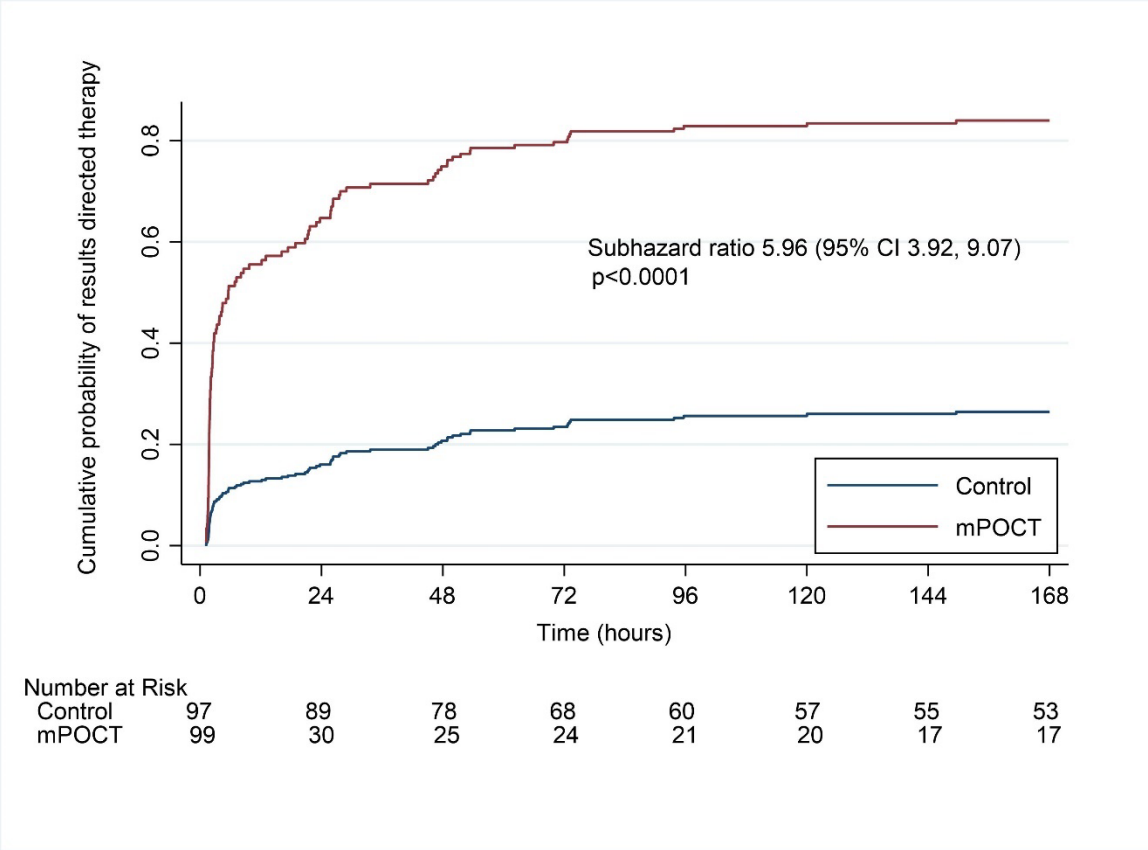


Figure 18: Time to results directed therapy

At recruitment, 58 (58%) of 100 patients in the mPOCT group and 58 (59%) of 99 patients in the control group were receiving potentially de-escalatable (i.e. inappropriately broad for the pathogens subsequently detected) empirical antimicrobial therapy (difference of -0.6%, 95% CI -14 to 13; p=0.93) and at 48 hours, this had reduced to 29 (29%) of 99 in the mPOCT group and 40 (42%) of 95 in the control group (difference of -13%, 95% CI -26 to 0.6; p=0.063), Table 15. The median (IQR) number of antimicrobial-free hours in 14 days following recruitment was 145.6 (77.4-233.4) in the mPOCT group and 170.9 (82.3-239.5) in the control group (difference of -24.9, 95% CI -65.4 to 15.6; p=0.23). Additional secondary outcomes relating to antimicrobials are presented in Table 36, supplementary appendix.

Table 15: Antimicrobial use

Outcome	mPOCT group n=100	Control group n=100	Absolute difference (95% CI)	p value
Primary outcome				
Results-directed therapy	80 (80)	29/99 (29)	51 (39-63)	<0.0001
Secondary outcomes				
Time to results-directed therapy, hours*	2.3 (1.8-7.2)	46.1 (23.0-51.5)	-43.8 (-48.9 to -38.6)	<0.0001
Results-directed de-escalation	42 (42)	8/98 (8)	34 (23-45)	<0.0001
Time to results-directed de-escalation, hours†	4.8 (2.4- 13.0)	46.5 (26.3-48.9)	-41.4 (-53.0 to -29.7)	<0.0001
Results-directed escalation	9 (9)	1/98 (1)	8 (2 to 14)	0.034
Time to results-directed escalation, hours‡	5.1 (2.7-26.0)	27.5 (27.5-27.5)	-22.4 (-165.0 to 120.3)	0.38
Ineffective antimicrobial therapy at recruitment	14/100 (14)	14/99 (14)	-0.1 (-10 to 10)	0.98
Ineffective antimicrobial therapy at 48 hours post recruitment	12/99 (12)	8/95 (8)	4 (-5 to 12)	0.40
Duration of ineffective therapy, hours§	71.5 (46.0-113.0)	60.5 (18.5-127.5)	26.8 (-35.0 to 88.5)	0.38
De-escalatable therapy at recruitment	58 (58)	58/99 (59)	-0.6 (-14 to 13)	0.93
De-escalatable therapy at 48 hours post-recruitment	29/99 (29)	40/95 (42)	-13 (-26 to 0.6)	0.063
Duration of all antimicrobial therapy, days¶	7.6 (5.0- 10.8)	7.0 (4.7-9.8)	0.6 (-0.7 to 1.9)	0.35
Number of antimicrobial classes used	2/94 (1-3)	2/94 (1-2)	0.1 (-0.2 to 0.2)	1.00
Antibiotic free hours in following 14 days¶	145.6 (77.4-233.4)	170.9 (82.3-239.5)	-24.9 (-65.4 to 15.6)	0.23

All data are presented as n (%) or median (IQR). mPOCT, molecular point-of-care testing. CI, confidence interval. Ineffective and de-escalatable therapy relate to pathogens subsequently identified through molecular testing and/or culture.
*Assessed in 79 patients in the mPOCT group and 29 patients in the control group. †Assessed in 42 patients in the mPOCT group and 8 patients in the control group. ‡Assessed in 9 patients in the mPOCT arm and 1 patient in the control arm. §Assessed in 12 patients in the mPOCT arm and 8 in the control arm. ¶Assessed in 94 patients in both mPOCT and control arms.

There was no difference between the groups in the proportion of patients that received; mechanical ventilation, 60 (65%) of 92 versus 66 (70%) of 94 (difference of -5%, 95% CI -18 to 8; p=0.44), inotropic/vasopressor support, 42 (45%) of 94 versus 47 (50%) of 94 (difference of -5%, 95% CI -20 to 9; p=0.47) or in the duration of mechanical ventilation and inotropic/vasopressor support, 7.5 (4.5-15.1) days versus 6.7 (2.7-12.8) days (difference of 1.0 days, 95% CI -2.7 to 4.7; p=0.59) and 7.2 (3.7-12.1) days versus 5.3 (3.4-13.6) days, (difference of 2.3 days, 95% CI -1.7 to 6.3; p=0.26), respectively. Safety outcomes are presented in Table 16. Competing risk regression for time to hospital discharge, time to critical care discharge and 30 and 60 day mortality did not demonstrate a difference between the groups (Table 38, appendix A). New cases of adverse drug reactions were observed in only three patients; one in the mPOCT group and two in the control group. There was only one new case of *C. difficile* infection, which occurred in the control group. 8 (8%) of 100 patients in the mPOCT group were prescribed antimicrobials based on advice relating to pathogens detected with the Filmarray Pneumonia panel but later cultured an organism phenotypically resistant to these agents. The empirical antimicrobial therapy at recruitment for 7 of 8 of these patients was also ineffective against the organisms subsequently grown (Table 39, appendix A).

Table 16: Clinical outcomes

Outcome	mPOCT group n=100	Control group n=100	Absolute difference (95%CI)	p value
30-day mortality	18/99 (18)	16/96 (17)	2% (-9 to 12)	0.78
60-day mortality	20/99 (20)	21/96 (22)	-2% (-13 to 10)	0.77
Time to hospital discharge from recruitment, days*	21.2 (10.3-37.0)	18.2 (11.3-36.0)	3.0 (-4.5 to 10.5)	0.43
Time to critical care discharge from recruitment, days†	10.0 (3.2-16.3)	7.1 (4.0-14.0)	2.8 (-0.7 to 6.3)	0.11
NIV after recruitment	37/91 (41)	29 /93 (31)	9% (-4 to 23)	0.18
Duration on NIV, days‡	1.8 (0.6-3.9)	2.5 (0.8-4.5)	-0.7 (-2.5 to 1.1)	0.44
Invasive ventilation after recruitment	60/92 (65)	66/94 (70)	-5% (-18 to 8)	0.47
Duration on invasive ventilation, days§	7.5 (4.5-15.1)	6.7 (2.7-12.8)	1.0 (-2.7 to 4.7)	0.59
Oxygen therapy	84/94 (89)	86/94 (91)	-2% (-11 to 6)	0.62
Duration of oxygen therapy, days¶	10.5 (4.6-17.5)	6.0 (3.0-14.1)	4.7 (1.1 to 8.3)	0.010
Inotropic/vasopressor support after recruitment	42/94 (45)	47/94 (50)	-5% (-20 to 9)	0.47
Duration on inotropes/vasopressors, days**	7.2 (3.7-12.1)	5.3 (3.4-13.6)	2.3 (-1.7 to 6.3)	0.26
Antimicrobial adverse drug reaction within 30 days	1/99 (1)	3/100 (3)	-2% (-6 to 2)	0.34
New *Clostridioides difficile* infection within 30 days	0/99 (0)	1/100 (1)	N/A	N/A

All data are presented as n (%) and median (IQR). mPOCT, molecular point-of-care testing. CI, confidence interval. NIV, non-invasive ventilation. *Assessed in 74 patients in the mPOCT group and 74 patients in the control group. †Assessed in 78 patients in the mPOCT group and 78 patients in the control group. ‡Assessed in 37 patients in the mPOCT group and 29 patients in the control group. §Assessed in 60 patients in the mPOCT group and 66 patients in the control group. ¶ Assessed in 84 patients in the mPOCT group and 86 patients in the control group. **Assessed in 42 patients in the mPOCT group and 47 patients in the control group.

3.4 Exploratory outcomes

3.4.1 Association between pathogen quantification and clinical outcome measures

At least one semi-quantifiable bacteria was reported by Filmarray testing in 123/198 (62%) patients. 76/123 (62%) were single pathogen detections, and 47/123 (38%) were co-detections. There was no significant association between bin values and procalcitonin level or any clinical outcome, when compared across all bin values, although there was a trend for a higher 30-day

mortality in those with higher bin values (p=0.060, Table 17). There was an association between increasing bin values and multiple-target detection (p=0.010).

Table 17: Relationship of Filmarray bin values to clinical outcomes

	Highest Filmarray Bin Value, copies/ml				
	10^4 (n=19)	**10^5 (n=36)**	**10^6 (n=30)**	**10^7 (n=38)**	**p value**
Filmarray result					
Single detection	15 (79)	27 (75)	14 (47)	20 (53)	0.010
Multi-detection	4 (21)	9 (25)	16 (53)	18 (47)	0.010
Recruitment characteristics					
Diagnosis					
CAP	8 (42)	18 (50)	8 (27)	8 (21)	0.015
HAP	7 (37)	7 (19)	15 (50)	15 (39)	0.23
VAP	4 (21)	11 (31)	7 (23)	15 (39)	0.21
Apache II score	17 (14-22)	18 (12-21)	15 (11-22)	21 (17-26)	0.022
C-reactive protein (mg/l)	97 (21-198)	120 (20-212)	147 (67-184)	125 (62-172)	0.75
Fraction inspired oxygen	0.5 (0.32-0.6)	0.5 (0.36-0.6)	0.48 (0.35-0.6)	0.5 (0.35-0.63)	0.95
Outcomes					
Procalcitonin (ng/ml)	0.29 (0.1-2.7)	0.29 (0.15-2.7)	0.34 (0.12-5)	1.0 (0.32-5.8)	0.16
30-day mortality	2/19 (11)	5/35 (15)	5/30 (17)	11/37 (30)	0.060
Time to ICU discharge (days)	9 (2-18)	10 (5-16)	8 (4-15)	10 (5-21)	0.74
Proportion receiving Invasive ventilation	14/19 (77)	22/30 (73)	16/25 (64)	29/37 (78)	0.75
Proportion receiving NIV	7/19 (37)	12/31 (39)	13/22 (59)	10/37 (27)	0.41

Data are median (IQR) and n (%).

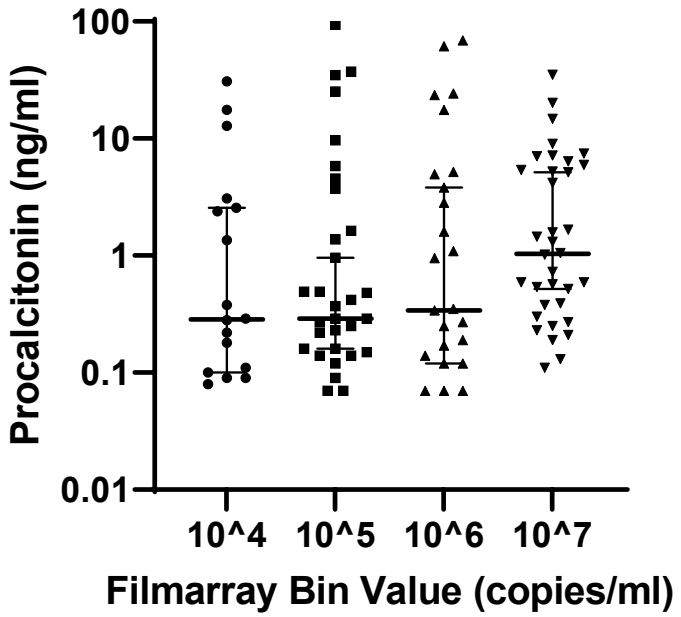


Figure 19: Scatter plot of Procalcitonin concentration vs Filmarray bin values for all bacteria detected

3.4.2 Utility of serum procalcitonin in differentiating between bacterial infection from non-bacterial infection and prediction of clinical outcome

188 patients had a recruitment PCT and molecular (Filmarray) testing of a lower respiratory tract sample. Of these 78 (41%) of 188 had CAP, 66 (35%) of 188 had HAP and 44 (23%) of 188 had VAP. The median (IQR) preceding duration of antibiotics prior to LRT sampling was 23 hours (14-39). 115/188 (61%) samples were positive for at least one bacterial target by Filmarray testing.

The area under the receiver operator characteristic (ROC) curve for PCT in determining presence of any bacterial pathogen by FA testing was 0.52 (Table 18) for all pneumonia and 0.51 for CAP. In patients with CAP, using a PCT cut off of 0.1ng/ml had a sensitivity of 90% for bacterial molecular detection and a specificity of 10%. This reduced to a sensitivity of 47% with a cut off of 0.5ng/ml and a specificity of 45% (Table 19). The sensitivity and specificity of PCT for predicting presence of pathogens at commonly used cut-offs are presented in Table 19.

The AUROC for all patients increased to 0.56 applying a minimum threshold of $>10^5$ copies/ml and when the highest threshold for molecular positivity (i.e. detection reported as the greatest Filmarray bin value of $\geq 10^7$ copies/ml) was applied, this increased to 0.63 for all patients (Figure 20) and 0.64 for CAP.

Table 18: Area under the ROC curve for recruitment PCT in determining presence of pathogen by different modalities

	AUROC	
	CAP (n=78)	All† (n=188)
Conventional diagnostic testing*	0.59	0.57
Any molecular detection	0.51	0.52
Bacterial abundance >10^5 copies/ml	0.57	0.56
Bacterial abundance >10^7 copies/ml	0.64	0.63

AUROC= Area under the receiver operator characteristic curve, CAP= Community acquired pneumonia, HAP= Hospital acquired pneumonia, VAP= Ventilator associated pneumonia*Not all participants had sputum culture †All = CAP, HAP and VAP

Table 19: Sensitivity and specificity of PCT for pathogen detection by different modalities at commonly used thresholds

Reference test		**Procalcitonin cut off**					
		0.1ng/ml		**0.25ng/ml**		**0.5ng/ml**	
		Sensitivity	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
Conventional testing alone	CAP	86%	9%	73%	36%	67%	54%
	All	88%	11%	72%	37%	59%	58%
Any molecular detection	CAP	90%	10%	66%	32%	47%	45%
	All	86%	8%	67%	33%	49%	51%
Bacterial abundance >10^5 copies/ml	CAP	93%	11%	80%	36%	53%	51%
	All	88%	11%	72%	36%	59%	56%
Bacterial abundance >10^7 copies/ml	CAP	100%	12%	89%	36%	67%	52%
	All	97%	14%	81%	38%	70%	56%

CAP, Community acquired pneumonia

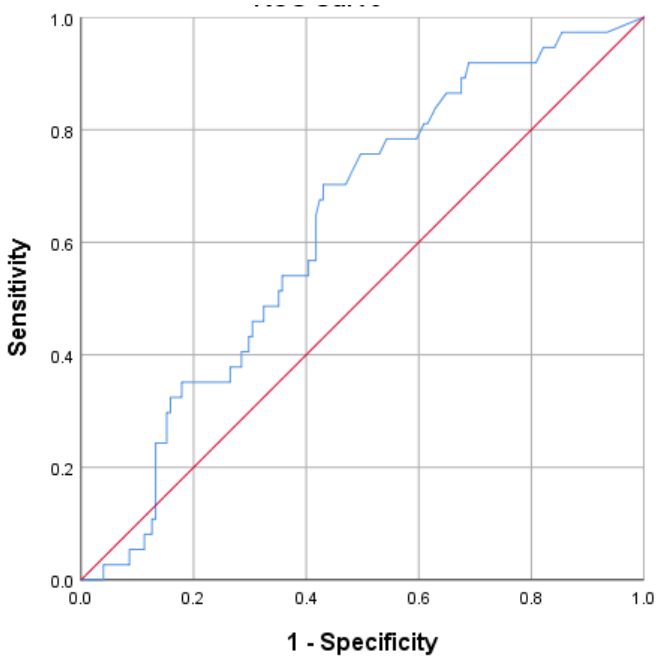


Figure 20: ROC curve for performance of PCT in determining very high level (>10^7 copies/ml) molecular bacterial pathogen detection

Outcomes according to recruitment PCT group for the 188 people who had both PCT at recruitment and molecular (FA) testing are presented in Table 20. 183/188 patients had a recruitment PCT and 30-day mortality data available, of these 31/183 died (17%). The AUROC for day 1 PCT in predicting 30-day mortality was 0.59, this was 0.57 at day 3 (where 94 patients had samples) and 0.42 at day 6 (where 58 patients had samples).

Table 20: Clinical outcome measures according to recruitment PCT

	Recruitment PCT, ng/ml				
	<0.1 (n=22)	**0.1 to <0.25 (n=40)**	**≥0.25 to <0.5 (n=34)**	**≥0.5 (n=92)**	**p value**
Time to ICU discharge (days)	13.5 (9.0-31.0)	8.1 (2.6-14.5)	11.1 (5.5-20.2)	9.3 (4.0-18.5)	0.027
Proportion mechanically ventilated	11/19 (58)	23/37 (62)	21/29 (72)	68/89 (76)	0.043
Duration of mechanical ventilation (days)	6.8 (2.7-11.4)	8.2 (2.8-13.9)	13.8 (7.0-22.0)	5.5 (3.2-13.5)	0.062
Proportion given NIV	4/20 (20)	12/37 (32)	13/29 (45)	34/89 (38)	0.16
Duration NIV (days)	2.9 (0.8-7.2)	1.1 (0.5-6.0)	2.3 (1.3-11.5)	2.5 (0.7-4.0)	0.68
30-day mortality	1/21 (5)	6/40 (15)	7/34 (21)	17/92 (18)	0.21

Data are median (IQR) and n (%). ICU, intensive care unit. NIV, non-invasive ventilation. PCT, procalcitonin

When patients with a negative bacterial result on the Filmarray were excluded, leaving 112 patients with a positive Filmarray bacterial result and PCT, there was a trend towards an association between PCT group and 30 day mortality, Table 21. In exclusively molecular positive patients, the AUROC for PCT at predicting 30 day mortality was 0.61 at day 1, 0.57 at day 3 (n=54), and 0.41 at day 6 (n=40). At day 1, using a cut-off of <0.1ng/ml, this translated into a sensitivity of 100% for 30 day mortality, and a specificity of 16%. Further excluding patients with a molecular detection below 10^6 copies/ml increased the AUROC to 0.65.

Table 21: All cause 30-day mortality by molecular (FA) detection and recruitment PCT

	Recruitment PCT, ng/ml				
	<0.1 (n=21)	**0.1 to <0.25 (n=38)**	**0.25 to 0.5 (n=32)**	**≥0.5 (n=92)**	**p value**
No bacteria detected	1/6 (17)	3/17 (18)	2/12 (17)	5/37 (14)	0.73
Bacteria detected	0/15 (0)	3/21 (14)	5/20 (25)	12/55 (22)	0.062

Data are n (%)

3.4.3 Pathogen detection in pneumonia occurring following macro-aspiration

67 patients had a history of likely preceding macro-aspiration. 53/67 (79%) had an organism detected by the Filmarray, compared to 78/131 (60%) of patients with no-preceding history of

aspiration (difference 20%, 95% CI 4.9-32, p=0.0068). Enterobacteriaceae were the most frequently detected organism group in both community-acquired and hospital-associated illness, occurring in 8/26 (31%) of community acquired cases and 23/41 (56%) of hospital associated cases (of which 30 had a diagnosis of HAP, and 11 a diagnosis of VAP). In both hospital acquired and community acquired cases, *H. influenzae* and *S. aureus* were the next most frequently detected pathogens. Enterobacteriaceae were more frequently detected in hospital associated aspiration pneumonia than community acquired cases, Table 22.

Table 22: Frequency of molecular pathogen detection in cases with known recent macro-aspiration event

	Community acquired (n=26)	**Hospital associated (n=41)**	**p value**
No pathogen detected	6 (23)	8 (20)	0.76
Enterobacteriaecae	8 (31)	23 (56)	0.049
H. influenzae	6 (23)	8 (20)	0.76
S. aureus	5 (19)	6 (15)	0.74
P. aeruginosa	4 (15)	6 (15)	0.99
S. pneumoniae	3 (12)	3 (7)	0.67
S. pyogenes	1 (4)	0	0.39
M. catarrhalis	0	4 (10)	0.15
S. agalactiae	3 (12)	2 (5)	0.37
Viral target	5* (19)	3† (7)	0.25

Data are n (%), *4x human rhinovirus and 1 parainfluenza virus, †1x human rhinovirus, 1x Human coronavirus

28/67 (42%) of patients who developed pneumonia following a macro-aspiration event were on regular proton pump inhibitor therapy, compared to 44/131 (34%) of patients with pneumonia not following a macro-aspiration event (difference 8, 95% CI 0 to 23, p=0.28).

3.4.4 Pathogen detection in patients with SARS-CoV-2 infection

In total, 27 participants were enrolled with known SARS-CoV-2 infection, 13 patients with CAP, and 6 patients had HAP and 8 patients with VAP. In patients with a community onset illness (i.e. primary COVID infection), 3/13 patients (23%) had a bacterial pathogen detected by Filmarray. This compared to 40/70 (57%) of CAPs without SARS-CoV-2 infection (difference 34, 95% CI 14-67, p=0.034, Table 23). Potential confounders for this data set are presented in Appendix A, Table 44.

Table 23: Bacterial detections in patients with known SARS-CoV-2 infection

Recruitment diagnosis	Proportion with bacterial target identified		Difference (95% CI)	p value
	SARS-CoV-2 infected	Non-SARS-CoV-2 infected		
CAP	3/13 (23)	40/70 (57)	34 (14-67)	0.034
HAP	3/6 (50)	41/63 (65)	15 (0 to 53)	0.66
VAP	8/8 (100)	29/38 (76)	24 (0 to 41)	0.32

Data are n/n (%). CAP, community acquired pneumonia. HAP, hospital acquired pneumonia. VAP, ventilator associated pneumonia.

The most frequently occurring bacterial detection in patients infected with SARS-CoV-2 were *P. aeruginosa* and *K. pneumoniae* group, Figure 21.

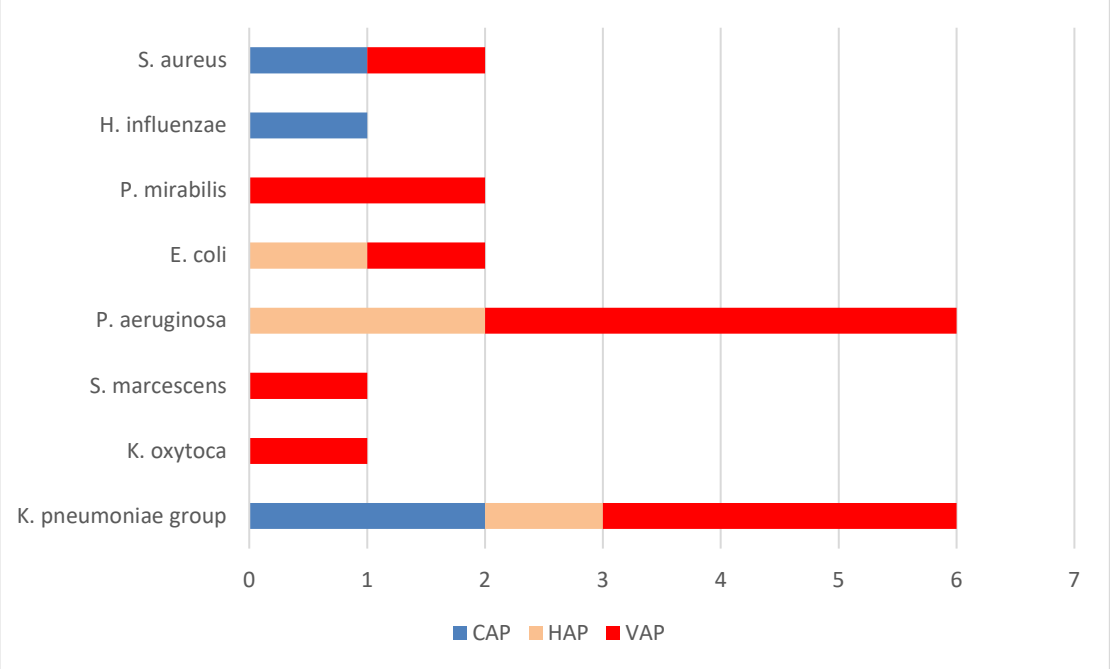


Figure 21: Frequency of bacterial detections in patients with COVID-19

3.4.5 Pathogen detection in relation to preceding duration of antibiotic therapy

175 patients had both lower respiratory tract bacterial culture and a FA pneumonia test performed during their illness. Samples were only taken prior to starting antibiotics in 4/175 (2%) patients tested by FA, versus to 39/175 (22%) of those tested by culture.

The percentage of samples positive for pathogens was greatest for both culture and molecular detection before starting antibiotics. The association between duration of antibiotics prior to sampling and rate of test positivity was significant for molecular testing (p=0.004) but not for

culture (p=0.28), Table 24. The difference in proportional positivity for molecular detection over culture was only significant between 12 and 24 hours of preceding antibiotics.

Table 24: Proportion of microbiological investigations which identified a bacterial cause relative to preceding duration of antibiotics

Duration antibiotics before sampling	**FA positive (n=175)**	**Culture positive (n=175)**	**Difference (95% CI)**	**P value**
Nil	4/4 (100)	20/39 (51)	49% (0 to 65)	0.12
<12 hours	19/26 (73)	13/29 (45)	28% (0 to 51)	0.055
12-24 hours	42/59 (71)	14/36 (39)	32% (10 to 51)	0.003
>24 hours	45/86 (52)	29/71 (41)	12% (0 to 27)	0.20

Data are n/n (%). Excludes FA detections for *S. agalactiae* and *L. pneumophilia* as these would not be reported in culture.

66 'typical' bacterial targets were detected by Filmarray testing in patients who had a paired LRT sample cultured. The proportion of bacterial detected targets which were grown was 2/3 (67%) if the samples were taken before antibiotics, 4/8 (50%) if the samples were taken in the first 12 hours of antibiotics, 14/36 (39%) if the samples were taken 12-24 hours after starting antibiotics and 8/19 (42%) if they were taken >24 hours after starting antibiotics, the trend was not significant p=0.49 (Figure 22).

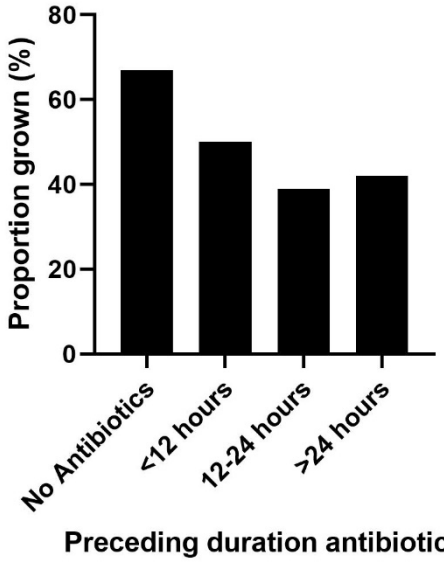


Figure 22: Proportion of pathogens detected by molecular methods which also grew by culture, according to preceding duration of antibiotics

3.5 Diagnostic performance of Pneumonia panel

3.5.1 Diagnostic yield compared with pooled culture

175 patients had LRT microbiological culture and phenotypic sensitivity testing, and a FA pneumonia test. 110/175 (63%) patients had their FA sample taken at a different time to the sample taken for culture testing. The median difference in time for request was -12 hours from study sample collection (IQR -25 to 3 hours). 90/110 (82%) had an LRT sample obtained for culture by the same method (BAL, ETA etc) as those obtained for Filmarray testing.

When considering only on-panel targets, the Filmarray pneumonia panel result was fully concordant, defined as finding exactly the same organism(s) as the culture result, in 97/175 (55%). The FA result was the same as the culture result but molecular testing identified at least one additional bacterial pathogen in 71/175 (41%). The FA result was discordant, defined as not detecting a bacterial pathogen which grew in culture, in 7/175 (4%), Figure 23. The discordant pathogens were *K. pneumoniae* group (3), *E.coli*, *P. aeruginosa*, *K. oxytoca* and *S. aureus*.

100 additional typical bacteria were detected by the Filmarray and not detected by culture in 71 patients. The most frequent additional detections were *H. influenzae* (23), *S. aureus* (15) and *E. coli* (14) (Table 40, appendix A). 30/100 (30%) of these detections were at are 10^4 copies/ml level of detection, 39/100 (39%) at 10^5 copies/ml, 19/100 (19%) at 10^6 copies/ml and 12/100 (12%) at $\geq 10^7$ copies/ml.

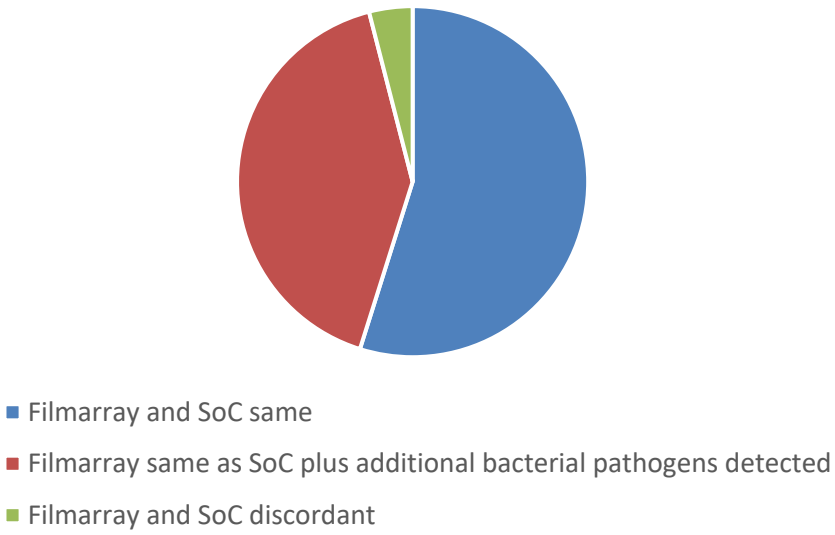


Figure 23: Bacterial identification using Filmarray molecular testing in comparison to culture

Total pooled diagnostic performance data for these patients are presented in Table 25. The total pooled PPA was 90.9% (95% CI 82.4-95.5) and NPA was 96.1% (95% CI 95.2-96.7).

Table 25: Diagnostic performance of FA pneumonia panel for on panel targets compared to culture (all)

Organism	Filmarray +		Filmarray -		PPA (%) (95%CI)	NPA (%) (95%CI)
	Culture +	Culture -	Culture +	Culture -		
A. baumanii clx.	0	0	0	174		100 (97.8-100)
E. cloacae clx	0	4	0	170		97.7 (94.2-99.1)
E. coli	8	13	1	152	88.9 (56.5-99.4)	92.1 (87-95.3)
H. influenzae	4	25	0	145	100 (51-100)	85.3 (79.2-89.8)
K. aerogenes	1	4	0	169	100 (51-100)	97.7 (94.2-99.1)
K. oxytoca	3	6	1	164	75 (30-98.7)	96.5 (92.5-98.4)
K. pneumoniae gp.	9	5	3	157	75 (46.8-91.1)	96.9 (93-98.7)
M. catarrhalis	0	5	0	169		97.1 (93.5-98.8)
P. mirabilis	4	1	0	169	100 (51-100)	99.4 (96.7-100)
P. aeruginosa	24	2	1	147	96 (80.5-100)	98.7 (95.2-100)
S. marcescens	4	6	0	164	100 (51-100)	96.5 (92.5-98.4)
S. aureus	11	15	1	147	91.7 (64.6-99.6)	90.7 (85.3-94.3)
S. agalactiae	0	7	0	167		96 (91.9-98)
S. pneumoniae	2	6	0	166	100 (17.7-100)	96.5 (92.6-98.4)
S. pyogenes	0	1	0	173		99.4 (96.8-100)
Overall	70	100	7	2433	90.9 (82.4-95.5)	96.1 (95.2-96.7)

PPA – positive percent agreement, NPA – Negative percent agreement. Clx, complex. Gp, group.

3.5.2 Diagnostic accuracy compared with paired culture

65/175 patients had paired samples (i.e. exactly the same sample tested). One patient was excluded as they had inadequate standard culture. In this patient specific organisms were not identified, and the culture result was reported as mixed coliforms only, so it is not possible to be certain how this correlated with the coliforms detected by the Filmarrray. As a result, 64 patient samples were analysed. When considering only on-panel targets, the Filmarray pneumonia panel

was fully concordant with culture in 37/64 (59%), concordant with culture but detected at least one additional pathogen in 25/64 (39%) and discordant in 2/64 (3%). There were 40 additional bacterial detections. The most frequently occurring additional detections were *S. aureus* (8), *E. coli* (6) and *H. influenzae* (5) (Table 41, appendix A). 16/40 (40%) of these detections were at 10^4 copies/ml, 16/40 (40%) at 10^5 copies/ml, 6/40 (15%) at 10^6 copies/ml and 2/40 (5%) at $\geq 10^7$ copies/ml. Diagnostic performance data is presented for these patients in Table 26. The total pooled PPA for all pathogens was 93.3% (95% CI 78.7-98.8) and NPA 95.4% (95% CI 93.8-96.6).

Table 26: Diagnostic performance of FA pneumonia panel for on panel targets compared to culture (paired samples only)

Organism	**Filmarray +**		**Filmarray -**		**PPA (%) (95%CI)**	**NPA (%) (95%CI)**
	Culture +	**Culture -**	**Culture +**	**Culture -**		
A. baumanii clx.	0	0	0	64		100 (94.3-100)
E. cloacae clx.	0	3	0	61		95.3 (87.1-98.7)
E. coli	5	6	1	52	83.3 (43.7-99.1)	89.7 (79.2-95.2)
H. influenzae	2	5	0	57	100 (17.8-100)	91.9 (82.5-97)
K. aerogenes	0	2	0	62		96.9 (89.3-99.4)
K. oxytoca	1	3	0	60	100 (5.1-100)	95.2 (87-98.7)
K. pneumoniae gp.	2	0	0	62	100 (17.8-100)	100 (94.2-100)
M. catarrhalis	0	2	0	62		96.9 (89.3-99.4)
P. mirabilis	2	1	0	61	100 (17.8-100)	98.4 91.4-100)
P. aeruginosa	12	3	1	48	92.3 (66.7-99.6)	94.1 (84-98.4)
S. marcescens	0	3	0	61		95.3 (87.1-98.7)
S. aureus	4	8	0	52	100 (51-100)	86.7 (75.8-93.1)
S. agalactiae	0	2	0	62		96.9 (89.3-99.4)
S. pneumoniae	0	2	0	62		96.9 (89.3-99.4)
S. pyogenes	0	0	0	64		100 (94.3-100)
Overall	**28**	**40**	**2**	**826**	93.3 (78.7-98.8)	95.4 (93.8-96.6)

NPA – Negative percent agreement, PPA – positive percent agreement. Clx, complex. Gp, group.

3.5.3 'Off panel' pathogens

5 bacterial species which were deemed significant pathogens that are not on the Filmarray pneumonia panel were detected by culture in 6/200 (3%) of patients (Table 27). The lack of detection of these did not lead to any negative change in treatment that could negatively impact outcome. Seven other organisms with pathogenic potential were also grown but were deemed colonising flora in the context of co-growth with clear pathogens (Appendix A, Table 42).

Table 27: Off panel pathogens identified in the SARIPOC trial

	Frequency of identification	
Pathogen	**mPOCT arm n=3**	**Control arm n=3**
Serratia ureilytica	1	0
Citrobacter koseri	1	1
Citrobacter freundii	1	0
Haemophilus parainfluenzae	0	1
Stenotrophomonas maltophilia	0	1

3.5.4 Quantitative diagnostic performance of typical bacteria compared with culture

66 organisms were grown or detected in 42/64 samples tested in parallel by FA pneumonia panel and culture. Of these, two were cultured and not detected by the FA, and 38 were detected by FA but not cultured. When scanty growth was reported, 3 of 11 (27%) FA detections were semi quantified in the scanty range (defined as either 10^4 or 10^5 copies/ml). When moderate growth was reported, 2 of 3 (67%) of samples were semi-quantified by FA in the moderate range (defined as 10^5 or 10^6 copies/ml). When heavy growth was reported, 12 of 14 (86%) of samples were semi-quantified in the heavy range (defined as $\geq 10^6$ copies/ml), Table 28. This is presented for individual organisms in Appendix A, Table 43.

Table 28: Quantification of FA pneumonia panel compared with culture

Organism culture concentration	Filmarray pneumonia bin value, copies/ml					Concordance
	Not detected n=2	10^4 n=17	10^5 n=17	10^6 n=18	≥10^7 n=12	
No growth		16	13	7	2	x
Scanty	1	1	2	5	2	27% (3/11)
Moderate	1			2		67% (2/3)
Heavy			2	4	8	86% (12/14)

3.5.5 BAL-like samples compared to sputum-like samples

197 samples were included in the study, the vast majority were endotracheal aspirates (125) and expectorated sputa (57). Only 15 samples were invasive 'BAL' like samples. Both sputum like samples and BAL-like samples had a median single target detected, with no difference observed between groups (p=0.78).

112 of 182 (62%) of sputum like samples were positive for any organism, compared to 11 of 15 (73%) of BAL like samples (difference 11, 95% CI 0 to 31, p=0.42). When considering only positive tests, there was no significant difference in the proportion that were positive for multiple organisms, when comparing sputum-like and BAL-like samples (39% vs 27% respectively, 95% CI 0 to 47, p=0.53).

Table 29: Number of different bacterial targets detected by sample type

	Number of bacterial targets detected					
	None	One	Two	Three	Four	Five
'Sputum like' (n=182)	70 (38)	68 (37)	29 (16)	10 (5)	4 (2)	1 (1)
ETA (n=125)	42 (34)	51 (41)	21 (17)	8 (6)	2 (2)	1 (1)
Sputum (n=57)	28 (49)	17 (30)	8 (14)	2 (4)	2 (4)	0 (0)
'BAL like' (n=15)	4 (27)	8 (53)	3 (20)	0 (0)	0 (0)	0 (0)
Undirected BAL (n=8)	4 (50)	2 (25)	2 (25)	0 (0)	0 (0)	0 (0)
BAL (n=7)	0 (0)	6 (86)	1 (14)	0 (0)	0 (0)	0 (0)

Data are n(%) ETA = endotracheal aspirate, BAL = bronchoalveolar lavage

3.5.6 Resistance genes

Resistance genes were detected in 6/198 (3%) of samples, Table 30. Only one of these samples was also directly tested by culture. This detected a *bla*$_{CTX-M}$ on the FA, but the organism did not

exhibit an ESβL phenotype on phenotypic sensitivity testing. Of the remaining five, four (80%) had non-paired, culture-based investigations during their admission and 2/4 (50%) demonstrated phenotypic sensitivity testing that was consistent with the resistance gene detected by the FA.

Table 30: Resistance genes detected by FA pneumonia panel

Gene	Study arm	Organisms detected by FA (bin value, copies/ml)	Was sample paired	Phenotypic result on Culture result	Clinical impact of FA identification
bla$_{CTX-M}$	Control	*K. pneumoniae* 10^7 (*S. aureus* 10^4 also present)	Unpaired	*K. pneumoniae* with ESβL phenotype cultured on prior sample	N/A
bla$_{CTX-M}$	Control	*P. aeruginosa* 10^5	N/A	No sample tested by culture	N/A
bla$_{CTX-M}$	Control	*E. coli* 10^7	Paired	Heavy *E.coli* growth without ESβL phenotype	N/A
mecA/C	Control	*S. aureus* 10^7 (*S. agalactiae* also present)	Unpaired	*S. aureus* with MSSA phenotype cultured	N/A
bla$_{IMP}$	mPOCT	*P. aeruginosa* 10^4 and *S. marcescens* 10^6	Unpaired	Fully sensitive *P. aeruginosa*	Antimicrobial therapy unchanged as felt to be clinically improving. Patient isolated
mecA/C	mPOCT	*S. aureus* 10^6 (*S. agalactiae* 10^5, *K. oxytoca* 10^4 and *P. aeruginosa* 10^4 also present)	Unpaired	*P. aeruginosa* and *Achromobacter xylosoxidans*. MRSA cultured subsequently.	Antimicrobial therapy unchanged as felt to be clinically improving. Patient isolated

mPOCT, molecular point of care testing. MRSA, methicillin resistant *Staphylococcus aureus.*

3.5.7 'Atypical' bacteria

Only one atypical organism was detected in the study: *Legionella pneumophilia* (serogroup one). The patient had a positive urinary antigen test result, *L. pneumophilia* was detected on the Filmarray pneumonia panel, and also grown on extended (prolonged) culture.

3.5.8 Additional identification of respiratory viruses as a result of syndromic testing

14 respiratory virus detections occurred in 14/100 (14%) patients in the mPOCT arm. The most frequently occurring viral detections were SARS-CoV-2 (6), rhinovirus/ enterovirus (4) and non-SARS-CoV-2 coronaviruses (2), Table 14. In the standard of care arm, 10 respiratory viruses were detected in 10/100 (10%) of patients. The most frequently detected viruses were SARS-CoV-2 (7), rhinovirus/enterovirus (2) and RSV (1).

On retrospective Filmarray testing of control samples, 'missed' respiratory viruses (those which were not identified prospectively by the clinical team) were identified in 7/100 patients (7%). These were rhinovirus/ enterovirus (4), human metapneumovirus (1), seasonal coronavirus (1) and parainfluenza virus (1).

3.6 Additional exploratory non-protocol outcomes

3.6.1 Impact of the SARS-CoV-2 pandemic on viral detections in critical care

14 respiratory viruses were detected by the Filmarray in the 62 patients (23%) recruited before the start of the COVID-19 pandemic, and 3 in 136 (2%) patients recruited afterwards, (difference -20, 95% CI -10 to -33, p<0.0001, Table 31).

Table 31: Impact of emergence of SARS-CoV-2 on other circulating respiratory viruses

Virus	**Recruitment period 1[*] (n=62)**	**Recruitment period 2[†] (n=136)**	**Difference (95% CI)**	**p value**
Human Rhinovirus/ enterovirus	7 (11)	3 (2)	9 (1-20)	0.012
Seasonal human Coronavirus[‡]	3 (5)	0 (0)	5 (0-14)	0.030
Parainfluenza viruses	2 (3)	0 (0)	3 (0-12)	0.10
RSV	2 (3)	0 (0)	3 (0-12)	0.10
Human metapneumovirus	1 (2)	0 (0)	2 (0-10)	0.31
Any non-SARS-CoV respiratory virus	14[§] (23)	3 (2)	20 (10-33)	<0.0001

Data are n(%). [*]Recruitment period 1: July 2019- March 2020 (pre- 1st SARS-CoV-2 national lockdown). [†]Recruitment period 2: 3rd July 2020- 5th May 2021. [‡]Includes HKU-1, 229-E, NL63, OC43 [§]One co-detection of seasonal coronavirus and RSV, i.e. 17 patients positive throughout study

3.6.2 Association between *S. pneumoniae* growth in blood and sputum, urinary antigen positivity, and molecular detection of *S. pneumoniae*.

No cases of invasive pneumococcal disease were identified in the study (i.e. no blood cultures or cultures of other sterile sites were positive for *S. pneumoniae*). 191 patients had *S. pneumoniae* urinary antigen testing (UAT), of which 2/191 (1%) were positive. Neither of these cases grew or detected *S. pneumoniae* in LRT samples by culture or molecular testing.

163 patients had both culture and UAT. 5/163 (3%) patients cultured *S. pneumoniae* which were all negative by UAT. 190 patients had FA testing and UAT. 11/190 (6%) of patients had *S. pneumoniae* identified by molecular testing and none of these were UAT positive.

Chapter 4 The SARIPOC trial discussion

4.1 Overview

Our study is the first to our knowledge to report on the clinical impact of molecular testing for pneumonia pathogens in patients with pneumonia in critical care, and the first to report on the use of such molecular panels at the point-of-care. It demonstrates that mPOCT led to the identification of a causative pathogen much more rapidly and in a greater proportion of patients than with current standard diagnostic testing. This was associated with more patients receiving antimicrobial treatment directed by a microbiological result, on average almost 2 days earlier than standard diagnostic testing.

4.2 Directing antimicrobial use

Studies evaluating the clinical impact of early appropriate antibiotic therapy have consistently reported that inadequate therapy is associated with higher mortality[195], and therefore avoiding this is a priority in pneumonia management. mPOCT led to more appropriate, microbiologically directed, escalations in antimicrobial therapy, on average 1 day earlier, than with standard diagnostic tests. Most antibiotic escalations involved changing narrower broad-spectrum agents (such as co-amoxiclav) to agents with even broader activity (such as piperacillin-tazobactam or carbapenems) on the basis of organism detection by mPOCT.

Expert consensus states that antibiotic de-escalation is desirable as it reduces drug-related side effects, costs and the development of antibiotic resistance[121]. mPOCT led to more de-escalations compared to standard diagnostic testing, and these took place on average 40 hours earlier. These antibiotic changes were mainly either changing a board spectrum anti-pseudomonal beta-lactam to a less broad-spectrum agent (e.g. changing piperacillin-tazobactam to co-amoxiclav), stopping additional antimicrobial agents (e.g. a macrolide used as a second agent) or stopping all antibiotic treatment completely (when ongoing clinical suspicion of pneumonia was deemed to be low).

Adjustment in empirical antimicrobial therapy occurred infrequently in the control group with standard diagnostic testing. Microbial sampling in pneumonia is recommended by guidelines in hospitalised patients to avoid undertreatment of resistant organisms and to facilitate de-escalation of inappropriately broad antimicrobial therapy[121] however our results suggest that the results of standard diagnostic testing do not achieve this aim, as they are typically available too late in the clinical course of pneumonia to influence antimicrobial prescribing decisions. On the rare occasion where antibiotic escalation or de-escalations occurred in the control group this was

generally before standard diagnostic test results were available and were made on the basis of clinical parameters.

The trial intervention did not demonstrate a reduction in the overall duration of ineffective antibiotic therapy. The lack of difference was due to a small number patients who had 'off panel' organisms grown (pathogenic organism that were not tested for by the molecular panel), or molecular detection of an organism with a phenotypic sensitivity which was harder to predict (and later found to be resistant), particularly Gram-negative isolates and especially *Pseudomonas aeruginosa*. We gave pragmatic advice in the event of detection of such organisms, which may reflect colonisation rather than infection in ICU patients[61], to escalate aggressively if the patient was deteriorating, or wait for phenotypic results if the patient remained clinically stable. It is our experience that this is how sputum culture results are interpreted in critical care in current practice. Molecular detection of resistance markers was rare, and as a result, had little impact on patient management. Growth of 'off panel' pathogens was also very unusual, most notably *Citrobacter* species and *Stenotrophomonas maltophilia*. The lack of detection of these organisms by the pneumonia panel did not adversely affect the patient's antimicrobial treatment in our trial, as none of these patients were de-escalated based on mPOCT from effective to ineffective therapy.

4.3 Safety

The use of mPOCT in this trial appeared to be safe, with no significant difference in any clinical outcomes including time in ICU, time on organ support, and mortality. There were no differences in drug-related adverse events or cases of *C. difficile*.

Patients in the mPOCT arm had a longer duration of additional oxygen support compared to controls, although the absolute proportion of patients who required oxygen support was the same. It does not seem biologically plausible that better directed antibiotics (more escalated and de-escalated therapies) would lead to additional requirement for oxygen in a subset of patients. Our initial explanation for this finding was the higher proportion of patients with underlying respiratory disease or a diagnosis of VAP in the mPOCT arm, two conditions which are associated with additional need for oxygen therapy. However a post-hoc regression analysis was performed to explore if the association remained after adjusting for VAP diagnosis or underlying respiratory disease which did not support this theory (regression co-efficient in adjusted model 5.7 (95% CI 2.5, 8.9, p=0.001). Another potential explanation for this finding is random chance, especially given the number of pre-defined secondary outcomes. If this genuinely reflected worse outcomes

as a result of tailored antimicrobial therapy, the results would be expected to be similar for multiple organ support modalities and mortality, which they are not.

4.4 Conclusions for key outcomes

Our study demonstrates that mPOCT for pneumonia pathogens in patients with pneumonia located in critical care units, led to the generation of actionable results much more rapidly and detected more pathogens compared with standard diagnostic testing. Embedding mPOCT within a stewardship intervention was associated with improvements in antimicrobial use including more patient receiving results-directed therapy and less frequent, unnecessary use of broad-spectrum antibiotic agents. If the findings of this trial are confirmed in larger multicentre trials and in other health care settings, including areas with higher rates of antimicrobial resistance, mPOCT for pneumonia pathogens should become the standard of care.

4.5 Study strengths

Other stewardship studies on ICU have highlighted that the addition of a highly sensitive test does not necessarily translate into improved prescribing, as clinicians override prescribing guidance[150]. A major strength of our study was that the stewardship intervention was not just a molecular test in isolation, but also included prescribing advice which considered clinical indices, molecular results, patient history and procalcitonin levels. The utility of procalcitonin has been extensively studied in lower respiratory tract infections in critical care[147,196] and in our experience was most helpful when levels were low , in the context of absent molecular detection, to support decisions to de-escalate or stop antibiotics. It is notable that initial PCT levels did not affect the impact of mPOCT on the primary outcome measure in our multivariate analysis. Other strengths of this study include that it was run over two and half years, both before and after the emergence of SARS-CoV-2, in a tertiary referral centre, making it highly generalisable to other similar UK and international centres in the post-pandemic era. Recruitment from three distinct critical care areas, all of whom have different responsible clinician groups with varying antibiotic prescribing practices increases the generalisability.

4.6 Study weaknesses

Our study has the weakness of being a single centre study, and one that was not powered specifically to detect differences in clinical outcomes. As a result of the nature of the study, we were unable to blind participants or clinical teams to the intervention.

A limitation to interpreting the impact of mPOCT specifically was the multi-faceted nature of the intervention in the trial. The mPOCT arm had four different interventions: PCT, molecular testing, pneumococcal urinary antigens and stewardship advice. The extremely low positive rate for urinary antigen testing (which also occurred in the control arm) was of little benefit, however it was difficult to assess the extent to which the other interventions influenced prescribing outcomes.

PCT testing became available as part of routine clinical care around April 2020, so was widely used in the standard care arm for the majority of the patients. As a result, the influence of including PCT as part of the stewardship intervention on outcomes would likely be small. Notably recruitment PCT did not impact the association between mPOCT and results directed therapy in the statistical regression model.

The impact of the stewardship advice on the effect of the intervention overall was particularly hard to unpick as this was highly dependent on the relationship and understanding between the advice provider and the clinician. A limitation of the study was that stewardship advice was not delivered in the same manner between both arms: in the intervention arm, any result prompted an immediate discussion, whereas in the control arm the patients were discussed at a pre-defined time (an afternoon stewardship ward round).

4.7 Exploratory outcomes

4.7.1 Association between pathogen quantification and clinical outcome measures

The relationship between molecular quantification of detected pathogens and clinical outcomes is currently unknown. Rand et al reported a correlation between bin values and sputum white cell count (as measured by direct microscopy using Gram's stain and Wright's stain for polymorphonuclear leukocytes (PMN)), and also with peak body temperature on the day of culture [187,197]. No significant associations were observed for clinical outcomes although there was a trend for an association between quantification and ICU length of stay and mechanical ventilation. The findings of these studies were not published when we designed our study, and we did not collect data on serial temperature measurements or Gram stain results to allow us to test these associations.

Our findings did not support an association between pathogen quantification and recruitment serum procalcitonin, or most clinical outcome measures including length of critical care stay, or need for mechanical ventilation or non-invasive ventilation. A signal was seen for higher 30 day all-cause mortality with rising levels of pathogen quantification. Our findings are limited by small

sample size, and several major confounders including the variety of different diagnoses (HAP, CAP and VAP), different sampling methods (i.e. ETA vs BAL), and the fact some participants received treatment interventions as part of the trial. The apparent relationship between pathogen abundance and 30-day mortality does warrant further investigation in larger prospective studies with appropriate adjustment for potential confounding factors.

4.7.2 Utility of serum procalcitonin in differentiating between bacterial infection from non-bacterial infection and prediction of clinical outcome

PCT is currently used in clinical practice to distinguish between bacterial and non-bacterial causes of pneumonia and to identify those in whom antibiotics can be safely withheld. The clinical impact and cost-effectiveness of this practice remains uncertain in most clinical settings. No optimum PCT threshold has been determined and validated. The most commonly used threshold in acute respiratory infection of 0.1ng/ml was reported by Self et al to have a sensitivity of 81% and a specificity of 46% for the presence of a bacterial pathogen[198] by conventional diagnostic testing. The utility of PCT in predicting molecular positivity is currently unknown. One other trial has previously combined PCT with multiplex PCR testing in non-pneumonic lower respiratory tract infection, however this did not include typical bacterial targets, instead utilising the Filmarray Respiratory Panel which tests for viruses and atypical bacteria only[199]. A meta-analysis of studies using PCT in addition to clinical criteria to diagnose HAP or VAP (according to the IDSA definition rather than culture positivity) reported an area under the summary receiver operating characteristic curve of 0.76[121].

We report that recruitment PCT appears to be a poor test to predict the molecular detection of bacterial pathogens at any threshold in the context of CAP, or all pneumonia on critical care (including HAP, CAP and VAP). Our study does not support its use in isolation for this purpose. The AUROC was greatest for predicting the highest levels (>10^7 copies/ml) of bacteria in patients with CAP, albeit improving the test performance only very modestly. An actively recruiting multicentre French RCT is using the FA pneumonia panel and PCT with guidance to withhold antibiotics if PCT is <1ng/ml and there is no molecular detection of bacteria[200]. There is no justification in the trial protocol for the choice of this cut-off. Their primary outcome is the number of days alive without any antibiotic from randomisation to day 28.

We report poor accuracy in predicting all cause 30-day mortality at any cut off. Similar to our findings, Scheutz et al reported an AUC of 0.6 for day 1 PCT predicting 30-day mortality[201]. Unlike their group however, we did not find additional benefit to measurement on subsequent days. The

impact of increasing the threshold for molecular detection above 10^5 copies/ml had only modest impact on the performance of PCT for prognostication, and still represented a poor test.

Of interest, 30-day mortality was consistent across all PCT concentrations when no bacterial pathogens were detected by the Filmarray but appeared to increase with greater concentrations when a bacterial pathogen was present. Our results are limited by the small sample size, with diminishing availability of serum samples as the study progressed as a result of patient discharge and inadequate resource to collect samples at weekends. There were several major confounders including the variety of different diagnoses (HAP, CAP and VAP), different sampling methods (i.e. ETA vs BAL), the potential for additional molecular detection of colonising flora at low levels, and the fact some participants received treatment interventions as part of the trial which may have directly affected these outcomes.

4.7.3 Pathogen detection in pneumonia occurring following macro-aspiration

The microbiology of aspiration pneumonia has long been considered to reflect that of CAP or HAP/VAP depending on the exposure of the patient to the healthcare setting (see 1.5.1). For the first time, to our knowledge, we report the microbiology of CAP and HAP/VAP aspiration pneumonia using molecular diagnostics. We found that in patients with prior macro-aspiration Enterobacteriaceae were predominantly detected, with *H. influenzae* and *S. aureus* also frequently detected. Marin-Corral et al recently compared the microbiology of CAP in patients with aspiration (termed ACAP) with those who had not aspirated. They divided the non-aspiration group into those with risk factors for aspiration (RF+) and those without (RF-). Those with severe CAP who had aspirated had higher rates of total Gram negative bacteria compared to those who had not aspirated, with or without risk factors (64.3% VS 44.3% VS 33.3%, respectively, P=0.021)[202]. This is in agreement with our finding of high levels of detection of Enterobacteriaceae in community acquired cases. The relatively frequent detection of *S. aureus* and *H. influenzae* reflects the findings of other studies using culture based methods[33]. Mier et al evaluated patients in a very similar cohort of patients to our study who were in critical care early in their disease course sampled using protected brush specimen. They reported the most frequently occurring organisms were *S. aureus*, Enterobacteriaceae, *H. influenzae* and *S. pnuemoniae*[203]: all of which were frequently detected in our study.

The finding of *P. aeruginosa* in community acquired aspiration is not typical[33], however three of four patients who had *P. aeruginosa* did have pre-existing respiratory disease which may pre-dispose them to persistent colonisation with this organism[204].

Long term proton pump inhibitor therapy has been theorised to be associated with an increased risk of pneumonia by increasing the gastric pH and hence allowing potential pathogenic bacteria to proliferate in the GI tract[33,205]. We observed higher rates of long-term PPI use in the aspiration group, however these patients were at greatest risk of confounding comorbidities that would increase their risk of aspiration.

Our results have several major limitations. Historically anaerobes were considered a major pathogen of aspiration pneumonia, although this is no longer the case, the Filmarray pneumonia panel does not detect anaerobes. No anaerobes were cultured during the study. The non-uniform method of sampling employed by the study (ETA, expectorated sputa and BAL) may identify more colonising bacteria than using an invasive method (for example just BAL). Additionally, the duration of time from aspiration to sampling was not uniform, and typically in excess of 48 hours, by which time the microbial landscape may have changed significantly under the influence of antibiotics. Lastly, the definition of macro-aspiration was a pragmatic one, albeit adjudicated by one investigator (SP).

4.7.4 Pathogen detection in patients with SARS-CoV-2 infection

Autopsy studies from previous influenza pandemics suggest that secondary bacterial infection was a major driver of mortality, being very frequently present in fatal cases[94]. The high mortality and lack of comprehensive rapid diagnostics mean that most patients admitted to hospital with COVID-19 receive antibiotics. The number of cases observed worldwide is now well in excess of 240,000,000 worldwide which equates to a massive antimicrobial consumption with subsequent impact on AMR. The extent to which SARS-CoV-2 is associated with secondary bacterial infection is not well known. A meta-analysis of 24 studies (using culture) identified a bacterial pathogen on admission in 3.5% of patients, and 14.3% subsequently developed a secondary bacterial infection[190]. A review of post-mortem studies of COVID-19 found histopathological changes consistent with potential superinfection in 32% of patients (200/621), but an organism was only recovered by culture in 8% (51/621)[206].

The use of a rapid, culture independent methods to rule out bacterial infection is therefore desirable for patients with COVID-19. We report that bacterial co-infection was uncommon in community acquired cases admitted to ICU when tested using the Filmarray pneumonia panel, and that the rate of detection was lower than for non-SARS-CoV-2 CAP. Several other groups have reported using the Filmarray pneumonia panel for secondary infections in COVID-19 in observational studies (mainly in secondary VAP diagnoses)[207,208], however these have typically reported only on which organisms were detected, rather than overall frequency. A meta-analysis

by Timbrook et al reported that culture identified a co-infecting bacteria in 18% of patients compared to 33% by molecular testing with the Filmarray pneumonia panel[209]. Our findings are limited by the small sample size, and proportionally greater numbers of BAL samples in the COVID-19 group, which is known to be related to detection of fewer organisms. Certainly, this finding warrants further investigation in larger prospective studies.

4.7.5 Pathogen detection in relation to preceding duration of antibiotic therapy

It is already well established that bacterial culture yield declines following the administration of antibiotics[192]. The rate of decline of molecular bacterial yield according to preceding duration of antibiotics has not been studied, although is theorised to be less than with culture as a result of the test detecting specific genome sequences rather than complete, viable, organisms.

We have shown from crude positivity rates in our trial data that the rate of molecular positivity decreased over time following the administration of antibiotics, within the first 24 hours. We did not observe a significant decrease in culture positivity, which would be expected based on previous studies[192], although this may reflect our relatively small sample size. We have shown that there is a significantly higher yield of pathogen detection with molecular testing compared to culture and the greatest difference was seen when patients had been exposed to between 12 and 24 hours of antibiotics. This supports the theory that the superior diagnostic yield of molecular tests is less influenced by preceding antibiotic therapy than culture. Additionally, in paired samples, the proportion of pathogens detected by molecular testing which were also cultured did appear to decline with increasing antibiotic exposure, although was not statistically significant, likely on account of inadequate sample size.

As with many of the exploratory analyses, the interpretation of these data are limited by many factors, not least the small sample size of patients tested, particularly prior to starting antibiotics. The effect of antibiotics can be highly variable, some patients may have been on ineffective therapy so effectively had no preceding treatment. Furthermore, the mechanism of action of antibiotic classes vary, some causing rapid cell death (for example beta-lactams) and others working more slowly by inhibiting bacterial replication (for example quinolones). Different organisms can also be more or less susceptible to antibiotics: for example *P. aeruginosa* readily forms biofilms making therapy more difficult. Future studies assessing the rate of decline of culture compared to molecular tests could avoid many of these pitfalls in their design. Ideally, a prospective observational study would collect samples from patients at regular intervals by the same method: before starting antibiotics, then 6 hours, 12 hours and 24 hours afterwards. These

would be tested by culture and Filmarray pneumonia panel in parallel, and rate of decline of positivity compared between both for specific organisms with the same therapy.

4.8 Diagnostic performance of the Filmarray Pneumonia panel

The study was not specifically designed to evaluate the diagnostic accuracy of the FA pneumonia panel: this has already been established by others in order to obtain regulatory approval for the test[159], and subsequently also in real world studies including that published by Murphy et al[175]. The diagnostic performance of the Filmarray pneumonia panel in comparison to sputum culture in our study was consistent with that reported in previously published diagnostic accuracy studies[175,176] and demonstrated excellent sensitivity. The test platform also appeared to be reliable and there were no run failures. As reported by others using molecular panels in pneumonia, pathogens were detected in a much higher proportion of patients compared with those tested using conventional diagnostic methods[8,176].

4.8.1 Typical bacteria

Filmarray molecular testing identified many more organisms than culture, with additional targets detected in around 40% of patients. The most frequently detected such organisms were *H. influenzae*, *S. aureus* and *E. coli*. These three organisms accounted for 50% of the additional detections. The clinical significance of these findings is unclear as they are all potential colonisers of the respiratory tract and also common causes of pneumonia[49,53]. 80% of additional detections in the direct comparison of molecular testing and culture, were present at a molecular quantity of 10^5 copies/ml or less, suggesting that the abundance of these organisms may recently have fallen below the threshold at which they would have cultured.

This could reflect the more fastidious nature of some organisms (for example *H. influenzae*), or the impact of recent antibiotic therapy which did impact culture positivity in our trial. We witnessed several cases that appeared to reflect the impact of antibiotics, for example the case described in Figure 24 below, where the Filmarray detected the presence of organism previously identified in culture but which no longer cultured.

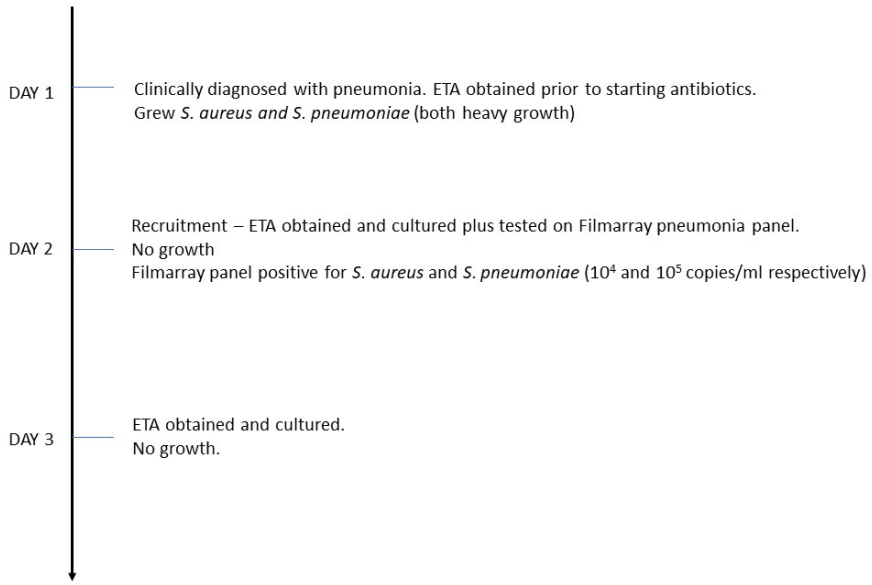


Figure 24: Case study of molecular positivity reflecting culture results prior to antibiotic therapy

The Filmarray pneumonia panel had a pooled PPA of 93.3% and NPA of 95.4% demonstrating the excellent performance in comparison to culture. The only two 'missed' on-panel targets (those which were on the Filmarray panel but not detected, in spite of a positive culture result) were one moderate growth of *P. aeruginosa* and one scanty growth of *E.coli*. Large data sets have not suggested an issue with detection of these targets[175,176], and the confidence intervals are wide on account of our relatively small sample size. Previous studies suggest this is a rare event, occurring as a result of levels of genome at or below the threshold for Filmarray positivity.[175] The pooled negative predictive value was 99.8%, highlighting its potential utility as a 'rule out test' for on-panel targets.

In formal laboratory quantification studies, the FA pneumonia bin values are consistent and highly accurate when compared with other quantitative molecular methods[175]. The relationship with culture quantification is less clear, and several groups have reported that the pneumonia panel consistently has higher bin values for molecular copy number than culture quantification values (CFU)[176,188,189]. All of these have noted concordance is especially low at lower culture concentrations (3%-42% below 10^6 CFUs/'few' colonies) and increases with organism abundance (90-100% at above 10^6 CFUs/ 'many' colonies). This is consistent with our experience, in paired samples at low concentration the concordance was 27%, rising to 86% at higher concentrations (Table 28). Discrepancy testing using other molecular assays in the above studies demonstrates that this difference is largely explained by the additional sensitivity of the Filmarray assay compared to culture, with organisms typically being present below the threshold for culture positivity in the lower bin values[175].

Molecular results appear to be reproducible, and when evaluating the proportional concordance of culture and molecular detection, the PPA and NPA are consistent when comparing all tested samples, and not just those tested in parallel.

One of the challenges to interpreting any lower respiratory tract microbiological result is the possibility of contamination at the point of sampling, particularly when the respiratory tract is home to multiple commensals on the Filmarray panel. We did not observe a significant difference in the number of targets identified by different sampling methods which has been noted in other large reports[175,176]. This result is limited by a very small invasive sample size, and by the fact these statistics comparing number of targets identified in different patients. The ideal study would compare the relative yield by sampling from the same patient, using two different methods (i.e. first ETA, then BAL) in a larger data set, ideally with a histopathological reference standard.

The 2016 IDSA/ATS guidelines for HAP and VAP collated studies which examined the performance characteristics of different sampling types compared to a histopathological reference standard[121]. The pooled sensitivity of ETA with any amount of growth was 75% (95% CI 58-88) and specificity 46% (95%CI 29-65), compared to quantitative BAL which had a sensitivity of 57% (95% CI 47-66) and a specificity of 80% (95% CI 71-88). It seems reasonable to assume that the greater sensitivity of molecular testing would improve this sensitivity further, possibly with an associated reduction in specificity. Our prescribing strategy in the event of multiple detections was to ensure cover for all organisms detected, therefore we are more likely to have covered the main pathogen as a result of the use of the Filmarray pneumonia panel.

4.8.2 Resistance genes

The Filmarray pneumonia panel has primers for genetic markers of resistance, so diagnostic accuracy studies should compare detection with a reference standard test which looks for these specifically, rather than crude antibiotic phenotypic sensitivity (as resistance can occur by many different mechanisms). It is a separate question of how detection of genetic resistance markers corresponded with phenotypic sensitivity. Only a very small number of resistance genes were detected in the study, and most of these were in samples that did not have standard of care culture (in the control arm) and so comparison was not possible.

4.8.2.1 Methicillin resistant *Staphylococcus aureus*

Penicillin resistance in *S. aureus* is almost always accounted for by the presence of a *mecA* gene, so molecular determination of resistance is theoretically reliable[210]. Two cases detected the presence of *mecA/mecC* and *MREJ* genes with a *S. aureus* detection, and neither of them had

parallel culture performed. One of these was extremely likely to have had MRSA present on their sample as it was frequently isolated in other respiratory samples around the same time. As a result of the molecular detection of the Filmarray, the patient was placed in isolation earlier than they would otherwise have been. The other case was never isolated and had only Methicillin sensitive *S. aureus* detected in their respiratory secretions by culture both before and after the FA sample was taken. It therefore seems feasible that this may not have represented a true MRSA infection. Of interest, this patient isolated *Staphylococcus epidermidis* in a catheter specimen urine from the same day as recruitment, suggesting they are colonised with this organism. These isolates are frequently carriers of *mecA* genes[211]. The Filmarray attempts to discriminate the origin of the *mecA* and *mecC* gene by requiring the additional presence of *MREJ,* the SCC*mec* right-extremity junction which links the genetic element carrying these resistance genes to the *S. aureus* genome (therefore is only present in *S. aureus*). It is possible, but unusual, that the Filmarray could detect *mecA* from the *S. epidermidis*, whilst also detecting an empty *MREJ* cassette on MSSA, and therefore represent a false positive. In a large multicentre validation, Murphy et al reported 19 'false positives' (as compared to molecular testing direct from specimen, rather than culture) in 320 samples. 18/19 were confirmed as genuine positives on discrepancy analysis using a separate molecular test[175]. A major multicentre validation study comparing FA detection to culture reported 4.5% (24/531) of *S. aureus* samples which were phenotypically MSSA were reported as MRSA by FA testing[176].

4.8.2.2 Extended spectrum beta-lactamase (CTX-M)

Three $bla_{\text{CTX-M}}$ genes were detected. One detection was undoubtably of clinical significance (in the control arm and therefore tested retrospectively) and the patient subsequently cultured a phenotypically ESβL producing *K. pneumoniae*. This patient was on an antibiotic that would not have been effective, and so if the Filmarray result had been known in real time it could have resulted in a change to appropriate antimicrobial therapy earlier than occurred following culture results.

A second $bla_{\text{CTX-M}}$ case was also detected in the control arm and did not have culture performed, and so the significance of this detection is unclear, however the patient improved on piperacillin/tazobactam which would not have activity against this resistance mechanism.

The final detection $bla_{\text{CTX-M}}$ was tested in parallel with culture, where *E. coli* was detected at $>10^7$ copies/ml. In culture there was heavy growth of *E.coli* but with a non-ESβL phenotype[212]. Unfortunately, there was inadequate sample remaining for discrepancy analysis, although the patient achieved symptomatic cure on piperacillin-tazobactam which would be hydrolysed by ESβLs. Early trials of accuracy using the FA pneumonia panel found $bla_{\text{CTX-M}}$ as one of the most

commonly occurring resistance mechanisms detected in clinical samples, albeit still relatively infrequently. Only one false positive was observed in 425 positive samples, and this was not detected by an independent molecular test[175], suggesting false positives are an infrequent occurrence. A potential explanation for this could be the presence of another organism with $bla_{CTX\text{-}M}$ below the $10^{3.5}$ limit of detection of the Filmarray.

4.8.2.3 Carbapenemase producing enterobacteriaeceae

Only a single CPE gene was detected during the study period which raised significant challenges. The Filmarray detected bla_{IMP} with *S. marcescens* at 10^6 copies/ml and *P. aeruginosa* at 10^4 copies/ ml. Culture sent the day prior to recruitment later grew a fully sensitive *P. aeruginosa* (which did not have a bla_{IMP} detected on FA). The patient was clinically improving on a beta-lactam, and had never previously had a CPE, although they were at risk of more resistant organisms by merit of a diagnosis of motor neurone disease causing respiratory impairment. Our pragmatic decision, in tandem with the infection control team, was to isolate and test the patient as per our CPE transfer policy (with at least 2 rectal swabs at least 24 hours apart looking for carriage) and continue the same antibiotic. Ultimately, the patient was discharged before the screening swabs were complete (negative). He was immensely frustrated by the inconvenience of his wife having to wear full PPE when visiting him daily, and being restricted in his movement around the ward, during a difficult hospital admission. bla_{IMP} detection is very rare in other large reported data sets[175,176], and validation data for accuracy submitted as part of the FDA approval process was largely on contrived, spiked samples[213]. This case highlights another issue with molecular testing: was the resistance gene on the *S. marcescens* genome, the P. aeruginosa genome, or another organism present below the limit of detection. If it is the latter of these, what is the clinical significance?

Taken together, the molecular detection of resistance genes did not clearly correlate with phenotypic resistance patterns, although there was inadequate detection of resistance markers to draw strong conclusions. There is a potential benefit to patients of increased detection of these markers of resistance, but this must be weighed against the cost of increased detection of uncertain significance.

4.8.3 'Off panel' organisms

We diagnosed only a small number of 'off-panel' pathogens, and the intervention did not lead to negative changes in antibiotics for any of these patients. 3/6 'off-panel' pathogens were in the intervention arm. These were *Citrobacter freundii, Citrobacter koseri* and *Serratia ureilytica*. The patients with *C. koseri* and *S. ureilytica* were on antimicrobial therapy that would have been

effective against them at recruitment. Both had coliforms detected by the Filmarray and remained on antibiotics that would have activity against the 'off panel' pathogen. The patient with *C. freundii* was on cefuroxime and metronidazole at recruitment which would have been ineffective. The Filmarray detected only a *S. aureus* but the same antimicrobials were continued on the basis that the patient had been unwell, had a PCT of >2 but was clinically improving. Antibiotics were escalated when the culture result became available. As a result, the trial did not negatively or positively impact this patient.

Of the 'off panel' organisms detected, it was notable that two would have been resistant for standard empirical therapies: *C. freundii* and *Stenotrophomonas maltophilia.* This would ultimately have meant that if prescribing practice had followed local guidelines, antibiotic de-escalations in these patients on the basis of lack of FA detection would not be detrimental to the patient compared to standard of care, as they were changing between ineffective therapies. These results reinforce the need to interpret all results in the correct clinical context. The diversity of bacterial pathogens and resistance mechanisms mean that no molecular panel could ever be completely comprehensive, and the FA pneumonia panel is no exception. As a result, culture is still an essential diagnostic tool.

4.8.4 Atypical bacteria and viruses

Only one atypical bacterium was identified during the trial period: a case of *L. pneumophilia* which was positive on the Filmarray, urinary antigen testing and extended culture. Authors have suggested that the rates of atypical infection may be under-diagnosed on account of the limitations of standard diagnostics[86], however we have not found evidence for this in our cohort. The additional sensitivity for the Filmarray in detecting atypical organisms facilitated early cessation of macrolide therapy in the study.

From a respiratory virus perspective, the recruitment period for the study was unusual with a very mild influenza season in 2019/20 and the advent of SARS-CoV-2. We originally envisaged key secondary outcomes would include isolation facility use in the context of influenza and RSV, however by March 2020, every patient was being treated as potentially infectious with heightened precautions. We consider the impact of the national lockdown and social distancing on existing seasonal respiratory virus detection in 4.9.1. Even factoring in the decline of seasonal respiratory viruses, syndromic testing using the Filmarray identified 'missed' respiratory virus infections in 7% of control arm patients. This highlights the benefit of syndromic testing: identifying infectious cases early so they are unable to transmit to other patients and healthcare providers.

4.9 Additional exploratory non-protocol outcomes

4.9.1 Impact of the SARS-CoV-2 pandemic on viral detections in critical care

In separate studies of adults admitted to the acute medical unit of Southampton General Hospital, we identified a decline in the detection of other respiratory viruses following the emergence of SARS-CoV-2 from an average of 54% to 4% in the first wave of the COVID-19 pandemic[97]. We have clearly demonstrated that this decline was carried through into patients in critical care, where the absolute rate of detection fell by 20% after March 2020. This was driven by a large drop in human rhinoviruses. The reason for this decline is likely to be due to reduced community and in-hospital transmission: extensive social distancing requirements introduced nationally to tackle the spread of SARS-CoV-2 by governments across the world led to marked decline in other highly transmissible diseases like measles[214], and are highly likely to have impaired transmission of other respiratory viruses.

4.9.2 Association between *S. pneumoniae* growth in blood and sputum, urinary antigen positivity, and molecular detection of *S. pneumoniae*.

There was no apparent relationship between pneumococcal urinary antigen testing and lower respiratory tract test positivity (including both culture or molecular tests) in our study. A systematic review by Sinclair et al of 12 studies of accuracy of the Binax now test compared to a composite of blood culture, sputum culture and pleural culture estimated the sensitivity at 68.5% (95% CI 62.2-74.2)[107]. There were insufficient positive cases in our study to calculate measures of diagnostic accuracy, however this would appear to be an over-estimate compared to what we observed. The explanation for this difference may be due to the antibiotics our patients had received before recruitment (median around 24 hours), and also that many of the studies in the paper exclusively looked at CAP. Molecular testing for *S. pneumoniae* had a PPA of 100% and NPA of 96% compared to culture.

Chapter 5 Clinical impact of molecular point-of-care testing for suspected COVID-19 in hospital (COV-19POC): a prospective, interventional, non-randomised, controlled study

5.1 Introduction

The management of suspected SARS-CoV-2-associated respiratory disease (COVID-19) is severely hampered by the long turnaround times associated with centralised laboratory PCR testing, which can take several days to generate results. In acute hospitals this leads to poor patient flow through clinical areas, as suspected patients are cohorted in assessment areas until their results are available. In addition, lack of single occupancy rooms means that COVID-19 negative patients in these assessment areas may acquire infection from positive patients before results are available. Hospital acquired infection is a hallmark metric for quality of care in hospitals and NHS data suggests that large proportions of COVID-19 cases diagnosed in hospital during the first wave were acquired nosocomially[215,216].

Rapid, accurate diagnostics that can be performed in admission areas are therefore urgently required. In previous work we have shown that the routine use of molecular point-of-care testing (POCT) for influenza and other respiratory viruses is associated with improvements in antiviral use and infection control measures, and that this impact is dependent on very short turnaround times, not achievable in centralised laboratories[178,217]. Several rapid molecular platforms that can test for SARS-CoV-2 at the point-of-care have now been developed and are likely to reduce time to results, but evidence for their clinical impact and real-world diagnostic accuracy are lacking[218]. The aim of this trial was to assess the clinical impact and real-world diagnostic accuracy of POCT using the QIAstat-Dx Respiratory SARS-CoV-2 Panel, in adults presenting with suspected COVID-19, during the first wave of the pandemic.

5.2 Methods

5.2.1 Study design and participants

We performed a single centre, prospective, interventional, non-randomised, trial with a contemporaneous control group, in secondary care. The study design was selected as a

randomised trial was felt likely to be unacceptable to many patients in the context of a pandemic due to an organism of unknown lethality at the time. The trial took place during the first wave of the pandemic, from 20th March to 29th April 2020. All patients were recruited from the Acute Medical Unit (AMU), Emergency Department (ED) or other acute areas of Southampton General Hospital, a large acute teaching hospital in the South of the UK serving a population of 650,000 for secondary care, run by University Hospital Southampton Foundation NHS Trust (UHSFT), who were the sponsor for the trial. The study was approved by the South Central - Hampshire A Research Ethics Committee: REC reference 20/SC/0138, on the 16th March 2020. The protocol is available at:

https://eprints.soton.ac.uk/439309/2/CoV_19POC_Protocol_v2_0_eprints.pdf

The protocol was amended a single time, changing the control group from a pre-implementation control group to a contemporaneous control group. This was due to recognition that the majority of patients tested for COVID-19 prior to the trial commencing were ambulatory community patients who were tested in hospital as part of the containment phase of the pandemic, and therefore not comparable to the patients presenting with acute respiratory illness who were recruited into the interventional arm of the trial.

5.2.2 Intervention group

Eligible patients were: aged 18 years or over; had the capacity to give informed, written consent or where capacity was lacking consultee assent was obtained; a provisional decision had been made to be admitted to hospital; located in either the AMU, ED or other acute areas; could be recruited within 24 hours of presentation; had an acute respiratory illness (ARI) or did not have ARI but were a suspected case of COVID-19 according to the current PHE case definition. An episode of acute respiratory illness was defined as a provisional diagnosis of acute pulmonary illness including pneumonia, bronchitis (non-pneumonic lower respiratory tract infection - NPLRTI) and influenza-like illness (ILI), or an acute exacerbation of a chronic respiratory illness (including exacerbation of COPD, asthma or bronchiectasis). Exclusion criteria were: patient declined nasal/pharyngeal swabbing, or patient previously included in the study and re-presenting within 14 days after previous enrolment. There was originally provision in the protocol for symptomatic members of hospital staff to be recruited however this was abandoned after only a single member of staff was enrolled (see below).

5.2.3 Procedures

Prior to recruitment starting on the 20th March, a brief validation phase took place where the QIAstat-Dx Respiratory SARS-CoV-2 Panel was evaluated using control material, under biosafety level 2 conditions within a class 2 medical safety cabinet, as per PHE guidance. The panel received CE marking on the 18th March. Patients were recruited from 20th March to 29th April by research staff, from 8am until 6pm, 7 days a week. Following obtaining informed consent, combined nose (mid-turbinate) and throat swabs were obtained from patients by research staff and placed directly into Sigma Molecular Medium to rapidly inactivate viruses. Samples were then tested on the QIAstat-Dx platform using the Respiratory SARS-CoV-2 Panel, in a dedicated testing hub located in the AMU, following local risk assessment and approval. In addition, laboratory PCR testing for SARS-CoV-2 on an additional combined nose and throat swab (collected contemporaneously) was performed on all patients, in the on-site Public Health England (PHE) microbiology laboratory. Initially laboratory PCR testing used the PHE RdRp gene assay alone and subsequently used the PHE RdRp and E gene assays combined. COVID-19 positive status was defined as PCR positivity for SARS-CoV-2 on either assay. Demographic and clinical data was collected at enrolment and outcome data collected retrospectively from case note and electronic systems. The data management systems ALEA and BC platforms were used for data capture and management.

5.2.4 The QIAstat-Dx respiratory SARS-CoV-2 Panel

The QIAstat-Dx panel was chosen for the study largely because it was the first 'sample to answer' platform offering molecular testing for SARS-CoV-2 with POCT potential to get FDA approval and CE marking. The analyser (Figure 26) uses multiplexed real-time PCR (RT-PCR) to detect 22 clinically important respiratory viruses and atypical bacteria. The targets are Influenza A, Influenza B, seasonal coronaviruses (229E, HKU1, NL63, OC43), SARS-CoV-2, Parainfluenza viruses (1-4), RSV, hMPV, Adenovirus, Rhinovirus/enterovirus, M. pneumoniae, C. pneumoniae and B. pertussis.

Figure 25: The QIAstat-Dx Respiratory panel test cartridge

Figure 26: The QIAstatDx analysers

5.2.4.1 How the panel works

The nasopharyngeal swab is dipped into molecular medium. 300µl of this mixture is moved using sterile, individually packaged pipettes into the test cartridge port (indicated by red star on Figure 25). After inserting the sample, it enters the lysis chamber of the cartridge where it is mixed with dried MS2 bacteriophage: a single stranded mRNA virus, which acts as the reaction internal control. This mixture is homogenised and lysed by the action of a high-speed rotor within the device. The lysate is moved through to the purification chamber by pneumatic pressure where nucleic acids are extracted by binding to a silica membrane. The purified nucleic acids are moved into the dried chemistry chamber, where PCR reagents including DNA polymerase are added. This master mix is moved into the eight PCR chambers at the bottom of the pouch which contain

target-specific primers and probes. One chamber contains the single plex internal control. Four chambers have four-plex reactions (i.e. four sets of distinct target primers and probes with four fluorescent dyes) which occur simultaneously. Two chambers have triplex reactions. The final chamber is a single-plex dual assay for SARS-CoV-2. This has primers for two gene targets, the Orf1b and the E gene, but only uses one dye, so reports fluorescence of the two together. Unlike the Filmarray, the Qiastat reports cycle threshold values (cT) as fluorescence is measured in real time.

5.2.5 Control group

A contemporaneous control group of patients was identified, consisting of adults ≥18 years old presenting to hospital with ARI and/or suspected COVID-19 to the ED or AMU, during the same time period as the study (20th March to 29th April 2020). These patients were eligible for inclusion into the study but were not enrolled due to the limited capacity of the research team as we had insufficient research staff to recruit all patients with suspected COVID during the day and did not have resources to deploy research teams overnight, and were tested only by laboratory PCR. Control patients were not consented and routinely obtained fully de-identified data including demographic, clinical and outcome data collected retrospectively from hospital systems after local data protection assessment and approval.

5.2.6 Outcomes

The primary outcome measure was the time to results, defined as time from COVID-19 testing being requested (for the POCT group this was the time of recruitment and for control patients the time laboratory testing was requested) to the result being available to clinical teams. Secondary outcomes included: time from admission to arrival in a definitive ward area (i.e. COVID-19 positive or negative ward), total number of bed moves before reaching definitive clinical area, proportion of COVID-19 positive patients enrolled into other clinical trials, time from admission to enrolment in other clinical trials, duration of hospitalisation, proportion of patients treated with antibiotics, proportion of patients with intensive care unit (ICU) admission, in-hospital and 30 day mortality, sensitivity, specificity, positive predicted value, negative predictive value, and overall diagnostic accuracy of QIAstat-Dx SARS-CoV-2 assay, and reliability of the QIAstat-Dx system (proportion of tests with run failures). All outcomes were measured for the duration of hospitalisation or up to 30 days (whichever is shortest) unless otherwise specified.

5.2.7 Sample size

The sample size of 500 patients in the POCT arm was chosen pragmatically based on the availability of the QIAstatDx Respiratory SARS-CoV-2 Panel test kits. The control arm consisted of all contemporaneously identified patients who presented in the same time period as the intervention and fulfilled the inclusion criteria in the same admission pathways. It was anticipated that the number included in the control arm would be similar, based on the time periods for recruiting to POCT and the proportion of potentially eligible patients who were recruited. These numbers were thought sufficient to provide power for comparisons between arms, and to estimate the diagnostic accuracy with acceptable precision. Although not formalised in the study design, this sample size corresponds to more than 90% power for a hazard ratio of 1.25 for turnaround time (equivalent to decreasing median time to results from 24 hours to less than 20 hours, or increasing the percentage of those with results within 24 hours from 50 to 58%). The likely prevalence of COVID-19 during the study was highly speculative at the time of study conception, and so a formal sample size calculation for evaluation of diagnostic accuracy was not undertaken. However, a sample size of 500 patients in the POCT arm would have 80% power to give an approximately 90% chance of achieving a 95% confidence interval width no larger than 10% based on sensitivity of 90% and prevalence of 30%.

5.2.8 Statistical analysis

Statistical analysis was performed by a dedicated medical statistician from the University of Southampton Clinical Trials Unit (Dr Sean Ewings), independent from the study team. Analysis was carried out using Prism version 7.0 (GraphPad Software Inc; La Jolla, California), and Stata version 16 (StataCorp, College Station, Texas). The use of multiple imputation was planned should missing data exceed 5% for the primary outcome or for key secondary outcomes but was not needed. Summaries of all baseline characteristics are presented.

Baseline characteristics and outcomes were compared between the groups using chi-square tests for equality of proportions for binary data and using independent-samples t-tests (when presented with means) or Mann-Whitney U test (when presented with medians), as appropriate for continuous data. Time to results and time to definitive ward arrival had no censoring. For length of stay deaths were right censored at 30 days. Median differences and corresponding confidence intervals were calculated using the Hodges-Lehmann estimate. Enrolment into other COVID-19 studies was only evaluated in COVID-19 positive patients.

For assessment of diagnostic accuracy (POCT group only), measures were calculated based on a composite reference standard of PCR positivity by any assay when confirmed by a second assay.

Therefore, where results were discordant between the POCT and laboratory PCR, further PCR testing was performed using two additional CE-marked SARS-CoV-2 assays (Primerdesign genesig COVID-19 RT-PCR assay and CerTest Viasure SARS-COV-2 RT-PCR) in another regional laboratory with operators blinded to the original results. Results are presented as sensitivity, specificity, likelihood ratios and predictive values. Confidence intervals for sensitivity, specificity and accuracy are 'exact' Clopper-Pearson confidence intervals and for the likelihood ratios are calculated using the 'Log method'.

Further analyses were carried out for the primary outcome (time to results) and key secondary outcomes (time to arrival at a definitive ward). Timing of events are presented graphically using the Kaplan-Meier failure function. In addition, multivariable analysis was carried out, based on a Cox proportional hazards model to adjust for confounding variables in view of the non-randomised nature of the study. Based on a directed acyclic graph, time of presentation (in light of consenting the POCT arm between 8am to 6pm) and severity of disease (based on NEWS2 score), alongside age and sex, were identified as confounding variables to be controlled for, represented using the R package dagitty (appendix B, Figure 30). These variables were identified prior to analysis among the research team, based on scientific rationale and clinical experience.

Confidence intervals for comparison of proportions are based on the Newcombe/Wilson method. Confidence intervals for individual proportions are based on the Wilson/Brown method except for measures of diagnostic accuracy as above.

This study was prospectively registered with the ISRCTN14966673 on the 18th March 2020.

5.3 Results

Between 20th March and 29th April 2020 500 patient-participants were recruited to the POCT arm and 555 contemporaneously tested patients were identified for inclusion into the control group. One participant in the POCT group was excluded as they were a member of staff rather than a patient presenting to ED with suspected COVID-19, Figure 27. The trial period included the upslope, peak and downslope of the first wave of the pandemic in our locality. Table 32 shows baseline characteristics for patients in the groups. Patients in the POCT group had a higher median (IQR) NEWS2 score (5 (3-6) versus 4 (2-6), difference of 1, 95% CI 0 to 1; p=0.041), a higher frequency of requiring supplementary oxygen (135/499 (35%) versus 128/555 (23%), difference of 12%, 95% CI 6 to 17;p<0.0001) and having infiltrates or consolidation on chest X-ray (277/488 (57%) versus 136/507 (27%), difference 30%, 95%CI 24 to 36; p<0.0001).

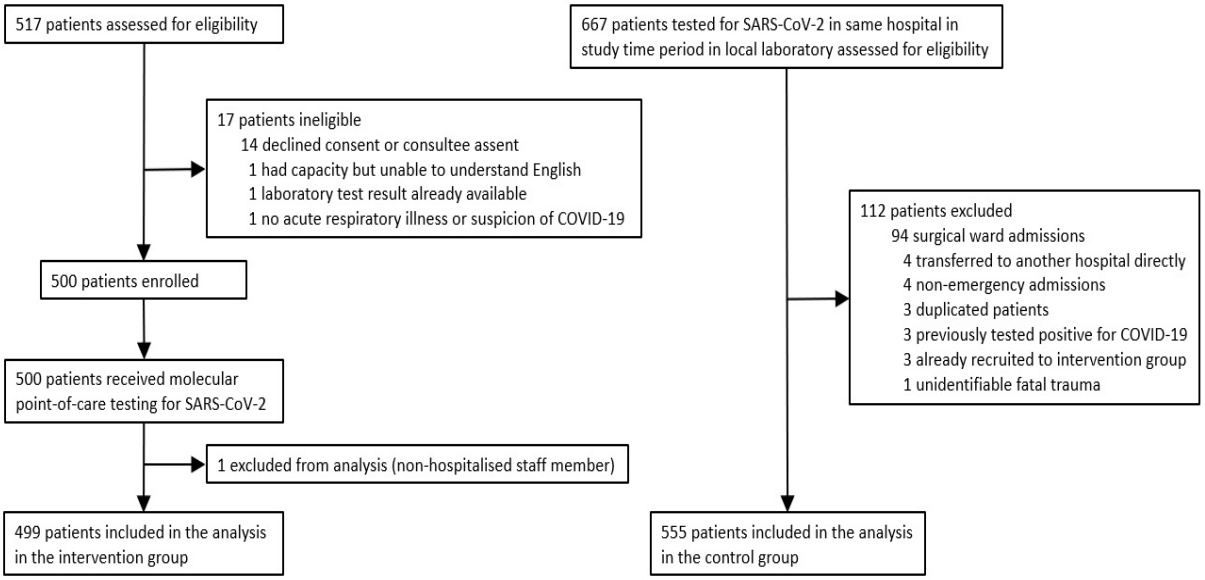


Figure 27: CoV-19 POC Trial design

Table 32: Baseline characteristics of patients in the CoV-19 POC trial

	mPOCT n=499	**Control n=555**	**Difference (95%CI)**
Age (years)	68 (51 to 81)	70 (51 to 81)	2 (-2 to 3)
<50	117 (23)	133 (24)	1 (-5 to 6)
50-59	67 (13)	66 (12)	1 (-2 to 6)
60-69	77 (15)	78 (14)	1 (-3 to 6)
70-79	99 (20)	124 (22)	2 (-2 to 7)
≥80	139 (28)	154 (28)	0 (-5 to 5)
Sex			
Male	262 (52)	303 (54)	2 (-4 to 8)
Female	237 (48)	252 (46)	
Ethnicity			
White British	406 (85)	442 (85)	0 (-4 to 4)
White other	19 (4)	23 (4)	0 (-2 to 3)
Black	13 (3)	9 (2)	1 (-1 to 3)
Asian	37 (8)	30 (6)	2 (-1 to 5)
South Asian	14 (3)	18 (3)	0 (-2 to 3)
Other Asian	23 (4)	12 (2)	2 (-1 to 4)
Other	2 (<1)	14 (3)	2 (1 to 4)

	mPOCT, n=499	Control, n=555	Difference (95%CI)
Pregnant			
Yes	4 (1)	5 (1)	0 (-1 to 2)
No	490 (99)	550 (99)	
Duration of symptoms, days	4 (1 to 10)	3 (1 to 7)	1 (0 to 1)
Comorbidity			
Hypertension	175 (37)	247 (45)	8 (2 to 14)
COPD	93 (19)	85 (15)	4 (-1 to 9)
Asthma	84 (18)	95 (17)	1 (-4 to 5)
Renal disease	38 (8)	85 (15)	7 (3 to 11)
Liver disease	24 (5)	43 (8)	3 (-1 to 6)
Diabetes mellitus	108 (22)	135 (24)	2 (-3 to 7)
Cancer	40 (8)	36 (6)	2 (-1 to 5)
Dementia	56 (12)	57 (10)	2 (-2 to 6)
Observations at admission			
Temperature, °C	36.8 (36.4 to 37.6)	36.7 (36.4 to 37.5)	0.1 (0 to 0.2)
Temperature ≥38°C	92 (19)	92 (17)	2 (-3 to 7)
Pulse rate, bpm	95 (82 to 109)	92 (78 to 106)	3 (0 to 5)
Respiratory rate, bpm	24 (20 to 28)	21 (18 to 26)	3 (0 to 2)
Oxygen saturations, %	96 (94 to 98)	96 (94 to 98)	0 (0 to 1)
Supplementary O_2 used	174 (35)	128 (23)	12 (6 to 17)
Systolic blood pressure, mmHg	134 (120 to 150)	133 (119 to 150)	1 (-3 to 4)
NEWS2 score	5 (3 to 6)	4 (2 to 6)	1 (0 to 1)
Laboratory and radiological parameters			
C-reactive protein, mg/L	52 (12 to 125)	55 (12 to 129)	3 (-4 to 6)
White cell count, $x10^9$/L	9.3 (6.8 to 13.2)	9.3 (6.7 to 13.2)	0.0 (-0.5 to 0.7)
Neutrophils, $x10^9$	7.1 (4.6 to 11.1)	7.0 (4.8 to 10.5)	0.1 (-0.5 to 0.6)
Lymphocytes $x10^9$	1.0 (0.7 to 1.6)	1.1 (0.7 to 1.7)	0.1 (-0.1 to 0.1)
CXR performed	488 (98)	507 (91)	7 (4 to 9)
Infiltrates/consolidation on CXR	277 (57)	136 (27)	30 (24 to 36)

Data are n (%) or median (IQR). mPOCT, molecular point-of-care testing. COPD, chronic obstructive pulmonary disease. NEWS2, National early warning score 2. CXR, Chest x-ray.

The turnaround time for laboratory PCR results prior to, and during the trial is shown in the appendix B, Figure 31. Median (IQR) time to results with POCT was 1.7 (1.6 to 1.9) hours versus 21.3 (16.0 to 27.9) hours with laboratory PCR in the control group (difference of 19.6 hours, 95%CI 19.0 to 20.3; p<0.0001, Mann-Whitney U test). Figure 28 shows the time-to-event curve for test results in the groups (Log rank test, p<0.0001). The large difference between groups remained after controlling for age, sex, time of presentation and severity of illness in a Cox proportional hazards regression model (hazard ratio [HR] = 4023, 95%CI 545 to 29696; p<0.0001), appendix B, Table 45.

197 (39.5%) of 500 patients in the POCT group were PCR positive for SARS-CoV-2 compared to 155 of 555 (28.0%) patients in the control group (difference of 11.5%, 95%CI 5.8 to 17.2; p<0.0001). Of those patients admitted to hospital for at least 24 hours, 313 of 428 (73.1%) in the POCT group versus 241 of 421 (57.2%) in the control group were transferred to the correct definitive clinical area according to their test results (i.e. COVID-19 positive or negative ward) (difference of 15.9%, 95%CI 9.3 to 22.2; p<0.0001). The median (IQR) time from presentation to arrival in a definitive clinical area was 8.0 (6.0 to 15.0) hours in the POCT group versus 28.8 (23.5 to 38.9) hours in the control group (difference of 20.8 hours, 95%CI 18.4 to 21.2; p<0.0001, Mann-Whitney U test). Figure 29 shows the time to event curve for time to arrival in definitive clinical area (Log rank test, p<0.0001). The hazard ratio for group was 10.2 (95%CI 8.0 to 13.0; p<0.0001) in favour of the POCT arm arriving at a definitive clinical area earlier, based on a Cox proportional hazards model controlling for age, sex, time of presentation and severity of illness (appendix B, Table 45). Further details of transfers to definitive ward areas are given in appendix, Figure 32.

The mean (SD) total number of bed moves from admission before definitive ward arrival was 0.9 (0.5) in the POCT versus 1.4 (0.7) in the control group (difference of 0.5, 95%CI 0.4 to 0.6; p<0.0001). 43 of 313 (13.7%) patients in the POCT group were transferred directly from ED to a definitive ward area without going to an assessment area, compared to 0 of 241 (0%) in the control group (difference of 13.7%, 95%CI 10.0 to 18.0; p<0.0001). 124 of 197 (62.9%) COVID-19 positive patients were recruited into other COVID-19 clinical trials in the POCT group versus 104 of 155 (67.1%) in the control group (difference of 4.2, 95%CI -5.9 to 14.0; p=0.42). Median time to enrolment into trials was 1.0 (1.0 to 3.0) days in the POCT versus 3.0 (2.0 to 4.5) days in the control group, (difference of 2.0 days, 95%CI 1.0 to 2.0; p<0.0001), Table 33. There was more antibiotic use, a longer length of stay, and a higher ICU admission rate in the POCT group compared to the control group, Table 34.

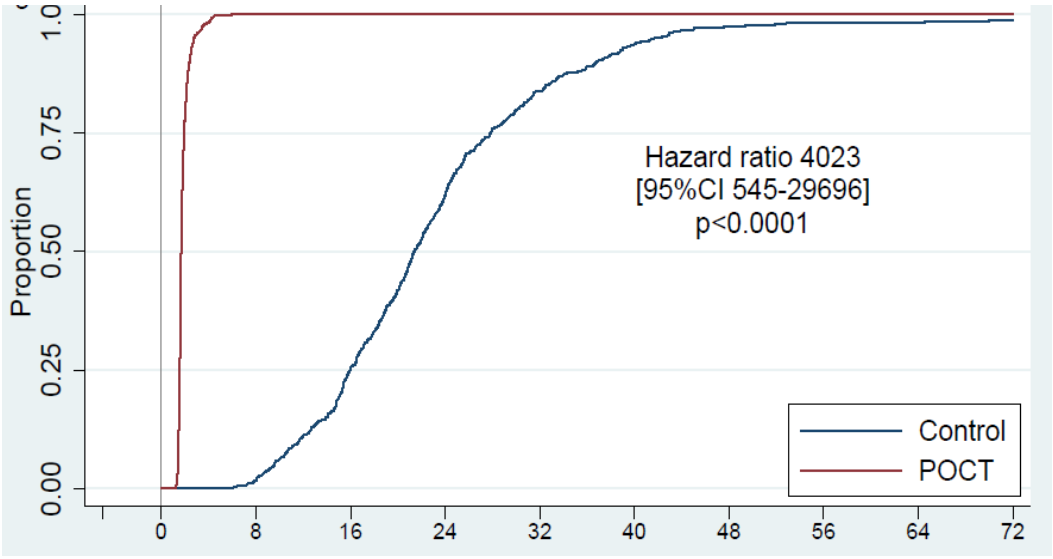


Figure 28: Time to event: time to results

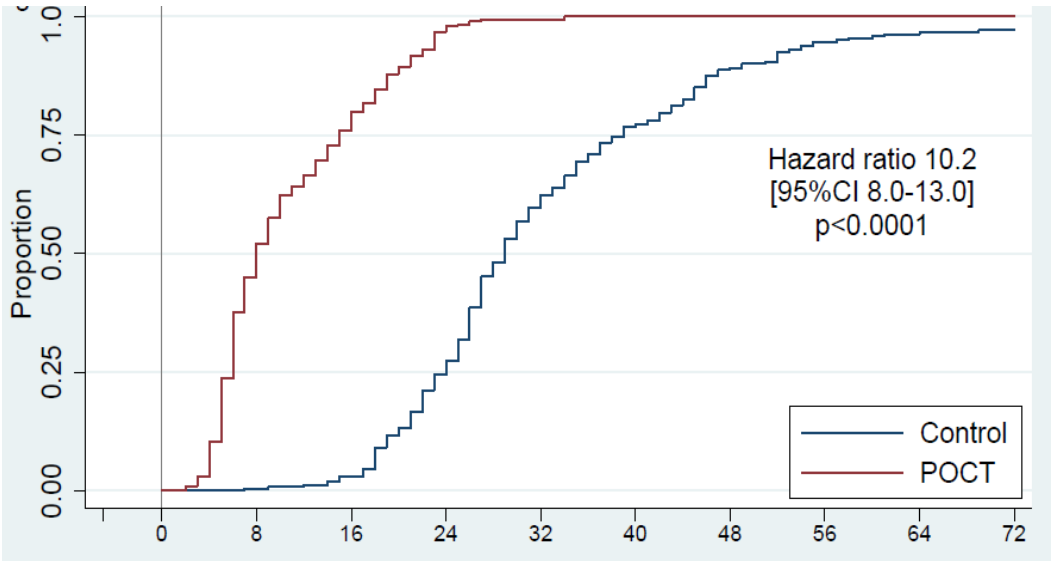


Figure 29: Time to event: time to definitive ward

Table 33: Primary and key secondary outcome measures

	mPOCT n=499	**Control n=555**	**Difference (95%CI)**	**p value**
Time to results (hours)	1.7 (1.6 to 1.9)	21.3 (16.0 to 27.9)	19.6 (19.0 to 20.3)	<0.0001
COVID-19 (SARS-CoV-2) positive	197 (39.5)	155 (28.0)	11.5 (5.8 to 17.2)	0.0001
Admitted for >24 hours	428 (85.8)	421 (75.8)	10.0 (5.0 to 14.7)	<0.0001
Transferred from assessment (cohort) area to definitive ward*	313 (73.1)	241 (57.2)	15.9 (9.3 to 22.2)	<0.0001
Time from admission to definitive ward arrival, hours	8.0 (6 to 15)	28.8 (24 to 39)	20.8 (18.4 to 21.2)	<0.0001
Number of bed moves once admitted (mean)	0.9 (0.5)	1.4 (0.7)	0.5 (0.4 to 0.6)	<0.0001
Bed moves				
0	43 (13.7)	0 (0)		
1	244 (77.9)	163 (67.6)		
2	26 (8.3)	56 (23.2)		
3	0 (0)	12 (5.0)		
4	0 (0)	4 (1.7)		
5	0 (0)	1 (0.4)		
COVID-19 positive patients enrolled into other COVID-19 trials†	124 (62.9)	104 (67.1)	4.2 (-5.9 to 14.0)	0.42
Time from admission to enrolment into other COVID-19 trials (days)	1.0 (1.0 to 3.0)	3.0 (2.0 to 4.5)	2.0 (1.0 to 2.0)	<0.0001

Data are n (%) or median (IQR) expect where stated otherwise. mPOCT, molecular point-of-care testing. CI, confidence interval. *n=428 in mPOCT arm and 421 in control arm. †n=197 in mPOCT arm and 155 in control arm.

Table 34: Additional secondary outcome measures

	mPOCT n=499	**Control n=555**	**Difference (95%CI)**	**p value**
Antibiotics used	418/496 (84.3)	387 (69.7)	14.6 (9.5 to 19.5)	<0.0001
Length of stay, days	5.1 (2.0 to 9.2)	4.2 (1.2 to 9.6)	0.9 (0 to 1.0)	0.017
ICU admission	64 (12.8)	42 (7.6)	5.2 (0.2 to 8.9)	0.004
In hospital mortality	67/494 (13.5)	69 (12.4)	1.1 (-2.9 to 5.2	0.58
30-day mortality	80/440 (18.2)	86 (15.5)	2.6 (-2.0 to 7.3)	0.26

Data are n (%) or median (IQR) expect where stated otherwise. mPOCT, molecular point-of-care testing. CI, confidence interval. ICU, intensive care unit.

5.3.1 Diagnostic accuracy

In the POCT group 24 patients did not have laboratory PCR performed and 6 samples were unavailable for discrepancy analysis, so a total of 469 were evaluated for diagnostic accuracy. The sensitivity of the QIAstat-Dx Respiratory SARS-CoV-2 Panel for detection of SARS-CoV-2 was 176/177 (99.4%, 95%CI 96.9 to 100) and specificity was 288/292 (98.6%, 95% CI 96.5 to 99.6) compared to the composite reference standard of detection by any PCR assay and confirmed by a second assay. The overall sensitivity of the laboratory PCR during the trial was 152/177 (85.9%, 95%CI 79.9 to 90.7) and specificity was 289/292 (98.9%, 95%CI 97.0 to 99.8). During the first 7 days of the study the sensitivity of the laboratory PHE RdRp assay was found to be very poor compared to QIAstat-Dx; 15/24 (62.5%, 95% CI 40.6 to 81.2). This assay was then optimised and a second gene target added (E gene, with detection of either gene target being considered positive) subsequently improving the sensitivity to 137/153 (89.5%, 95%CI 83.6 to 93.9) measured over the remainder of the study. Measures of diagnostic accuracy are given in table 35. 29 of 499 (5.8%) patients in the POCT group had other respiratory pathogens detected by the panel (appendix B, table 46). Due to reagent shortages PCR for other respiratory viruses was not performed in the control group. Overall there were 26 of 499 (5.2%) initial run failures on the QIAstat-Dx.

Table 35: Diagnostic accuracy measures for the QIAstat-Dx SARS-CoV-2 assay and Laboratory PCR in the POCT group, n=469

	QIAstat-Dx SARS-CoV-2 assay		**Laboratory PCR**	
	n/n	**% (95%CI)**	**n/n**	**% (95% CI)**
Prevalence	177/469	37.7 (33.3 to 42.3)	177/469	37.7 (33.3 to 42.3)
Sensitivity	176/177	99.4 (96.9 to 100)	152/177	85.9 (79.9 to 90.7)
Specificity	288/292	98.6 (96.5 to 99.6)	289/292	98.9 (97.0 to 99.8)
Positive Likelihood ratio	-	72.6 (27.4 to 192.1)	-	83.6 (27.1 to 258.1)
Negative Likelihood ratio	-	0.01 (0.0 to 0.04)	-	0.14 (0.1 to 0.21)
Positive predictive value	176/180	97.8 (94.3 to 99.2)	152/155	98.1 (94.3 to 99.4)
Negative predictive value	292/293	99.7 (97.6 to 99.9)	289/314	92.1 (88.9 to 94.3)
Overall accuracy	464/469	98.9 (97.5 to 99.7)	441/469	94.0 (91.5 to 96.0)

CI, confidence interval

5.4 Discussion

The long delays associated with centralised laboratory PCR testing are recognised as a major challenge for hospitals in effectively responding to the COVID-19 pandemic and mitigation strategies are urgently required in preparation for a likely second wave this winter. To our knowledge this is the first study to assess the clinical impact of molecular POCT for COVID-19 for acute admissions and demonstrates that routine use of POCT can deliver rapid, accurate, and actionable results to clinical and infection control teams. The use of POCT led to large reductions in time to availability of results compared with laboratory PCR and this was associated with improvements in infection control measures and patient flow, with patients spending around one day less in assessment areas and having fewer bed moves before arriving in definitive COVID-19 positive or negative clinical areas. Less time spent in assessment areas means that non-infected patients would spend less time unknowingly exposed to infected patients and are less likely to acquire nosocomial infection. In addition the rapid identification of COVID-19 patients in assessment area means that health care workers would be less likely to be exposed and infected, as positive patients were rapidly moved to positive areas rather than staying in assessment areas for over 24 hours, where PPE recommendations were less stringent[219]. The fewer number of bed moves in the POCT group equates to a cost and time saving for hospitals as each bed space must

be decontaminated after a patient has vacated it, and cleaning staff are less likely to be exposed to heavily contaminated environments. Some patients tested by POCT received their results whilst still in the ED and were transferred directly to definitive clinical areas, bypassing the assessment cohort wards entirely. It is likely that if an even quicker turnaround time for results could be achieved, all patients could have their results returned whilst still in the ED so that assessment cohort areas would become unnecessary.

COVID-19 patients in the POCT group were recruited 2 days earlier into other clinical trials. Recruitment of COVID-19 patients into trials is an international priority and the early identification of patients for inclusion is vital as antiviral therapies are most likely to be effective when given early in the course of the disease[179]. The utility of routine POCT in facilitating early enrolment into clinical trials has not been fully recognised and should be highlighted. Whilst there were no approved therapeutic agents available during the COV19-POC trial, subsequently both the antiviral agent remdesivir and the corticosteroid dexamethasone have been proven to be efficacious in treating COVID-19 pneumonia patients requiring supplementary oxygen or respiratory support[220,221]. Routine POCT will enable the early identification of COVID-19 patients as they are being admitted to hospital, facilitating rapid directed therapy with these agents in a 'test and treat' paradigm maximising therapeutic benefit.

There are many potential 'use case's for point-of-care testing in addition to testing symptomatic acute admissions to hospital, including elective hospital admissions, primary care patients, hospital staff, care home staff and residents, airport screening, school screening and even population level screening. Due to the lack of availability of suitable POCT platforms for all these use cases, prioritisation must be undertaken and should initially be given to acute admission to hospitals to prevent nosocomially acquired infection.

In this study the diagnostic accuracy of the QIAstat-Dx SARS-CoV-2 assay was found to be high and initiating POCT alongside laboratory PCR alerted us to the poor sensitivity of the nationally recommended PHE RdRp screening assay, early in the course of the first wave, preventing the release of many additional false negative results. Multiple groups across the world have now reported on the insensitivity of the RdRp as a gene target in PCR assays for SARS-CoV-2[222,223]. The findings of this study highlight the shortcoming inherent to instituting PCR assays for a novel virus using a single gene target and without the availability of robust quality assurance systems. Not all POCT platforms that are currently available have been shown to be sufficiently sensitive for use in secondary care where the consequences false negative result may be very serious. POCT platforms with appropriate levels of accuracy must be selected based on the intended use case.

We would also point out that POCT must be undertaken under a robust overarching governance structure that includes all element so of the testing process including pre and post analytics steps.

The detection of other respiratory viruses by the QIAstat-Dx Respiratory SARS CoV-2 Panel was infrequent during this study, presumably due to reduced circulation of viruses resulting from social distancing measures or due to viral interference from SARS-CoV-2. In Europe COVID-19 incidence was low in the summer when this article was written, however a second wave was expected the following winter with concern this may occur in tandem with seasonal epidemics of other viruses including influenza and RSV. Therefore the use of syndromic POCTs that test for SARS-CoV-2 and other viruses was anticipated to be vital for hospitals to rapidly differentiate the cause of acute respiratory illness and manage patients appropriately.

This study has a number of limitations, the most important of which is its non-randomised nature. There were differences between the groups at baseline in terms of their respiratory symptoms and signs and NEWS2 score that are explained by the higher prevalence of COVID-19 in the POCT group. Similarly the longer length of stay and higher rate of antibiotic use and ICU admission in the POCT group are also likely to be explained by this. Patients in the POCT group were recruited during the day by research staff and eligible patients were highlighted initially by clinical staff in the ED. It is likely that patients considered to be at high likelihood of COVID-19 were prioritised for POCT by clinical staff, leading to these differences.

We have attempted to control for bias through the use of multivariable analyses for key outcomes. The multivariable analyses were based on a directed acyclic graph representing the research team's knowledge of variables related to group assignment and time to results or destination, allowing us to identify and control for confounding variables while avoiding spurious association between group and outcome. However, it is possible that other unrecognised confounders may exist that impact the relationship between group and outcome. We believe the plausibility and magnitude of the effect for the outcomes make it highly unlikely that the process of group assignment would significantly alter the conclusions of the study. Whilst the result of this study are compelling we do acknowledge that as a non-randomised study they are not fully definitive and ideally should be confirmed with a randomised trial. This would, however, be difficult to conduct currently in the UK due to the low incidence of COVID-19. In addition there remain uncertainties around the ideal implementation model for POCT in hospitals. There are several different models for deployment including nurse delivered POCT and laboratory technician delivered testing and the most appropriate and cost effective of these will vary between health care institutions.

The same swab could not be used for both the POCT and laboratory testing so a second swab was obtained contemporaneously for laboratory testing and this could have contributed to the differences seen in diagnostic accuracy in terms of swabbing technique. Our estimates of diagnostic accuracy are also complicated by the use of the PHE RdRp assay as our comparator. Due to the poor sensitivity of RdRp we cannot be sure that the QIAstatDx did not generate false negative results that were also not detected by RdRp but would have been detected by a more sensitive assay. In addition several POCT positive samples could not be tested by RdRp as samples were not sent to the laboratory, which could have affected the overall measures of performance. Finally, as this study was performed in symptomatic adults presenting to hospital, the impact of POCT in other patient groups such as children, community dwelling adults and those who are asymptomatic or pauci-symptomatic, is currently unknown.

In summary, routine use of POCT for emergency admissions was associated with a large reduction in time to results and improvements in infection control measures, patient flow and recruitment into other clinical trials, compared with laboratory PCR testing. The QIAstat-Dx SARS-CoV-2 assay had high diagnostic accuracy for the detection of COVID-19. Resources should be urgently made available to support the implementation of appropriate POCT platforms in emergency departments and admission units in hospitals, in preparation for the next phase of the pandemic.

5.5 My contribution to this work

Prior to opening recruitment, I designed and road-tested the electronic case report form, in collaboration with Nicole Vaughan-Spickers (Southampton Clinical informatician). I screened patients, recruited them and collected prospective clinical data. I swabbed and took blood from recruits and then tested nasopharyngeal swabs on the QIAstat analyser.

I led data extraction and analysis: both direct from the ALEA platform, and then in collaboration with Hang Phan and Florina Borca (clinical data scientists) using automated retrospective extraction from hospital clinical IT systems into the BG insight platform. Using the same methods with HP, FB and Tristan Clark I identified the control group and extracted data. I designed and performed the bed-moves analysis. I contributed to the manuscript and was joint first author.

Chapter 6 Conclusions and Future work

6.1 Summary of findings

The overarching theme of this work was assessing the clinical utility of rapid molecular diagnostics in Pneumonia. I participated in two large clinical trials to investigate these impacts from different perspectives, and with slightly different goals. The SARIPOC trial primarily utilised rapid molecular diagnostics to improve antimicrobial stewardship, whereas the CoV-19 POC study used rapid molecular diagnostics to reduce in-hospital transmission of SARS-CoV-2. Our work has shown that molecular point-of-care testing for pneumonia is reliable and feasible in routine practice with substantial clinical impact.

The SARIPOC trial (Chapter 2, 3 and Chapter 4) recruited 200 adults with pneumonia between July 2019 and May 2021. We demonstrated that use of molecular point of care testing returned results much more rapidly than conventional testing and identified causative pathogens in a greater proportion of patients. This led to a much greater proportion of patients receiving microbiologically directed therapy, and safely facilitated many more de-escalations and escalations in therapy than conventional diagnostics, without compromising patient safety.

The CoV-19 POC trial (Chapter 5) recruited 500 adults presenting to hospital between March and May 2020. We demonstrated that adults tested with a point-of-care molecular test for respiratory viruses (including SARS-CoV-2) received their results much earlier than those tested by standard laboratory PCR, and as a result, spent almost 20 hours less in cohort assessment areas where they could have potentially been exposed to SARS-CoV-2.

6.2 COVID-19 impact

My thesis was initially planned exclusively around my work in the SARIPOC study, however the emergence of SARS-CoV-2 14 months into my studentship required me to become involved in COVID-19 studies and opened up new opportunities for research.

6.2.1 SARIPOC substantial study amendment and supporting resource prioritisation

I was unable to screen or recruit any patients to the SARIPOC study between 14th March 2020 and 3rd July 2020 in order to prioritise work on the CoV-19 POC study for the direct benefit of patients. My recruitment was further paused from 3rd July until 27th August 2020 in order to prioritise data collection and analysis for CoV-19 POC.

The pandemic led to many local resources being re-deployed for development of vaccine and therapeutics for SARS-CoV-2 and this significantly reduced my nursing support until the end of the study. In line with re-prioritisation of critical care research resources nationally, we performed an unplanned interim analysis which reduced our target sample size to 200 patients from 300, in a substantial amendment. This reduced the power of the study for assessing secondary outcomes. The pandemic also 'moved the goalposts' in terms of our infection control outcomes for the study. We had initially envisaged isolation of patients with transmissible organisms as a key metric of the study, however the lines of what constituted isolation became too blurry to make any meaningful conclusions when every patient with a respiratory illness was being managed as potentially having COVID-19. Furthermore, the introduction of routine mPOCT from the CoV-19 POC trial meant that a very high proportion of patients reaching ICU with community onset pneumonia were already tested with a comprehensive respiratory virus panel.

The emergence of SARS-CoV-2 as a new, frequently occurring hazard category 3 organism raised a significant new challenge in deploying the Filmarray near-patient and required me to carry out new risk assessments in the CL2 laboratory for me to be able to use the Filmarray and mini-VIDAS in line with national infection control guidance[184].

6.2.2 The CoV-19POC study

The impact of the pandemic on my research was not all negative, as it resulted in the COV-19POC study. The nature of collaborative working in the pandemic, and the reallocation of research resources as described above, meant that the study went from first design to publication in less than 8 months.

6.2.3 International conference presentations

I presented a poster at the European Society of Clinical Microbiology and Infectious Disease Conference on Coronavirus disease (ECCVID) which was changed to a virtual conference (September 2020). The title was: the burden and clinical characteristics of viral respiratory disease before and during the SARS-CoV-2 pandemic. The same abstract was selected as an oral presentation at the European Scientific Working group on Influenza (ESWI) conference (December 2020) which was also a virtual conference.

6.2.4 Additional clinical responsibilities

After the conclusion of the CoV-19 POCT study, the AMU POCT hub was formally transitioned into a routine clinical service. I took on responsibility and oversight for this service whilst continuing to

recruit for SARIPOC until July 2021. This included maintenance and repair of modules, validation of new units, supply (which was particularly challenging when reagents were in high demand) and troubleshooting.

6.3 Skills obtained during my candidature

As a result of this work, I have developed many new skills in research. Setting up the SARIPOC trial has given me an understanding of the regulatory framework that underpins research in the UK, including the work of the HRA, trial sponsors and ethics committees. I have a greater understanding of research ethics, and the processes in place to protect them. In recruiting critically unwell patients who were frequently lacking capacity to decide upon their participation in trials, I have developed my understanding of the mental capacity act with regards to research, and the human tissues act. I have learned and developed my skills in writing scientific manuscripts by writing a trial protocol and multiple research papers. I have also improved significantly in my presentation of research to scientific audiences, both in oral presentations and posters. I have learned a great deal about the use and handling of research data, including database management, automated extraction and statistical methods.

6.4 Future work

6.4.1 Understanding low level molecular detections

A challenge that I have discussed in this thesis with molecular testing for pneumonia is the possibility of additional, extra detection of non-clinically relevant colonising flora. This is a limitation of any microbiological testing for pneumonia as sampling of the area of interest (alveolar tissue) has to occur indirectly, via washings or sputa from a non-sterile region. The next step in understanding the significance of many of these detections, for example the additional *S. aureus* and *H. influenzae* frequently found by molecular testing, would be to measure how well upper respiratory tract samples compare to tissue samples collected at autopsy soon after death. This would be a difficult trial to structure, as patients would be required to provide assent when they are at the end of life, and would not benefit from the trial themselves.

6.4.2 Biomarkers in pneumonia

Hellyer et al validated a combination of inflammatory cytokines that ruled out the presence of any organism in sputum with a negative predictive value of 100% (compared to a reference standard of culture). To date, the association between molecular detection of organism, sputum

inflammatory profile, and clinical outcome has not been explored. We have a number of banked residual frozen sputa from patients from the SARIPOC trial with paired blood samples. Our collaborative work during the pandemic involved multiplex cytokine testing of patients with SARS-CoV-2 from the CoV-19 POC study[224]. We had a grant application for a study exploring this association rejected after shortlisting.

6.4.3 Other novel rapid diagnostics for pneumonia

A major limitation of the Filmarray Pneumonia panel with regards to its use as a stewardship tool (discussed in 4.8.2) is predicting phenotypic antibiotic sensitivity. Diagnostic tests are being developed which provide rapid organism identification and phenotypic susceptibility data, most notably the Accelerate Pheno system (Accelerate Diagnostics Inc., Tucson, AZ, USA). The Pheno system uses fluorescence in situ hybridisation to rapidly identify pathogens, and then morphokinetic cellular analyses to provide phenotypic sensitivity data in around 8 hours[225]. A blood stream infection panel is already CE marked, and a pneumonia panel is in development. The clinical impact of the Pheno system is an appealing potential future avenue of research.

6.4.4 Health economic analyses

Health and social care systems like the NHS are always resource limited, and any new therapy or investigation needs to prove that its use is cost-effective. A full health economic analysis for both testing strategies in this thesis, balancing the financial costs against the clinical impact is urgently needed. In the context of bacterial pneumonia in critical care, this would need to be performed on a larger scale than the SARIPOC study, and across multiple sites. In the CoV-19POC study, we collected data showing dramatically improved patient flow and operational capacity with POCT, which suggests that routine mPOCT would be cost-effective. A limitation to the generalisability of such a study would be the changing costs and availability of reagents as the pandemic progresses, in a volatile and changing financial market.

Appendix A SARIPOC Supplementary appendices

Patient trial ID:

University Hospital Southampton NHS
NHS Foundation Trust

Participant Consent Form: adults with capacity

Pragmatic randomised controlled trial of molecular point-of-care testing for respiratory pathogens versus routine clinical care in adults with pneumonia (SARIPOC). Chief Investigator: Dr Tristan Clark

Please put your INITIALS in each box and sign at the bottom to indicate your consent to the following:

1.	I confirm that I have read and understand the Participant Information Sheet, Version_____, dated _____ for the above study. I have spoken to_____ and had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason and without my care or legal rights being affected.	☐
3.	I give permission for my GP to be informed about my participation in the study.	☐
4.	I agree to have a sputum sample taken to be analysed by a 'point-of-care' rapid diagnostic test for respiratory viruses, bacteria and resistance genes.	☐
5.	I understand that the results of this point-of-care test, which will be shared with my treating doctors and nurses, may alter my treatment as it may indicate that I have a specific infection.	☐
6.	I give permission for the research team to have access to my medical notes, including electronic records, for the purposes of this study.	☐
7.	I give permission for relevant sections of any of my hospital records and research data collected during the study to be looked at later by responsible individuals from regulatory authorities, the University of Southampton and University Hospital Southampton NHS Foundation Trust for the purposes of data analysis, audit and monitoring.	☐
8.	I understand that I may be asked for a sputum samples, urine samples and blood samples. If I have a procedure that allows collection of a sample of fluid from my lungs as part of my hospital treatment, a sample of this may be used for research. (I have the right to opt out of all or any of these, should it be asked).	☐
9.	I understand that any samples collected from me may be kept for the purpose of further ethically approved research.	☐
10.	I agree to take part in this study.	☐

RESEARCH PARTCIPANT SIGNATURE:.....

NAME (IDEALLY PRINT): ..... DATE:.....

RESEARCH STAFF: SIGNATURE:.....

NAME:..... DATE:.....

ROLE (PLEASE CIRCLE): Doctor / Nurse

For use with adults with capacity or adults who have regained capacity to consent
When completed: original to site file; photocopies for participant and notes

REC number 17/SC/0110 Study number RHM MED1387 Version 2.0, Date 26/02/2019
IRAS Project ID: 216585

University Hospital Southampton NHS
NHS Foundation Trust

Participant Information Sheet: adults with or regained capacity

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

Chief Investigator: Dr Tristan Clark BM MRCP DTM&H MD
Associate Professor and Consultant in Infectious Diseases

Research Study
You are being invited to take part in the research study named above. Before you decide if you want to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this information sheet.

What is the purpose of this study?
Pneumonia (chest infection) is a serious illness which is usually caused by bacteria. When identified, it warrants swift treatment with antibiotics and sometimes requires high-dependency or intensive care unit admission.

The choice of which of the many antibiotics to use in patients with pneumonia is 'best-guess': it is guided by what bacteria are likely be present. As a result we use antibiotics which kill many different bacteria. We know that use of these antibiotics promotes antibiotic resistance. The World Health Organisation have identified antibiotic resistance as one of the biggest threats to global health today.

Current tests looking for bacteria in sputum take several days to generate results so do not allow doctors to be more targeted with their antibiotic use. Rapid 'point-of-care' tests for pneumonia have been developed which can provide accurate results in about 1 hour rather than several days. We wish to explore if using a rapid test improves the use of antibiotics and improves patient care.

Why have I been asked?
The symptoms and clinical signs that you display are consistent with pneumonia and so we would like to potentially test you with a rapid test for a wide variety of bacteria and respiratory viruses which might have caused your illness. The test will also look for certain genes in the bacteria that are responsible for antibiotic resistance. We would then collect data from your hospital case notes to see if testing people with this new method makes any difference to their medical care and how quickly they recover.

We are therefore asking for volunteers over the age of 18 who are patients in intensive care or respiratory high dependency who have pneumonia to be part of this study.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You will receive a copy of your consent form. If you decide to take part, you are still free to

withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

After you have finishing reading this, you will have the opportunity to discuss the study in more detail with a member of the research team. If you are happy to take part, then you will be asked to read and sign a consent form. Certain details will be briefly asked of you, including what symptoms you have had. A computer program will then assign you randomly to Group A or Group B. Group allocation is completely random: neither the research team nor anyone else can influence which group you are placed in.

For all patients (both Groups A and B)

All patients in the study will be asked for a sputum sample. If you have a procedure as part of your standard hospital care that allows collection of a sample of fluid from your lungs (such as a bronchoscopy or endo-tracheal tube), then we shall ask for some of that too, but only if there is enough left after the tests your doctors want done. No patient taking part will have extra procedures purely for the purposes of the study.

A blood sample will also be requested (about one and a half tablespoons) for additional research that has been ethics-committee approved.

A urine sample will be requested next time you pass water. As with the sputum sample, no procedure will be done purely to obtain this for the study. If you have a catheter (urine draining tube) urine will be collected from the waste bag.

Subsequent blood tests will be requested from everyone who takes part in the study at roughly 12, 24, 48, 72 hours and 5 days after consenting to take part. Many patients in intensive care have special cannulas from which blood tests can be taken.

For those allocated to Group A:

This sputum is taken immediately to be analysed for many bacteria and viruses that can cause chest problems. The blood sample will be tested for the presence of a protein which is produced when chest infections are caused by bacteria. The urine sample will be tested for the presence of bits of protein from certain bacteria that cause chest infections.

The results will be available in about 2 hours and you will be informed of the results, as will the doctors looking after you. The research team are also practicing Infection specialist doctors so may give suggestions about your antibiotic treatment either immediately or in the following days, based on the results of the tests, to the doctors who are looking after you.

You have the right to discuss the results with the research team and the clinical team looking after you.

For those allocated to Group B: you will receive standard care from the doctors and nurses looking after you. The research team will ask you for samples as described above when you initially agree to the study but these will be tested at a later date and will not influence your care in hospital. Additionally, the follow up blood tests described above will still be requested.

The flow chart at the end of this document summarises all that you may be involved in if you agree to take part.

You have the right to decline all or any of these further research tests, should you wish, and this will not affect you being part of this study or the care you receive. If you lose the ability to consent and the research team wish to take any more samples from you, then we would have to get written permission from someone close to you or one of the doctors looking after you, and you would have to agree that we could use them once you recovered.

Once you have been discharged a researcher will look at your hospital case notes to see if having the test done, or not, has affected your medical management during your hospital stay. This includes how quickly you were discharged, if you were put into a side room or not, and what antibiotics you received.

What about confidentiality?

We take participant confidentiality very seriously. Only a very limited amount of personal identifiable information is requested from you, and when we come to look at and publish any results then information is presented anonymously – i.e. your details and personal information are never made available. With your permission, your GP will be informed that you are participating in this study, but no further information is given to them. The doctors and nurses treating you are told the results of the point-of-care test for respiratory viruses for those in Group A.

How will you look after my data?

The Research and Development department (R&D), University Hospitals Southampton NHS trust (UHS) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. UHS R&D will keep identifiable information about you for 15 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. You can find out more about how we use your information at:

http://www.uhs.nhs.uk/ClinicalResearchinSouthampton/Public-and-patients/Get-involved/General-Data-Protection-Regulation.aspx

What are the risks?

The risks of having a sputum sample taken are minimal. There is no risk associated with the production of a urine sample. If you have a medical tube that goes into an artery or vein already, then you may not need to have any needles for us to take the blood test. Otherwise the blood test is only mildly uncomfortable and any bruising is usually very mild and short lived. Study activities are insured via the hospital and University of Southampton.

What happens when the research study stops?

The study will end when we have recruited 300 people like you. By this time we hope to have collected all the information we need to decide if the research question has been answered. The samples collected during the study are stored without any of your details on them and are only used in further ethically approved research under the direction of the chief investigator of this study.

What will happen to the results of the research study?

We intend to publish the results of our research in medical journals and to present the results at scientific meetings. The information from these journals may be available on the internet. All of your results are completely anonymous in these publications or presentations. We would like any useful results to form the basis of other studies looking at this and also change how medical professionals treat patients for the better.

Who is organising and funding this research?

Money from the NHS and the University of Southampton is funding this research. The NHS research and university authorities may examine the data we have collected from you to ensure it is accurate. The company who manufacture the equipment and test kits used in this study have provided them to us for free but have not been involved in the design of the study and will not be involved in the conduct of the study or the analysis and presentation of the results. They do not have any access to your information.

Who has approved this study?

The Health Research Authority, including an ethics committee, has reviewed the design of this study and approved it to go ahead. The local NHS Research and Development department has approved this study too.

Who can I talk to further?

The research team are very happy to answer your questions and discuss things further with you. You are welcome to talk to your doctors and nurses, family and friends, should you wish, about participating. Should you have any specific concerns you are welcome to discuss these with a research doctor, or the chief investigator, or Patient Support Services, about how you might take your concerns further.

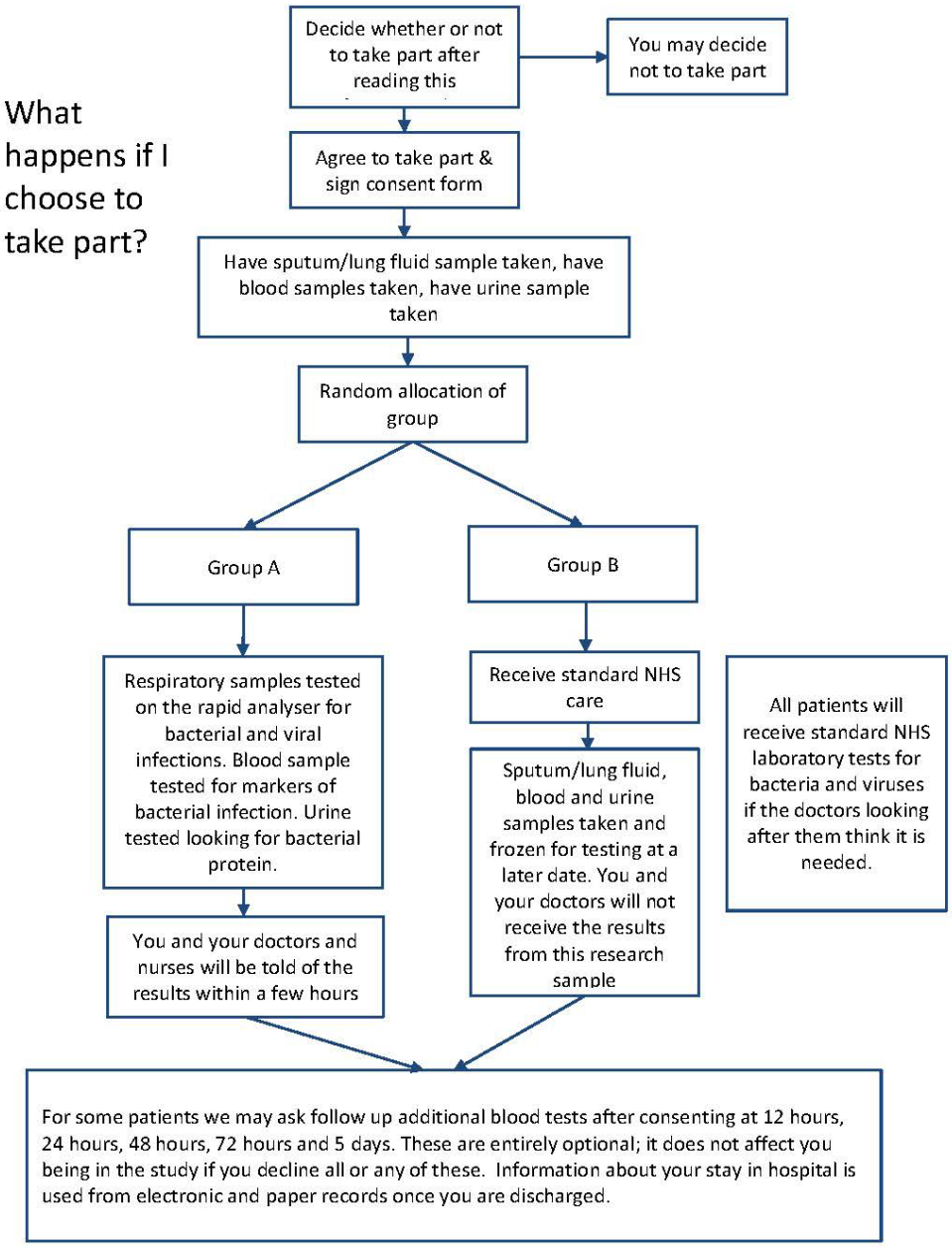
Dr Tristan Clark, Chief investigator
Associate Professor and Consultant in Infectious Diseases, University of Southampton and University Hospital Southampton NHS Foundation Trust
Tel: 02381218410. T.W.Clark@soton.ac.uk

Dr Stephen Poole
Clinical Research Fellow, Infectious Diseases and Microbiology Specialist Registrar, University Hospital Southampton NHS Foundation Trust
Tel: 02381204989. S.Poole@soton.ac.uk

Patient Support Services, University Hospital Southampton NHS Foundation Trust
Tel: 023 8120 6325. patientsupportservices@uhs.nhs.uk

University Hospital Southampton NHS
NHS Foundation Trust

What happens if I choose to take part?



Patient Trial ID: _____

Regarding Patient: _____

University Hospital Southampton NHS
NHS Foundation Trust

Consultee Agreement Form: adults without capacity

Pragmatic randomised controlled trial of molecular point-of-care testing for respiratory pathogens versus routine clinical care in adults with pneumonia (SARIPOC). Chief Investigator: Dr Tristan Clark

Please put your INITIALS in each box and sign at the bottom to indicate your consent to the following:

Regarding Patient: _____

1. I confirm that I have read and understand the Consultee Information Sheet, Version_____, dated _____ for the above study. I have spoken to _____ and had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I confirm that the participation is voluntary and I know of no reason why the above patient would not wish to take part in the study and once they have recovered and regain capacity they are free to withdraw at anytime, without giving any reason and without their medical or legal rights being affected. ☐
3. I confirm that I am acting as consultee for the above named patient who is temporarily incapacitated and I also confirm that, as far as I am aware there is no advance statement that would prevent them from taking part in the study. ☐
4. I give permission for his or her GP to be informed about participation in the study. ☐
5. I agree to sputum samples being taken from the participant, which will be analysed by a 'point-of-care' rapid diagnostic test for respiratory viruses, bacteria and certain resistance genes. ☐
6. I understand that the results of this point-of-care test, which will be shared with the participant's treating doctors and nurses, may alter his or her treatment as it may show a specific infection. ☐
7. I give permission for the research team to have access to participant's medical notes, including electronic records, for the purposes of this study. ☐
8. I give permission for relevant sections of any of participant's hospital records and research data collected during the study to be looked at later by responsible individuals from regulatory authorities, the University of Southampton and University Hospital Southampton NHS Foundation Trust for the purposes of data analysis, audit and monitoring. ☐
9. I understand that sputum samples and/or urine samples and/or a blood sample may be taken and stored for further tests. If the participant has a procedure that allows collection of a sample of fluid from their lungs as part of their hospital treatment, a part of this sample may be used for research. (I have the right to opt out of all or any of these, should it be asked). ☐
10. I understand that any samples collected may be kept for the purpose of further research. ☐

When completed: original to site file; photocopies for participant and notes

Patient Trial ID: _____

Regarding Patient: _____

University Hospital Southampton NHS
NHS Foundation Trust

11. I agree to discuss the study with the patient when they regain capacity. ☐

NAME OF CONSULTEE: _____

RELATIONSHIP TO THE PARTICIPANT: _____

SIGNATURE: _____

DATE: _____

RESEARCH STAFF: SIGNATURE:.....

NAME:..... DATE:.....

ROLE (PLEASE CIRCLE): Doctor / Nurse

When completed: original to site file; photocopies for participant and notes
REC number 17/SC/0110
IRAS Project ID 216585 Study number RHM MED1387 Version 2.0, Date 26/02/2019

Consultee Information Sheet: adults without capacity

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

Chief Investigator: Dr Tristan Clark BM MRCP DTM&H MD
Associate Professor and Consultant in Infectious Diseases

We are aware this is a very anxious time for you, as your close relative/friend or person you are representing has been admitted to the intensive care or high dependency unit with a serious medical problem. We would be very grateful if you could take the time to read the following information.

Research Study
We would like to invite the person you are representing to take part in this study and to ask you whether, in your view, they may have any objections. Before you decide if you want him/her to take part, it is important for you to understand why the research is being done and what it will involve. Please ask us to clarify anything that's not clear or if you would like more information. Please take time to read the following information carefully and discuss it with others if you wish. Thank you for reading this information sheet.

What is the purpose of this study?
Pneumonia is a serious illness which is usually caused by bacteria. When identified, it warrants swift treatment with antibiotics and sometimes requires high-dependency or intensive care unit admission.

The choice of which of the many antibiotics to use to treat patients with pneumonia is 'best-guess': it is guided by what bacteria are likely be present. As a result we use antibiotics which kill many different bacteria. We know that use of these antibiotics promotes antibiotic resistance. The World Health Organisation have identified antibiotic resistance as one of the biggest threats to global health today.

Current tests looking for bacteria in sputum take several days to generate results so do not allow doctors to be more targeted with their antibiotic use. Rapid 'point-of-care' tests for pneumonia have been developed which can provide accurate results in about 1 hour rather than several days. We wish to explore if using a rapid test improves the use of antibiotics and improves patient care.

Why have I been asked?
As your relative/ friend or the person you are representing is currently unable to consent for this study, we are approaching you to confirm that you do not know of any reason why they would not want to take part in this study. However once they have recovered, we will approach them directly to clarify if they are happy to provide consent for their continued participation. If they do not wish to be part of the study, they have the option of having their personal data removed from the study.

We would like to potentially perform a rapid test for a wide variety of respiratory viruses and bacteria which might have caused their illness. We would then collect data from

hospital case notes to see if testing people with this new method makes any difference to their medical care and how quickly they recover.

We are therefore asking patients over the age of 18 who are patients in intensive care or respiratory high dependency who have pneumonia to be part of this study.

Do we have to take part?

Taking part in the study is entirely voluntary. If you chose that your relative/ friend or the person you are representing should not be involved, please be assured the standard of care received will not be affected in anyway.

We are more than happy to take you through the information sheets and consent procedures in more detail. We can provide you with additional information if needed. If you agree for them to take part, you will be given this information sheet to keep and be asked to sign a consent form. The person you are representing is free to withdraw from the study at anytime without giving a reason. This will not affect the standard of care provided.

What will happen to me if I take part?

After you have finishing reading this, you will have the opportunity to discuss the study in more detail with a member of the research team. If you are happy for the person you are representing to take part, then you will be asked to read and sign a consent form. Certain additional details may be required, including what symptoms they have had. A computer program will then assign them randomly to Group A or Group B. Group allocation is completely random: neither the research team nor anyone else can influence which group someone is placed into. Broadly speaking, group A will get the new test plus NHS standard care and group B will just get the current standard NHS care.

For all patients (both Groups A and B)

All patients in the study will be asked for a sputum sample. If the person you are representing has a procedure as part of their standard hospital care that allows collection of a sample of fluid from their lungs (such as a bronchoscopy or endo-tracheal tube), then we shall ask for some of that too, but only if there is enough left after the tests your doctors want done. Nobody in the study will have extra procedures purely for the purposes of the study.

A blood sample will also be taken (about one and a half tablespoons) for additional research that has been ethics-committee approved. If the patient already has a plastic device in a vein or artery that allows blood to be taken we will use this to draw the blood.

A urine sample will be taken. As with the sputum sample, no procedure will be done purely to obtain this for the study. If the person you are representing has a catheter (urine draining tube) urine will be collected from the waste bag.

Subsequent blood tests will be requested from everyone who takes part in the study at roughly 12, 24, 48, 72 hours and 5 days after consenting.

For those allocated to Group A:

This sputum is taken immediately to be analysed for many bacteria and viruses that can cause chest problems. The blood sample will be tested for the presence of a protein which is produced when chest infections are caused by bacteria. The urine sample will be tested for the presence of bits of protein from certain bacteria that cause chest infections.

The results will be available in about 2 hours and you will be informed of the results, as will the doctors looking after your relative/friend. The research team are also practicing Infection specialist doctors so may give suggestions about antibiotic treatment either immediately or in the following days, based on the results of the tests, to the doctors delivering the care.

For those allocated to Group B: The person you are representing will receive standard NHS care from the doctors and nurses. The research team will collect samples as above but these will be tested at a later date and will not influence care in hospital. Additionally, the follow up blood tests described may be requested. The flow chart at the end of this document summarises all that may be involved.

Am I the right person to make this decision?

If you feel that you might not be the right person to make a decision, you could ask/nominate another family member, friend, or a person who may know the patient's wishes/opinions better. We are more than happy to discuss these issues.

What about confidentiality?

We take participant confidentiality very seriously. Only a very limited amount of personal identifiable information is requested from you, and when we come to look at and publish any results then information is presented anonymously – i.e. their details and personal information are never made available.

Their GP, with your permission, would be informed about participation in this study, but no further information is given to them. The doctors and nurses treating them are told the results of the point-of-care test for respiratory viruses and bacteria for those in Group A.

How will you look after collected data?

The Research and Development department (R&D), University Hospitals Southampton NHS trust (UHS) is the sponsor for this study based in the United Kingdom. We will be using information from the medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after private information and using it properly. UHS R&D will keep identifiable information about the person you are representing for 15 years after the study has finished.

A persons rights to access, change or move their information are limited, as we need to manage it in specific ways in order for the research to be reliable and accurate. If a participant withdraws from the study, we will keep the information that we have already obtained. To safeguard people's rights, we will use the minimum personally-identifiable information possible. You can find out more about how we use personal information at:

University Hospital Southampton NHS
NHS Foundation Trust

http://www.uhs.nhs.uk/ClinicalResearchinSouthampton/Public-and-patients/Get-involved/General-Data-Protection-Regulation.aspx

What are the risks?

The risks of having a sputum sample and a urine sample are minimal. If the person you are representing has a plastic device in that allows us to take blood directly we will use this. If not, a blood test is usually of minimal discomfort, any bruising is usually very mild and the volume taken is small. The volume of these blood samples is far less than taken normally for routine care and the extra samples will not result in the need for a blood transfusion. Study activities are insured via the hospital and University of Southampton.

What happens when the research study stops?

The study will end when we have recruited 300 people. By this time we hope to have collected all the information we need to decide if we have answered the research question. The samples collected during the study are stored without any of personal details on them and are only used in further research under the direction of the chief investigator of this study.

What will happen to the results of the research study?

We intend to publish the results of our research in medical journals and to present the results at scientific meetings. The information from these journals may be available on the internet. All of the results are completely anonymous in these publications, presentations and online. We would like any useful results to form the basis of other studies looking at this and also change how medical professionals treat patients for the better.

Who is organising and funding this research?

Money from the NHS and the University of Southampton is funding this research. The NHS research and university authorities may examine the data we have collected from patients to ensure it is accurate. The company who manufacture the equipment and test kits used in this study have provided them to us for free but have not been involved in the design of the study and will not be involved in the conduct of the study or the analysis and presentation of the results. They do not have any access to your or patient information.

Who has approved this study?

The Health Research Authority, including an ethics committee, has reviewed the design of this study and approved it to go ahead. The local NHS Research and Development department has approved this study too.

Who can I talk to further?

The research team are very happy to answer any of your questions and discuss things further with you. You are welcome to talk to your doctors and nurses, family and friends, should you wish, about participating. Should you have any specific concerns you are welcome to discuss these with a research doctor, or the chief investigator, or Patient Support Services, about how you might take your concerns further.

Dr Tristan Clark, Chief investigator

Associate Professor and Consultant in Infectious Diseases, University of Southampton and University Hospital Southampton NHS Foundation Trust
Tel: 02381218410. T.W.Clark@soton.ac.uk

Dr Stephen Poole
Clinical Research Fellow, Infectious diseases and Microbiology Specialist Registrar, University Hospital Southampton NHS Foundation Trust
Tel: 02381204989. S.Poole@soton.ac.uk

Patient Support Services, University Hospital Southampton NHS Foundation Trust
Tel: 023 8120 6325. patientsupportservices@uhs.nhs.uk

University Hospital Southampton NHS
NHS Foundation Trust

What happens if I choose to take part?

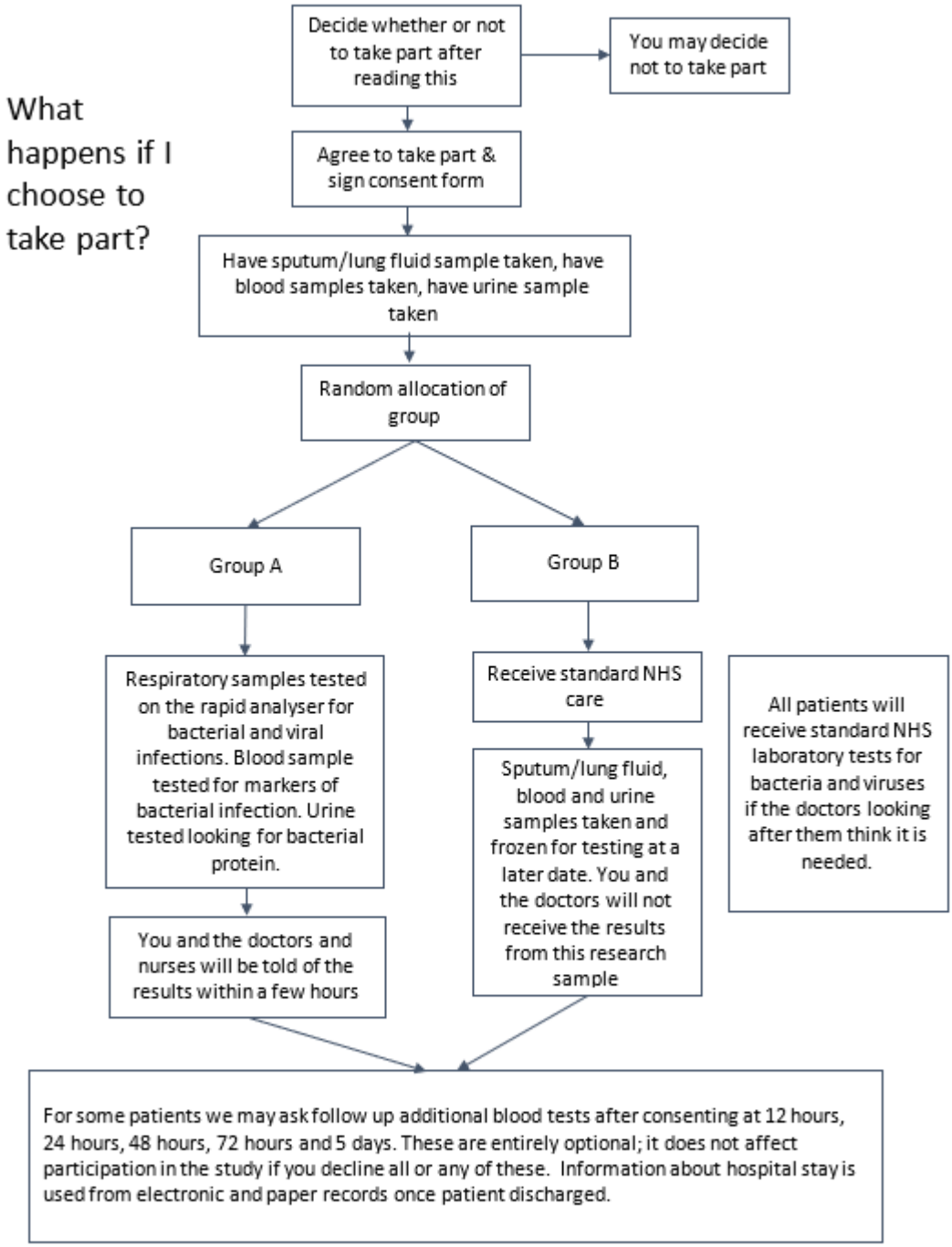


Table 36: Additional antibiotic related outcomes

Outcome	**mPOCT n=100**	**Control n=100**	**Absolute Difference (95% CI)**	**P value**
Pathogen directed therapy*	56 (56%)	18 (18%)	38 (25-50)	<0.0001
Received piperacillin-tazobactam after recruitment†	44 (47%)	52 (55%)	-9% (-23 to 6)	0.24
Duration of piperacillin-tazobactam, hours‡	90.5 (31.5-146.0)	108.5 (47.0-157.5)	-20.0 (-62.9 to 22.9)	0.36
Received carbapenem after recruitment†	25 (27%)	18 (19%)	7 (-5, 19)	0.23
Duration of carbapenem given, hours§	70.0 (43.0-136.0)	114.2 (70.0-184.0)	-46.2 (-104.8 to 12.4)	0.12

All data n (%) or median (IQR). CI, 95% confidence interval. *Assessed in 99 patients in control group. †Assessed in 94 patients in both mPOCT and control groups. ‡Assessed in 44 patients in mPOCT group and 52 patients in control group. §Assessed in 25 patients in mPOCT group and 18 patients in control group.

Table 37: Multivariate model for primary outcome

Outcome measure	Odds ratio (95% CI)	p value
Group		
Control	Reference	
Intervention	9.9 (4.9, 20.1)*	<0.0001
Age, years	1.0 (1.0, 1.0)	0.34
Sex		
Male	Reference	
Female	1.0 (0.4, 2.1)	0.90
Diagnosis		
CAP	Reference	
HAP	0.7 (0.3, 1.6)	0.42
VAP	0.8 (0.3, 2.1)	0.65
COVID-19 (Yes vs No)	0.6 (0.2, 1.8)	0.40
Recruitment location		
GICU	Reference	
NICU	0.8 (0.3, 1.7)	0.34
rHDU	0.5 (0.1, 2.5)	0.11
Time from antimicrobials to recruitment, hours	1.0 (1.0, 1.0)	0.96
SOFA score	1.0 (0.9, 1.1)	0.75
Day one procalcitonin level, ng/ml	1.0 (1.0, 1.0)	0.96

*This equates to an absolute difference of 50% (37 to 62) in the fully adjusted model. CAP, Community acquired pneumonia. HAP, Hospital acquired pneumonia. VAP, Ventilator associated pneumonia. GICU, General intensive care unit. NICU, Neurological intensive care unit. rHDU, Respiratory high dependency unit. SOFA, Sequential organ failure assessment

Table 38: Competing risks regression model

Outcome	SHR (95% CI)	p value
Time to results directed therapy	5.96 (3.92, 9.07)	<0.0001
Time to hospital discharge	0.96 (0.69, 1.32)	0.79
Time to critical care discharge	0.92 (0.67, 1.26)	0.62
30-day mortality	1.03 (0.54, 1.95)	0.94
60-day mortality	0.98 (0.53, 1.81)	0.94

SHR, Subhazard ratio. CI, 95% confidence interval. For 'time to results directed therapy', 'time to hospital discharge' and 'time to critical care discharge', the competing risk is death. For 30 and 60-day mortality, the competing risk is discharge from hospital within 30 and 60 days, respectively.

Table 39: Cases where antimicrobial prescribing advice based on molecular detection, was subsequently reviewed due to discordant culture results

Case number	FA detection	PCT, ng/ml	Antimicrobial advice from FA result (clinical action)	Sputum culture	Subsequent clinical management	Clinical outcome
1	*P. aeruginosa* and *S. marcescens*	<0.05	No change (P-T continued)	*P. aeruginosa* (P-T resistant)	No change as organism considered likely to represent colonisation	FSR
2	Human coronavirus only	0.11	De-escalate (antibiotics stopped)	*K. pneumoniae*	No change as patient was clinically improving	FSR
3	*H. influenzae* and *E. coli*	0.07	De-escalate (Azithromycin stopped, Co-amoxiclav continued)	*E. coli* (Co-amoxiclav Resistant)	Changed to Ciprofloxacin and Doxycycline	FSR
4	*E.coli, H. influenzae, S. aureus* and *M. catarrhalis*	5.16	No change (Co-amoxiclav continued)	*E. coli* (Co-amoxiclav resistant)	Changed to Ciprofloxacin.	FSR
5	*P. aeruginosa, P. mirabilis, E. cloacae* complex, *E. coli, S. agalactiae*	0.13	No change (Co-amoxiclav continued)	*P. aeruginosa* (P-T sensitive)	Changed to P-T	FSR
6	Nil detected	4.95	De-escalate (Doxycycline stopped, Co-amoxiclav continued)	*P. aeruginosa* (P-T sensitive)	Changed to P-T	FSR
7	*P. aeruginosa*	1.02	Escalate (changed from P-T to Meropenem)	*P. aeruginosa* (Meropenem resistant, P-T sensitive)	Changed back to P-T	FSR
8	*S. pneumoniae* and *H. influenzae*	0.21	No change (Co-amoxiclav continued)	*S. pneumoniae* and *H. influenzae* (Co-amoxiclav resistant)	Changed to P-T	FSR

FA detection, Filmarray pneumonia panel detection, PCT, Procalcitonin, P-T, Piperacillin-tazobactam, FSR, Full symptomatic recovery.

Table 40: Additional bacteria identified by Filmarray pneumonia panel compared to sputum culture

	Filmarray bin value (copies/ml)				**Total**
Organism	**10^4**	**10^5**	**10^6**	**10^7**	
E. cloacae complex	1	1	2	0	4
E. coli	4	8	2	0	14
H. influenzae	6	8	3	6	23
K. aerogenes	0	3	1	0	4
K. oxytoca	2	2	1	1	6
K. pneumoniae	0	1	2	1	4
M. catarrhalis	1	2	1	1	5
P. aeruginosa	2	2	1	0	5
P. mirabilis	0	0	1	0	1
S. agalactiae	3	3	0	1	7
S. aureus	5	7	2	1	15
S. marcescens	3	0	3	0	6
S. pneumoniae	2	2	0	1	5
S. pyogenes	1	0	0	0	1
Total	30	39	19	12	100

Table 41: Additional bacteria identified by the Filmarray pneumonia panel compared to culture in direct testing

	Filmarray bin value (copies/ml)				**Total**
Organism	**10^4**	**10^5**	**10^6**	**10^7**	
E. cloacae complex	1	0	2	0	3
E. coli	3	3	0	0	6
H. influenzae	2	2	1	0	5
K. aerogenes	0	2	0	0	2
K. oxytoca	0	2	1	0	3
M. catarrhalis	1	1	0	0	2
P. aeruginosa	2	1	0	0	3
P. mirabilis	0	0	1	0	1
S. agalactiae	1	1	0	0	2
S. aureus	3	3	1	1	8
S. marcescens	3	0	0	0	3
S. pneumoniae	0	1	0	1	2
Total	16	16	6	2	40

Table 42: Organisms with pathogenic potential deemed colonising flora that were grown in the presence of another likely pathogen

Organism	**Frequency**
Enterobacter bugandensis	1
Hafnia alvei	1
Aspergillus flavus	1
Providencia rettgeri	1
Achromobacter xylosoxidans	1
Group C Streptoccoci	1
Pseudomonas montelii	1

Table 43: Individual analyte quantification by FA compared to culture

Organism culture concentration	Filmarray pneumonia bin value, copies/ml				
	Not detected	**10^4**	**10^5**	**10^6**	**≥10^7**
E. coli					
No growth	52	4	2		
Scanty	1	1		2	
Moderate					
Heavy					2
P. aeruginosa					
No growth	48	2	1		
Scanty			1	1	1
Moderate	1				
Heavy			2	1	6
S. aureus					
No growth	52	3	3	1	1
Scanty				2	
Moderate				1	
Heavy				1	
K. pneumoniae gp.					
No growth	62				
Scanty					
Moderate				1	
Heavy				1	
K. oxytoca					
No growth	60		2	1	
Scanty					
Moderate					
Heavy					1
K. aerogenes					
No growth	62		1	1	
Scanty					
Moderate					
Heavy					

Organism culture concentration	Filmarray pneumonia bin value, copies/ml				
	Not detected	10^4	10^5	10^6	≥10^7
*H. influenzae**					
No growth	57	2	2	1	
Scanty					1
Moderate					
Heavy					
E. cloacae complex					
No growth	61	1		2	
Scanty					
Moderate					
Heavy					
S. marcescens					
No growth	61	3			
Scanty					
Moderate					
Heavy					
P. mirabilis					
No growth	61			1	
Scanty			1		
Moderate					
Heavy				1	
S. pneumoniae					
No growth	62		1		1
Scanty					
Moderate					
Heavy					
M. catarrhalis					
No growth	62	1	1		
Scanty					
Moderate					
Heavy					

*one H. influenzae detection not included as culture result not quantified

Table 44: Confounding variables for bacterial detection in SARS-CoV-2

	Proportion sputum like samples	**Proportion BAL-like samples**	**Pre-existing respiratory disease**	**Proportion positive by FA**
CAP				
SARS-CoV-2 infected (n=13)	11 (85)	2 (15)	3 (23)	3 (23)
Non SARS-CoV-2 (n=70)	67 (96)	3 (4)	30 (41)	40 (57)
HAP				
SARS-CoV-2 infected (n=6)	6 (100)	0 (0)	2 (33)	3 (50)
Non SARS-CoV-2 (n=63)	58 (92)	5 (8)	15 (24)	41 (65)
VAP				
SARS-CoV-2 infected (n=8)	7 (87)	1 (13)	0 (0)	8 (100)
Non SARS-CoV-2 (n=38)	35 (92)	3 (8)	8 (21)	29 (76)

Data are n(%). CAP, community acquired pneumonia. HAP, hospital acquired pneumonia. VAP, ventilator associated pneumonia. BAL, broncho-alveolar lavage. FA, Filmarray.

Appendix B Supplementary materials for the CoV-19 POC study

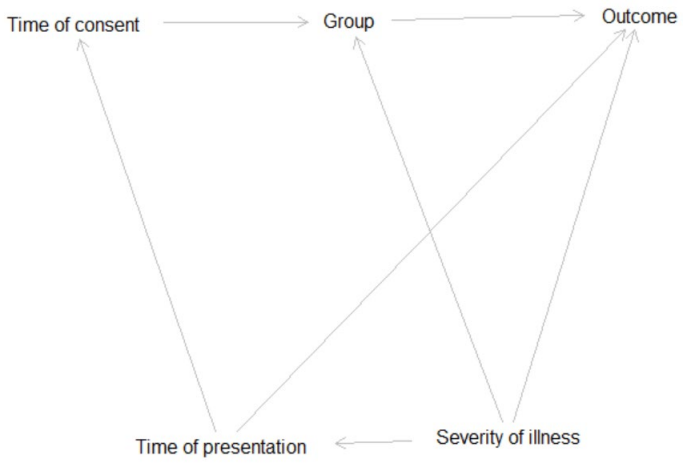
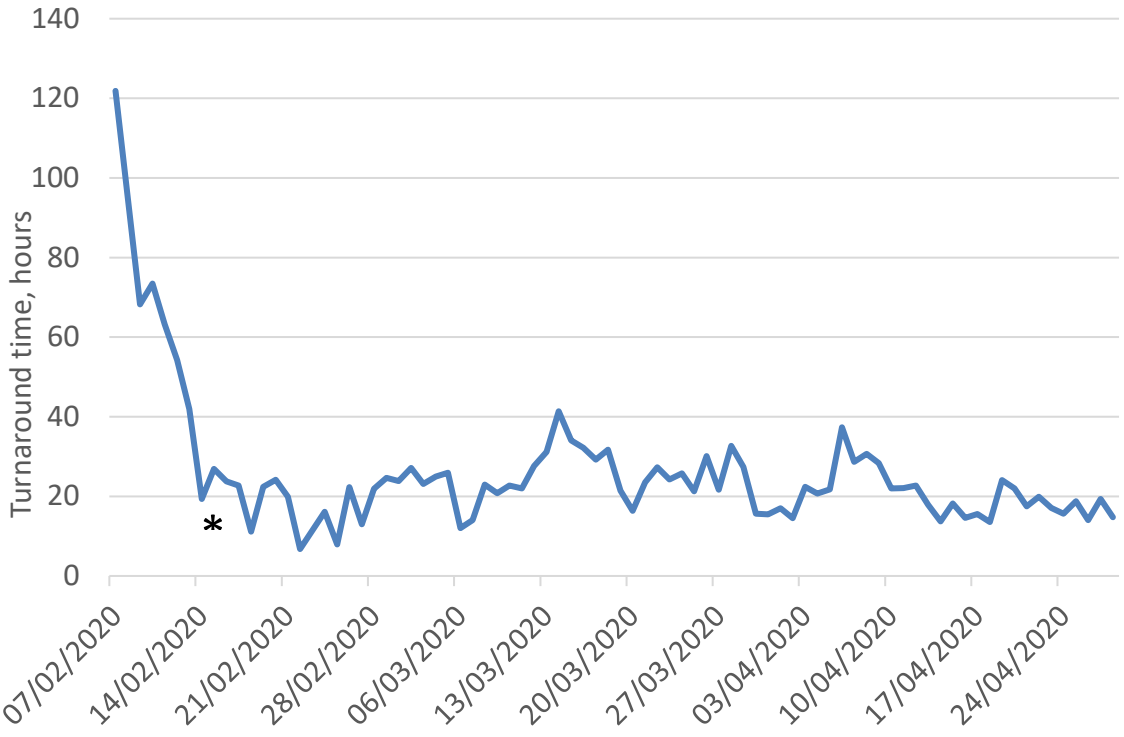


Figure 30: Directed acyclic graph representing theorised associations between variables related to group and outcomes



*Initially all PCR testing was performed off site at the PHE reference laboratory in Colindale, UK and on the 14th February in-house laboratory testing was introduced in the on-site PHE microbiology laboratory.

Figure 31: Median turnaround time for laboratory PCR before and during the study

Table 45: Multivariable analysis, results of Cox proportional hazards model for the outcomes time to results and time to destination

	Hazard ratio (95%CI)	**p value**
Time to results (n=1022)		
Group (ref: control)	4023 (545 to 29696)	<0.0001
Sex (ref: female)	1.04 (0.92 to 1.18)	0.53
Age*	1.00 (1.00 to 1.00)	0.56
NEWS2*	0.98 (0.96 to 1.00)	0.052
Time of presentation (ref: period from 6pm to 8am)	0.99 (0.87 to 1.12)	0.87
Time to destination (n=541)		
Group (ref: control)	10.2 (8.04 to 13.0)	<0.0001
Sex (ref: female)	1.04 (0.88 to 1.24)	0.64
Age*	1.00 (0.99 to 1.00)	0.97
NEWS2*	1.03 (1.00 to 1.06)	0.030
Time of presentation (ref: period from 6pm to 8am)	1.35 (1.13 to 1.60)	0.001

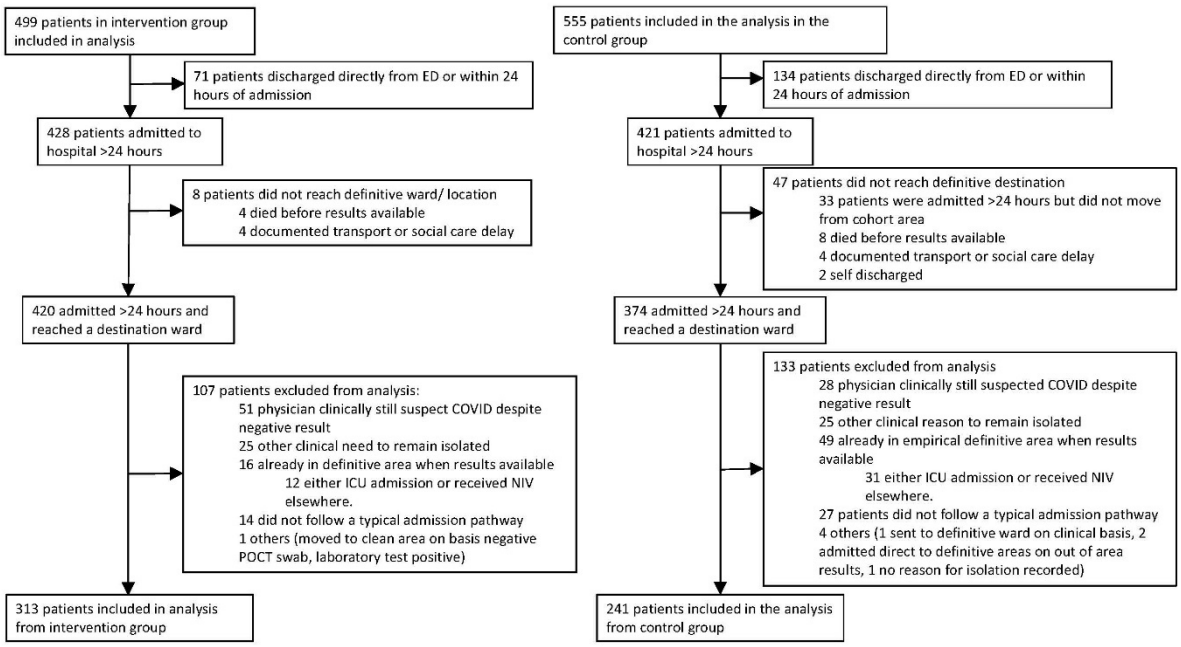


Figure 32: CoV-19 POC Bed moves profile

Table 46: Detection of respiratory viruses by QIAstat-Dx Respiratory SARS-CoV-2 Panel in the POCT group, n=499

SARS-CoV-2 Respiratory Panel Result	**Number, (%)**
Negative	278 (55.7)
Positive for any pathogen	221 (44.3)
Positive for SARS-CoV-2	197 (39.5)
SARS-CoV-2 only	192 (38.5)
SARS-CoV-2 + *Mycoplasma pneumoniae*	3 (0.6)
SARS-CoV-2 + Adenovirus	1 (0.2)
SARS-CoV-2 + Human Coronavirus-HKU1	1 (0.2)
Positive for non SARS-CoV-2 pathogen only	24 (4.8)
Rhino/enterovirus	11 (2.2)
Mycoplasma pneumoniae	5 (1.0)
Human Coronavirus*	4 (0.8)
Human metapneumovirus	3 (0.6)
Mycoplasma pneumoniae + Rhino/enterovirus	1 (0.2)

*HCoV-OC43 x3, HCoV-NL63 x 1

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