University of Southampton

Faculty of Medicine

Clinical and Experimental Sciences

Molecular point-of-care testing for respiratory viruses

by

Nathan James Brendish

Thesis for the degree of Doctor of Philosophy

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Abstract

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Respiratory virus infection is a common cause of hospitalisation in adults. New molecular platforms have been developed that can be deployed as point-of-care tests (POCT). Use of molecular POCTs for respiratory viruses may improve clinical care. This thesis explores the clinical impact of molecular point-of-care testing for respiratory viruses in adults hospitalised with acute respiratory illness.

We did a large pragmatic, open-label, randomised controlled trial of routine molecular POCT for respiratory viruses, compared with routine clinical care, in adults presenting with acute respiratory illness to a large teaching hospital in Southampton over two winter seasons (the ResPOC trial).

We recruited 720 patients of which 714 were included in the modified intention-to-treat analysis (360 assigned to POCT and 354 to routine care). The proportion of patients who received antibiotics was 84% in the POCT group compared with 83% in the control group ($p=0.84$). Of those who received antibiotics, 17% in the POCT group received a single dose or <48 hours of antibiotics compared with 9% in the control group ($p=0.0047$). Mean length of stay was shorter in the POCT group (5.7 days) than in the control group (6.8 days; $p=0.443$). Appropriate antiviral treatment of influenza-positive patients was more common in the POCT group (91%) than in the control group (65%; $p=0.0026$).

We went on to show that POCT with a turnaround time to results of <1.6 hours was associated with higher rates of early hospital discharge and early discontinuation of antibiotics compared to longer turnaround times.

To further explore attitudes to neuraminidase inhibitor use in influenza treatment, an online, cross-sectional questionnaire-based survey of self-reported prescribing practice using clinical scenarios was distributed to frontline clinicians. There were 237 respondents. Adherence to national treatment guidelines in the clinical scenarios ranged from 56% to 72% with considerable variability between specialities.

Using ResPOC trial data, I also examined the reliability of pneumonia diagnoses on discharge documentation. 28% of patients with a diagnosis of pneumonia had no radiological evidence of pneumonia. 35% of patients with clinico-radiological evidence of pneumonia did not have a diagnosis of pneumonia recorded.

In conclusion, while routine molecular POCT for respiratory viruses did not reduce the proportion of patients treated with antibiotics, POCT led to higher use of single doses or brief courses of antibiotics. POCT was also associated with a reduced length of stay and antiviral use and appeared to be safe. Rapid testing turnaround times were associated with better outcomes and unlikely to be achievable with centralised laboratory testing; this suggests that viral diagnostics should be performed at the point-of-care. The trial data also showed frequent misclassification of pneumonia diagnosis on discharge documentation and this may have clinical, financial and research data implications. A large survey of frontline hospital physicians suggested that guideline adherence in influenza treatment was sub-optimal but strategies that promote rapid diagnostic testing such as molecular POCT may improve adherence.

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Research Thesis: Declaration of Authorship

Print name:	Nathan James Brendish

Title of thesis:	Molecular point-of-care testing for respiratory viruses

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.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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Brendish NJ, Clark TW. Molecular point-of-care testing for influenza to improve early neuraminidase inhibitor treatment and outcomes in hospitalised adults. *Clin Infect Dis* 2019; 68:2154-2155

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Brendish NJ, Malachira AK, Armstrong L, Houghton R, Aitken S, Nyimbili E, Ewings S, Lillie PJ, Clark TW. Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial. *Lancet Respir Med* 2017; 5:401-411.

Brendish NJ, Malachira AK, Clark TW. Molecular point-of-care testing for respiratory viruses versus routine clinical care in adults with acute respiratory illness presenting to secondary care: a pragmatic randomised controlled trial protocol (ResPOC). *BMC Infect Dis* 2017; 17:128.

Brendish NJ, Schiff HF, Clark TW. Point-of-care testing for respiratory viruses in adults: The current landscape and future potential. *J Infect* 2015; 71:501-10.

Signature:		Date:	

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Definitions and Abbreviations

95% CI: 95% Confidence Interval

AE: Adverse Event

AMU: Acute Medicine Unit

ARI: Acute Respiratory Illness

CAP: Community-Acquired Pneumonia

CE: Conformité Européene or European Conformity

CI: Chief Investigator

COPD: Chronic Obstructive Pulmonary Disease

CT: Computed Tomography Scan

ED: Emergency Department

FDA: Food and Drug Administration

IECOPD: Infective Exacerbation of COPD

IRAS: Integrated Research Application Service

GCP: Good Clinical Practice

hMPV: Human Metapneumovirus

ILI: Influenza-Like Illness

IQR: Inter-Quartile Range

LRTI: Lower Respiratory Tract Infection

MHRA: Medicines and Healthcare Products Regulatory Agency

NAI: Neuraminidase Inhibitor

NHS: National Health Service

NPLRTI: Non-Pneumonic Lower Respiratory Tract Infection

PCR: Polymerase Chain Reaction

PI: Principal Investigator

PIV: Parainfluenza virus

POCT: Point-of-Care-Test

PPE: Personal Protective Equipment

qPCR: Quantitative (real-time) PCR

R&D: Research and Development

RCT: Randomised Controlled Trial

REC: Research Ethics Committee

RSV: Respiratory Syncytial Virus

RT-PCR: Reverse Transcription PCR

SAE: Serious Adverse Event

SD: Standard Deviation

SOP: Standard Operating Procedure

TAT: Turnaround time

QALY: Quality Adjusted Life Year

Chapter 1 Introduction

1.1 Overview

Acute respiratory tract infections are responsible for around 3 million deaths worldwide each year.[1] Although bacteria have previously been considered to be the principal aetiological agents of severe respiratory infection, the global importance of respiratory viruses in all age groups has been increasingly recognised in recent years.[2–4] Diagnostic technology for respiratory virus detection has evolved rapidly over the last two decades from viral culture and immunofluorescence to the current standard of molecular detection by polymerase chain reaction (PCR). The following literature review focuses on the clinical impact of respiratory viruses, the currently available molecular diagnostic platforms for respiratory virus detection with potential for use as point-of-care tests (POCT) in adults and their potential for clinical impact.

1.2 Respiratory viruses: clinical and economic burden of disease

In adults, respiratory viruses have been detected by molecular diagnostic techniques in approximately 20-40% of community acquired pneumonia (CAP) cases,[4–9] 50-70% of asthma exacerbations,[10] and 30-50% of chronic obstructive pulmonary disease exacerbations.[3,11] In hospitalised adults with acute respiratory illness, viruses are the most commonly detectable pathogen (being detected in around 50%) with bacterial detection being much less frequent, although antibiotic use is close to universal.[3] In children, up to 72% of cases of community acquired pneumonia,[12,13] over 90% of infants with bronchiolitis,[14] and approximately 85% of asthma exacerbations,[15] are associated with respiratory virus detection.

Furthermore, preceding respiratory viral infection is thought to be a key predisposing event to secondary bacterial infections in the respiratory tract. This co-pathogenesis is characterised by complex interactions between co-infecting pathogens and the host, leading to disruption of physical barriers, dysregulation of the host immune responses and delays in a return to homeostasis.[16–18]

Respiratory viruses including influenza have also been implicated in precipitating non-respiratory illnesses such as myocardial infarction, venous thrombo-embolism, stroke and loss of diabetic control.[19–25] The increase in incidence of ischaemic stroke after respiratory virus infection is particularly evident in older adults and the increased risk duration lasts for up to 28 days but the risk period is more transient for acute myocardial infarction, suggesting potentially different

underlying mechanisms.[19,26] Myocarditis and encephalitis are other well-described non-pulmonary complications of influenza and some other respiratory virus infections.[27,28]

One notable recent finding was the incidence of admission for acute myocardial infarction was six times as high during the first seven days after laboratory-confirmed influenza infection. The incidence of acute myocardial infarction was also elevated after non-influenza respiratory virus infection.[25] A previous study suggested that 10% of patients admitted with acute myocardial infarction had an unrecognised influenza virus infection.[29]

Infection by respiratory viruses, similar to many other infections, can lead to an acute undifferentiated febrile illness. This may lead to diagnostic confusion and potentially unnecessary antibiotic treatment and investigations, or potentially the opposite, that is, failure to treat potentially rapidly lethal infections, such as meningococcal disease, as was noted during the 2009 influenza pandemic.[30,31]

Based on the 2003 population size, seasonal influenza epidemics resulted in an average of 610,660 life-years lost, 3.1 million hospital days and 31.4 million outpatient visits in the USA.[32] US direct medical costs averaged US$10.4 billion annually, and projected lost earnings due to illness and loss of life amounted to US$16.3 billion annually. The total economic burden of annual influenza epidemics using projected statistical life values amounted to US$87.1 billion.[33] The common cold also causes a significant economic burden with a US-based study estimating that non-influenza, viral respiratory tract illnesses (mostly common colds) cost around US$40 billion in 2001.[34]

Over 28,000 hospitalisations and over 7000 deaths are estimated to be attributable to influenza-related respiratory disease in a mean season in the UK.[35] Mortality attributable to seasonal influenza appears to vary significantly year to year within the UK.[36,37] During the 2014-2015 influenza season, around 27,500 deaths in England were attributed to influenza virus infection; most deaths were in adults over 65 years old.[37] Respiratory syncytial virus infection in England and Wales is associated with about 5000 to 7000 deaths each winter season, with the elderly again accounting for most of these deaths.[38] Influenza is estimated to be associated with over 850,000 primary care visits per year in the UK.[39] Cost data associated with respiratory virus infection in the UK is scarce. An independent review into the cost of the 2009-2010 UK influenza pandemic found that the total cost to the UK Government alone of preparedness and response was about £1.2 billion.[40]

1.2.1 Influenza

There are four types of influenza virus: A, B, C and D. Influenza A and B cause clinically important disease in humans and seasonal epidemics. Influenza A was traditionally thought to cause more severe disease than influenza B but this view is now broadly changing to one of equals in this regard, although influenza A virus infection is generally more common.[41-44]

Influenza C virus infection appears to be very rare and is associated with mild or clinically silent infection.[45] Human influenza D virus infection has only been shown from serological studies, notably from occupational exposure to host livestock.[46,47] Lacking any relevance to this work, influenza C and D are not considered elsewhere in this thesis and any reference to influenza unspecified is considered to apply only to influenza A and B.

Influenza A viruses are classified by two large proteins found on the outside of the viral particles. Haemagglutinin mediates viral binding to host cells for entry and neuraminidase is key for releasing virions from host cells, giving rise to the 'H' and 'N' in classification respectively.[47]

Currently circulating influenza strains in humans are influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B viruses (B/Victoria and B/Yamagata lineages).[47] A typical seasonal pattern in reported influenza virus infections in the UK is displayed in Figure 1. Minor acquired mutations in viruses occurring between influenza seasons contribute to winter annual epidemics in temperate climates and are known as antigenic drift. Infection in tropical and subtropical regions occurs year-round. Antigenic shift describes influenza A viruses when a more substantial change in viral proteins occurs, often from genome reassortment with a circulating strain from animals.[47,48] As immunity to the new strain is less common, the new virus spreads rapidly, often with more severe clinical disease, and in the worst cases results in a pandemic (severe global epidemic).[48] The last pandemic occurred in 2009 to 2010 with a novel H1N1 virus from a triple reassortment of avian, swine and human influenza virus combined with a swine-origin virus.[49,50]

The influenza virus causes seasonal epidemics in temperate climates leading to excess hospitalisations and death mainly in the elderly and in patients with co-morbidity.[51,52] It causes up to 650,000 deaths worldwide each year.[53] Annual seasonal influenza vaccine is recommended in high risk groups,[54] however vaccine uptake is variable and sometimes limited,[48,55] and vaccine efficacy in the elderly is sub-optimal.[56]

The rate of hospitalisation in adults with influenza in developed countries has been estimated at 5 to 20 per 100,000 overall population,[57,58] and may be as high as 1200 per 100,000 in those over 85 years old.[59] In adults hospitalised with laboratory confirmed influenza, 10-30% are admitted to

critical care units and 3-15% die in hospital,[60,61] with outcome broadly predicted by comorbidities.[62]

A Canadian study estimated that only around 1 in 14 emergency department visits due to influenza virus infection were correctly attributed to influenza.[63] In addition, hospital admission coding data, particularly in the frail elderly most susceptible to influenza, often omits a diagnosis of influenza or other virus, but describes the presenting complaint that the respiratory virus infection precipitated instead, such as falls, dehydration, and altered mental state.[22] Combined with the non-respiratory presentations of respiratory virus infection discussed previously and suggested by a recent *Lancet*-published estimate of global seasonal influenza mortality,[53] it is highly likely that the burden of influenza is severely under-estimated.

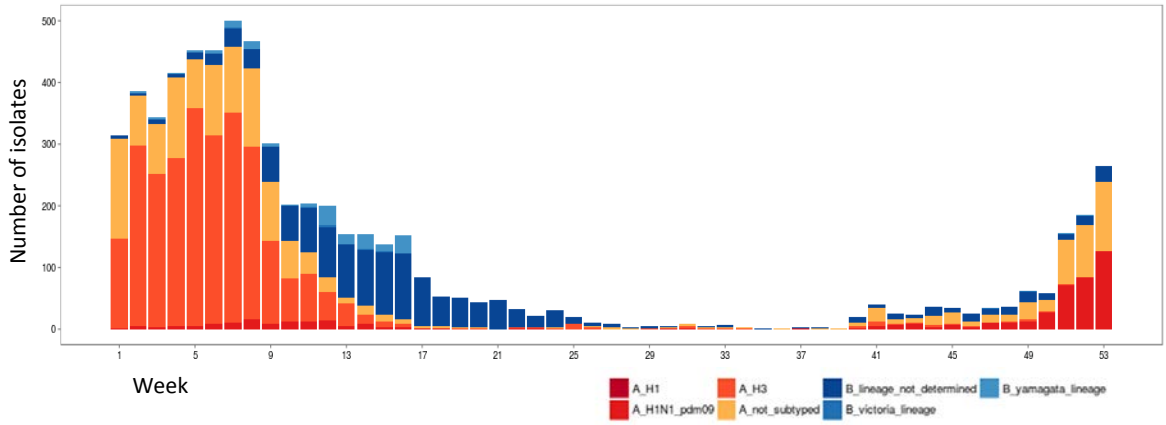


Figure 1: Circulating influenza viruses reported to WHO through laboratory surveillance for the UK in 2015

Data from WHO FluNET Interactive (https://pmacp.shinyapps.io/Influenza_isolates/). This demonstrates the winter seasonality of UK influenza infections: this graph of 2015 infers that in winter 2014-2015 influenza A H3N2 was the dominant virus (left peak), whereas in winter 2015-2016, influenza A H1N1pdm09 was more prevalent (right peak). For 2015, the peak of influenza B came after the peak of influenza A as is typical.

1.2.2 Respiratory Syncytial Virus

Respiratory Syncytial Virus (RSV) is the principal cause of bronchiolitis in infants but is now increasingly recognised as a major cause of respiratory illness in adults, with some studies suggesting a disease burden similar to that of influenza.[21,64] RSV affects all age groups and a study of hospitalised children and adults that calculated disability adjusted life years (DALYs) concluded that influenza and RSV were consistently the greatest causes of disease across all age groups.[65]

Adults at high risk of severe RSV disease include the frail elderly, those with chronic cardio-respiratory disease and the immunocompromised. The mortality rate of RSV infection in adults and the elderly is similar to that of influenza (7-8%) but may reach 30-70% in the heavily immunocompromised,[66] contrasting with the comparably negligible RSV-related mortality in infected children.[67] There appears to be no difference in clinical severity or magnitude of viral load between the two types of RSV, A and B.[68]

1.2.3 Rhinovirus

There are three species of rhinovirus, A, B, and C. There is no successful vaccine predominantly due to significant antigenic heterogeneity; there are over 150 serotypes.[69] The three rhinovirus species are now classified in the genus *Enterovirus* in the family *Picornaviridae* (picornaviruses).[70]

Rhinoviruses are responsible for the majority of common colds and adults typically suffer two to four symptomatic episodes per year.[71] They are also responsible for the majority of exacerbations of asthma in adults and a significant proportion of exacerbations of COPD.[10,11,72] They are detected in about one quarter of all infant bronchiolitis cases.[70] Common colds cause an estimated 20 million lost workdays per year in the US, with an estimated annual cost of around US$410 million for rhinovirus-related asthma exacerbations.[73]

1.2.4 Other respiratory viruses

Adenovirus infections are a common cause of mild acute respiratory illness in children, but also cause epidemics among adults in close proximity to each other and can cause serious infections in the immunocompromised and occasionally in immunocompetent adults.[74,75] Adenovirus is the only respiratory virus discussed here that has a DNA genome; all the other viruses are RNA-based.[76] Human bocavirus is a DNA-based respiratory-route virus but its clinical significance is very much debated, especially in adults with severe disease, where there is limited evidence of causal pathogenicity.[77,78]

There are four non-pandemic-potential human coronaviruses. The clinical impact of human coronaviruses 229E and OC43 infection has only recently been explored, with early data suggesting a prevalence in adults hospitalised with acute respiratory illnesses of between 3% and 11%.[3,79] Coronavirus NL63 was found in 13% of young adults with acute respiratory illness in a US community setting, whereas coronavirus HKU1 was found in less than 1%.[80]

A US multi-centre study showed that the prevalence of human metapneumovirus (hMPV) infection in hospitalised adults with respiratory symptoms was 2.6% and patients had similar

clinical characteristics to those infected with RSV infection with increasing age being a risk factor for emergency department visit and hospitalisation.[81] Parainfluenza viruses seem to be of less importance in adults compared to paediatric populations, however they can cause influenza-like illness in adults and are detected in adults hospitalised with acute respiratory illness at low frequency.[3,65,81,82]

1.2.5 The significance of respiratory virus detection

Respiratory viruses can be detected in asymptomatic adults and children. Although respiratory virus shedding typically lasts a few days, as detected by modern molecular techniques, occasionally viral shedding can be prolonged for three weeks or more. These longer durations are more often found with rhinovirus infections and viral infections in children rather than in adults. Adults with children in their household are at notable risk of asymptomatic and symptomatic viral infection compared to adults living alone.[83]

Some commentators have therefore suggested that detection of a respiratory virus by modern molecular techniques in an adult with an acute respiratory illness does not prove that the detected virus is the cause of that acute illness. While discussions about distinguishing between bacterial colonisation and active infection are not new, the recent introduction of sensitive molecular tests for respiratory viruses has added a viral dimension to this discussion.[84] Similarly, a patient who has a respiratory virus detected by a rapid molecular technique, could inadvertently have antibiotics withheld for a condition for which antibiotics may be needed (e.g. secondary bacterial pneumonia or incidental virus co-infection), and could therefore come to harm because of a positive virus test result. This reinforces the need to view new rapid molecular diagnostic tests for pathogens as a tool to aid overall clinical judgment, just as a chest radiograph or C-reactive protein test might add to patient care, rather than replacing clinical acumen.

A recent systematic review and meta-analysis of case-control studies in older adults (≥65 years old) provided significant evidence for respiratory virus infection being associated with acute respiratory illness. The study summarised over five thousand cases of acute respiratory illness across 16 studies, including studies in hospitalised adults. The study detailed pooled odds ratios (OR) and virus-specific attributable fractions among the exposed (AFE) as a quantification of the aetiological role of each virus in patients with acute respiratory viruses, compared to asymptomatic or healthy older adults, for multiple respiratory viruses. The virus-specific ORs for detection of influenza virus, RSV, and hMPV were all between 8.3 and 9.8 and AFEs for these three viruses plus parainfluenza viruses, adenovirus and rhinovirus were all between 86% and

100%, compared with asymptomatic or healthy older adults. Even rhinovirus detection had an OR of 7.1.[85]

This meta-analysis of studies complements the aforementioned evidence in prior sections of this chapter associating respiratory virus infections with a multitude of acute medical conditions, adding further credence to respiratory virus infections as significant causes of acute illness.

1.3 Laboratory PCR for detection of respiratory viruses

Nucleic acid amplification techniques such as reverse transcription polymerase chain reaction (RT-PCR) have now largely superseded cell culture and direct fluorescent antibody testing as the gold standard and method of choice for routine laboratory diagnostic testing for respiratory viruses. This is primarily due to their superior diagnostic accuracy and faster turnaround time. RT-PCR (not to be confused with real-time or quantitative PCR) is used to clone expressed genes by reverse transcribing the RNA of interest into its DNA complement through the use of reverse transcriptase. Subsequently, the newly created complementary DNA is amplified using traditional PCR. Laboratory RT-PCR is highly sensitive and specific but generally has a turnaround time of at least 24 hours and requires specialist laboratory facilities and expertise.[86,87]

1.4 Rapid antigen detection tests for respiratory viruses

There are several commercially available Food and Drug Administration (FDA) approved and European Conformity (CE) marked rapid diagnostic tests for respiratory viruses including influenza and RSV, which use antigen detection by either immune-chromatographic assay or immunofluorescence. The time to result is often around fifteen minutes. The tests are easy to use, do not require laboratory support, and generally involve visual inspection of test lines in addition to a control line, although some have a machine that interprets these for the operator (known as a Digital Immunoassay (DIA)).[88]

Unfortunately the clinical utility of rapid antigen based detection for respiratory viruses has been limited by their unacceptably poor sensitivity, often around 50% for influenza and lower for RSV in adults, meaning that their use in practice is severely limited and they cannot be used to rule out infection.[89-92] A large randomised controlled trial and meta-analysis found no clinical benefit of using rapid antigen tests for influenza in hospitalised adults.[92]

In addition, given the wide spectrum of viruses that can lead to acute respiratory illness, very few rapid antigen detection kits can detect a wide range of viruses.[93]

There is only one antigen-based system that detects more than three respiratory viruses (e.g. more than influenza A and B and RSV); the MariPOC (ArcDia Laboratories, Turku, Finland) is a CE marked, multi-analyte immunofluorescence-based antigen detection platform that can simultaneously detect eight respiratory viruses (influenza A and B, RSV, adenovirus, hMPV, and parainfluenza types 1, 2, and 3) in addition to *Streptococcus pneumoniae*. When compared to RT-PCR in children with acute respiratory illness, diagnostic accuracy was generally moderate although sensitivity was as low as 12.5% for some viral targets.[93-95]

1.5 Molecular platforms for the detection of respiratory virus with point-of-care test (POCT) potential

There are multiple rapid molecular test platforms that do not require specialist laboratory expertise and produce a result in around an hour or less. In general, they retain the sensitivity of laboratory PCR and some offer multiplex capability.

The FilmArray Respiratory Panel was used in the ResPOC study described in this thesis; it offers both rapid results and a multiplex detection of respiratory viruses allowing syndromic testing for respiratory viruses.

1.5.1 FilmArray Respiratory Panel

1.5.1.1 Platform Overview

The FilmArray Respiratory Panel (BioFire Diagnostics, Salt Lake City, UT, USA, a subsidiary of bioMérieux) is an FDA approved and CE marked platform that uses nested real-time PCR to detect 20 respiratory pathogens (17 viral targets and 3 bacteria). An unprocessed clinical sample (e.g. nose and throat swab in viral transport medium) is subjected to nucleic acid purification, reverse transcription, a high-order nested multiplex polymerase chain reaction and amplicon melt curve analysis. The FilmArray requires two minutes of “hands on” time and produces a test result in about one hour.[96]

Several studies have shown superiority of the FilmArray system in terms of ease-of-use and turnaround times compared with traditional laboratory PCR and other detection methods.[97-99]

1.5.1.2 Pathogen targets

The viral pathogens detected by the FilmArray Respiratory Panel are: Influenza A (untyped, A/H1, A/H1-2009, A/H3), Influenza B, Adenovirus, Coronaviruses (HKU1, NL63, 229E, OC43) Human Metapneumovirus, Human Rhinovirus/Enterovirus, Parainfluenza (types 1, 2, 3, 4) and Respiratory

Syncytial Virus. Three bacterial respiratory pathogens are also detected: *Bordetella pertussis, Chlamydophila pneumoniae and Mycoplasma pneumoniae*.[96,97] All targets are also listed in Appendix D. As rhinovirus show both considerable diversity but also considerable similarity to other viruses in the enterovirus family, many PCR-based systems cannot distinguish between rhinoviruses and other enteroviruses. However, the clinical entities caused by these different groups of viruses are generally distinct, and for the purposes of this research all positive targets for rhinovirus/enterovirus are considered to have been due to rhinovirus.

1.5.1.3 Sensitivity, specificity, and workflow

Sensitivity and specificity are broadly comparable to laboratory PCR (and with confirmatory cell culture and sequencing). Initial pooled sensitivity for viral targets compared with PCR was around 90%, lower than expected principally due to poor sensitivity in adenovirus detection, however, following improvements in the adenovirus assay, this has risen to over 95%.[91,92,100-102] It is notable that these studies were all conducted within a laboratory, rather at the point-of-care, and a large proportion of these studies were conducted using samples from children rather than adults. A notable limitation of the system is the workflow as only a single specimen can be tested at any one time on each analyser. A subsequent FilmArray platform designated the FilmArray Torch has the capacity to run up to 12 specimens simultaneously in a random-access manner.

1.5.1.4 How the FilmArray platform works

A standard operating procedure explaining the operation of the FilmArray platform is included in Appendix C. The consumables required for operation are shown in Figure 2. In brief, the procedure is that the operator first injects a FilmArray pouch in the dedicated hydration slot with a pre-filled hydration vial syringe. The operator squeezes the sample buffer into the sample injection vial, followed by pipetting three hundred microlitres of viral transport medium into the same sample injection vial, that is then introduced into the pouch via a dedicated sample slot. The prepared pouch is then placed into the FilmArray machine, patient and operator details are then added, and the lid of the machine closed prior to starting the run. The machine and associated laptop are shown in Figure 3. The run process takes about an hour to give a result (example given in Appendix D).[96] Further specific details relating to methods are given in the next chapter.

Once a run is started, the pouch is heat sealed. The sample, together with freeze-dried positive control yeast cells are moved into the large cell lysis blister (as seen in Figure 4). In this blister, the sample is mechanically agitated, and cells lysed, by ceramic beads. The nucleic acids are isolated by moving the sample lysate over silica-magnetic beads, which are then washed three times. The nucleic acids are then resuspended ready for reverse transcription and first stage PCR. Reverse

transcription occurs during three minutes at 54°C. The first stage PCR consists of 26 cycles of 94°C for four seconds followed by 60°C at 19 seconds. After a 225-fold dilution, the second (or 'nested') PCR occurs, consisting of cycles of 94°C for four seconds and 63°C for 19 seconds for 30 cycles. After the final PCR cycle, the amplicon melt analysis occurs where fluorescent images and corresponding temperature data in each well are collated creating a melt curve, which is then analysed to give a 'positive' or 'negative' result depending on the presence of target found in that well. Each target is replicated in triplicate, requiring two or three wells to be positive in order for a virus to be considered detected. For rhinovirus and influenza A, which have multiple targets due to the breadth of rhinovirus diversity and subtyping for influenza A, the final result is based on a combination of positive targets. The two controls (one positive, one negative) must also return appropriate results in order for the run to be valid and give results for the target viruses.[96]

Nested PCR is an adaptation of PCR, which overcomes the problem of non-specific or incorrect binding and subsequent amplification of non-target DNA. It does this by having a target amplified using two sets of primers used in two sequential runs of PCR. This allows amplification of a low number of target sequences in the first run of amplification, limiting non-specific products. The limitation of non-specific products is notably useful in samples where there is a high amount of background DNA, as may be found in nose and throat swabs with DNA from the host and commensal bacteria.

In the first step of nested PCR, a specific template is amplified using a pair of 'outer' primers. This PCR product is then diluted and subjected to a second step amplification using different primers located within the first PCR amplicon. The second step PCR product can then be detected by real-time or end-product (in this instance) analysis. Nested PCR is more sensitive than conventional PCR due to the ability to perform up to 50 or 60 total cycles of PCR. The specificity of nested PCR is similar to probe-based assays as all four primers must match the template. Although nested PCR was developed very early in the history of PCR, it has not been widely used in diagnostic settings as the apparatus typically used was an open-tube system which was highly prone to self-contamination. Several seals within the FilmArray pouch prevent self-contamination in this system.[96]

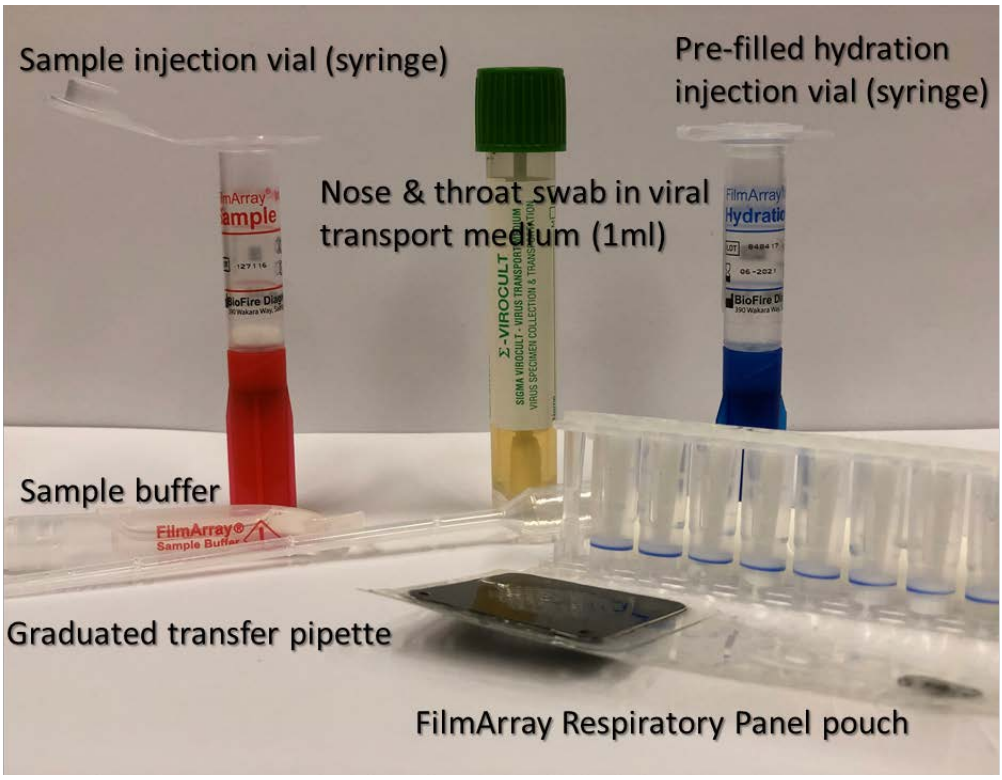


Figure 2: Consumables required for a FilmArray run

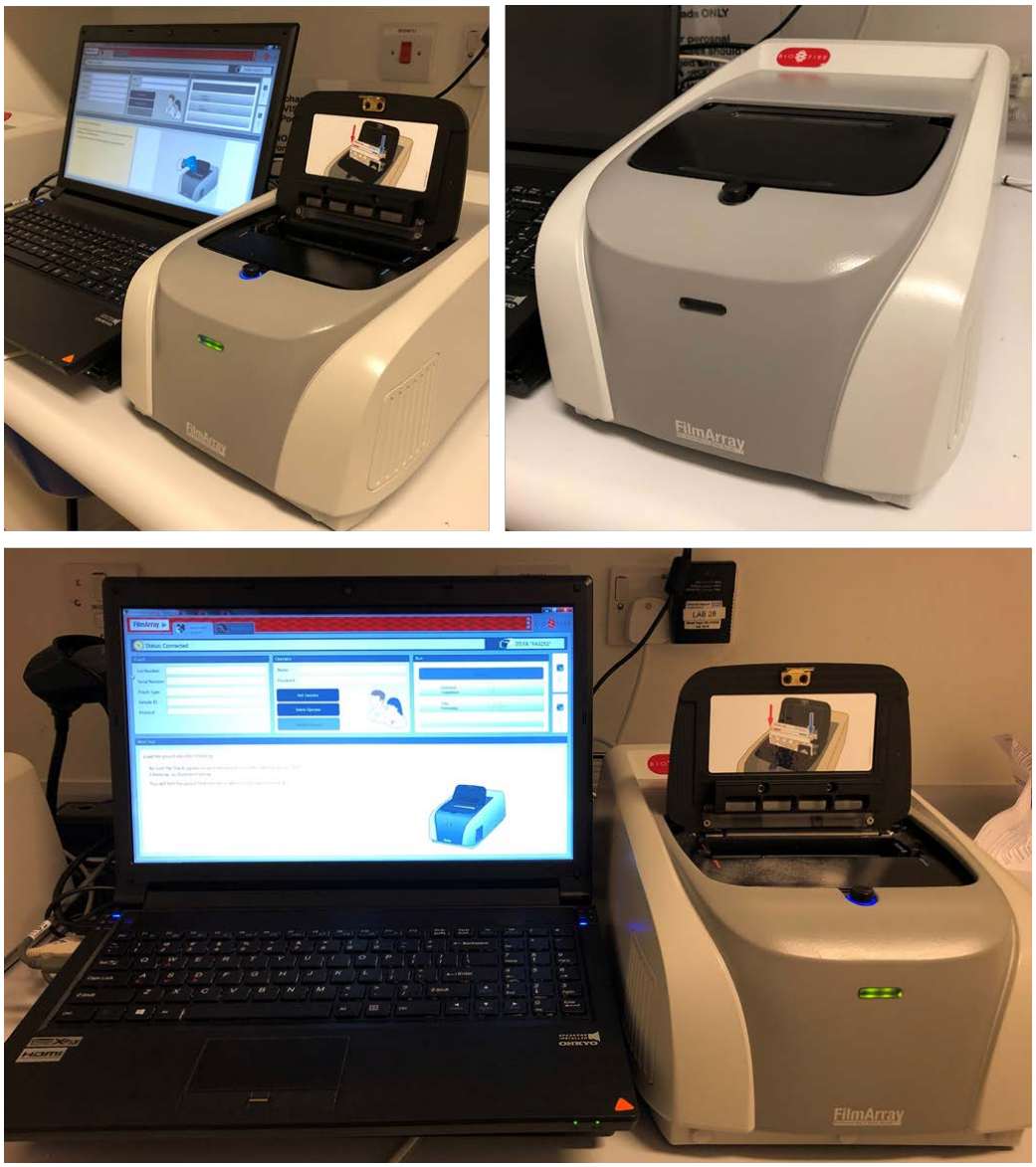


Figure 3: FilmArray machine and laptop

Unit deployed in the Acute Medicine Unit, Southampton General Hospital.

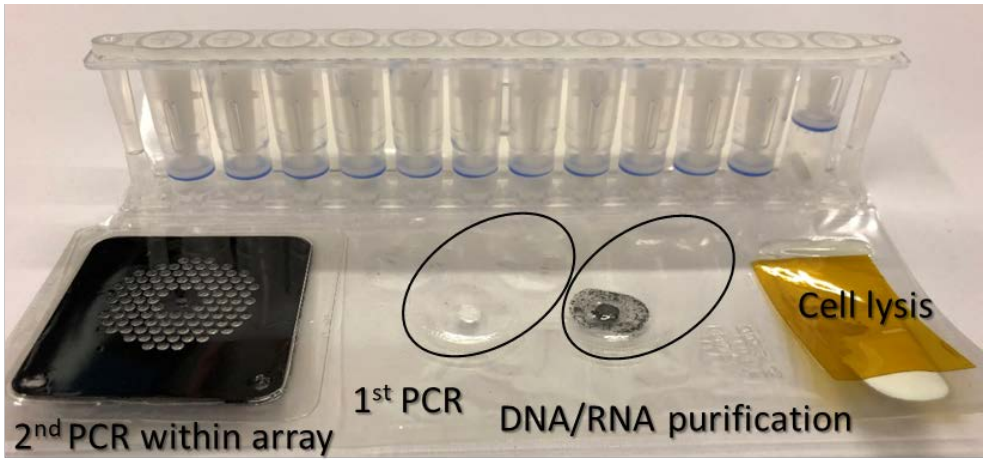


Figure 4: FilmArray Pouch

1.5.1.5 Studies with the FilmArray Respiratory Panel

A single study has examined clinical outcomes in children hospitalised with acute respiratory illness and tested with the FilmArray respiratory panel compared with standard laboratory PCR.[103] This was not a randomised controlled trial but examined outcomes pre- and post- intervention, and the FilmArray respiratory panel was not used as a POCT but was housed within the existing laboratory. This study demonstrated that in patients tested with the FilmArray, the test result was available to clinicians after a mean time of six hours versus around 24 hours with standard laboratory PCR. The duration of antibiotic usage was shorter in those tested with the FilmArray although this was dependent on the clinicians receiving the test results within four hours. The duration of inpatient stay and the time in isolation facilities were shorter in those tested with the FilmArray if the results were positive for viruses. Prior to the study described in this thesis, there have been no randomised controlled trials in children or adults published examining the potential clinical benefits of using this system as a POCT. Two relevant studies published after this work are compared to my findings in sections 4.17 and 4.18.

1.5.1.6 Newer version

Released after the start of the study that forms the basis of this thesis, the FilmArray Respiratory Panel 2 has a quicker run time of around 45 minutes and improved sensitivity compared to the original Respiratory Panel.[104]

1.5.2 Other platforms with POCT potential

This field is currently expanding rapidly.

There are several uniplex platforms, or platforms that detect a small range of viruses (typically influenza A and B +/- RSV), that are designed with point-of-care potential.

One such platform is the Alere i Influenza A&B (Alere, San Diego, CA, USA). This is an FDA approved and CE marked isothermal nucleic acid amplification-based system that uses a fluorescence-based molecular signal to detect influenza A and B. Results are generated within 15 minutes, with around 2 minutes of “hands on” time. The testing kits and analyser have been specifically designed to be used by non-laboratory clinical staff in an acute care environment. In a study examining diagnostic accuracy involving 545 respiratory specimens from symptomatic patients (85% children and 15% adults), the sensitivity and specificity of the Alere i Influenza A&B assay was 99.3% and 98.1% for influenza A, and 97.6% and 100% for influenza B compared to viral culture and PCR.[105] However, a Swiss study of 436 participants (broadly two-thirds children and one-third adults) showed a lower pooled influenza A and B sensitivity of 82.3% (mostly influenza A

rather than B) compared to PCR.[106] Another study using samples predominantly from adults, showed an even lower sensitivity for influenza A at 73.2%.[107] A UK-based diagnostic accuracy study showed a pooled sensitivity of 75.8% but despite this sensitivity, the study hypothetically modelled a substantial cost saving in terms of isolation and antiviral costs.[108] The high specificity demonstrated, simplicity of use and very fast turnaround time make the Alere i Influenza A&B test a promising prospect for point-of-care use. However, there have been no clinical trials evaluating clinical or health economic outcomes and the lower sensitivity in adults for influenza A and the limited range of pathogens detected are likely to markedly limit its usefulness in clinical practice.

The cobas Liat (Roche Diagnostics, Indianapolis, USA) platform uses PCR to give a result in under 20 minutes. It has the potential to detect influenza A and B, or influenza A and B and RSV with a high degree of specificity and sensitivity compared to laboratory PCR (values typically >96%).[109,110] It has been evaluated in a non-randomised, point-of-care study in an emergency department setting, but the study design, which tested changes in physician management, was open to significant potential bias.[111]

A systematic review and meta-analysis of novel rapid influenza tests, including the Alere and Liat systems found that these uniplex molecular systems had pooled specificities of >99% detection compared with laboratory PCR; however, while the Liat had pooled sensitivities for both influenza A and B >97%, the Alere system had a pooled sensitivity of only 84% for influenza A detection and 87% for influenza B detection. Concerningly, industry sponsored studies of these rapid molecular tests for influenza had a 17% higher pooled sensitivity for influenza B detection, and 6% higher pooled sensitivity for influenza A, than non-industry sponsored studies.[89]

The Xpert Flu (Cepheid, Sunnyvale, CA, USA) real-time PCR test cartridge is an FDA approved and CE marked test for use on the integrated, automated GeneXpert platform and detects influenza A and B with a turnaround time of about 75 minutes and reported “hands on” time of 2 minutes.[112] The modular multiple port system allows on-demand, random-access testing so that up to 16 tests can be run simultaneously (depending on the number of ports in the testing unit). The system has been evaluated in a prospective trial using samples from 300 adults with acute respiratory illness in emergency departments. In this group the sensitivity for detection of influenza was 95.3% (84.2%-99.4%) with a specificity of 99.2% (95% CI: 97.0%-99.9%) compared to laboratory PCR.[113] Although a comparatively easy to use test, there are currently no published trials evaluating its use as a point-of-care test or evaluating the potential clinical or health economic benefits of its use in emergency departments. The combined Xpert Flu/RSV cartridge has been evaluated in a retrospective study using adult and paediatric samples and demonstrated a sensitivity of 97% for influenza A, 100% for influenza B and 98% for RSV, with a specificity of

100% for all three viruses compared to laboratory PCR.[114] Although Xpert Flu and Xpert Flu/RSV have excellent sensitivity and point-of-care potential, the restricted range of viruses currently detected and relatively long turn-around time are limiting features of this system. One potentially useful feature of the GeneXpert series is that they provide a cycle-threshold (CT) value which is a good surrogate measure of viral load. This may allow some interpretation of the disease process underlying a patient's illness and facilitate clinical decision making and future research studies into magnitude of viral load compared with disease severity.

The Xpert Xpress Flu/RSV version of this Cepheid test became available in 2017, and although has no wider range of viruses detected, it does complete a test run in about 30 minutes and retains excellent accuracy compared to laboratory PCR.[115]

The ePlex Respiratory Pathogen Panel made by Genmark Diagnostics (Carlsbad, California, USA) uses a digital microfluidic technology to facilitate rapid thermal cycling to generate a result in about 90 minutes. It detects a wide range of respiratory viruses similar to the FilmArray Respiratory Panel (i.e. is a syndromic panel) and has broadly similar detection attributes to the FilmArray system too.[116-118]

1.6 Respiratory virus point-of-care testing in the wider context

The UK Department of Health commissioned reports into England's pathology services in the mid-2000s noted the importance of developing clinically relevant point-of-care diagnostic tests to reduce turnaround times and improve patient pathways.[119] Despite this, POCT for infectious diseases in the UK and globally have not advanced far beyond dipstick testing for urinary tract infection, with *in vitro* diagnostic tests for infection remaining confined to large centralised laboratories. The associated slow turnaround times mean that results are only available to clinicians many hours to several days after the patient has presented, and long after antimicrobial decisions have been made, perpetuating the current paradigm of empirical antimicrobial use rather than pathogen directed use. The Infectious Diseases Society of America policy paper "Better Tests, Better Care: Improved Diagnostics for Infectious Diseases" acknowledges the ongoing culture of empirical antimicrobial use and the unmet need for rapid accurate tests for infectious diseases to allow appropriate pathogen directed therapy.[120]

The World Economic Forum has stated that antimicrobial resistance is arguably the greatest threat to global human health and current high-profile initiatives on combating resistance have focused attention on antimicrobial stewardship, which seeks to preserve existing antimicrobial agents and slow the development of resistance. One of the strategies to achieve this goal is the development of rapid diagnostic tests and biomarkers so that antimicrobial use is pathogen-

directed rather than empirical and only used in those where there is clear evidence of benefit.[121,122]

The UK government and Wellcome Trust sponsored "Review on Antimicrobial Resistance" repeatedly cites the implementation of diagnostic technology as a key step in tackling the problem.[123] This review strongly promotes pathogen-directed antibiotic use, including use of point-of-care testing. Specifically, it recommends all antibiotic use in high income countries should be directed by rapid diagnostic testing by 2020, where a test is available.

The increasing recognition of the huge global burden of respiratory viruses has led to the creation of the WHO's global Battle against Respiratory Viruses Initiative (BRaVe) initiative which includes development of improved diagnostic tests including the development and use of cheap, accurate and easy-to-use POCTs.[124]

1.7 Potential clinical benefits of point-of-care testing

1.7.1 Reduction in antibiotic use

The current culture of empirical antimicrobial use in patients with suspected infection is no longer considered sustainable due the rise of antibiotic resistance. Antibiotic use in hospitalised patients with acute respiratory illness is near universal despite the predominance of viruses and the low frequency of bacteria detection in much of this group.[3] Furthermore, patients with acute respiratory illness syndromes that are known to be principally virally induced, such as asthma exacerbations and acute bronchitis, are often treated with antibiotics despite guidelines discouraging their use and the lack of evidence for benefit.[125-127] One recent UK study showed that almost 60% of patients hospitalised with an exacerbation of asthma received at least one dose of an antibiotic while in hospital.[3] Demonstrating to clinicians that the patient's symptoms are explained by the presence of a virus may, therefore, reduce antibiotic use. Patients with uncomplicated influenza-like illness caused by respiratory viruses including influenza are often treated with antibiotics due to diagnostic confusion with bacterial infection. In these patients the early detection of a respiratory virus in the absence of evidence of concomitant bacterial infection may prevent unnecessary antibiotic use.

The evidence base for respiratory virus testing reducing unnecessary antibiotic use is limited and mainly consists of trials using rapid antigen-based testing for influenza. A Health Technology Assessment, including a randomised controlled trial and meta-analysis found no benefit to antibiotic use in using rapid antigen tests compared with laboratory testing.[92]

However, one small non-RCT of hospitalised adults using rapid antigen testing for influenza demonstrated reductions in antibiotic use (74% vs 99%) with no increase in adverse events in those where antibiotics were withheld.[128] Studies in children, including several small RCTs, have evaluated the impact of routine rapid antigen testing on antibiotic use, with inconsistent results.[129-132]

For molecular tests, the evidence base is even more limited and trials tend to use molecular platforms within the laboratory rather than as POCT, with the associated prolonged turnaround times. The impact of next-day laboratory-based multiplex PCR respiratory virus testing in adult outpatients with acute respiratory illness was assessed in a small RCT. Antibiotic prescribing was significantly reduced compared to those treated with standard care.[133]

The FilmArray respiratory platform has been clinically evaluated in a single centre paediatric study where the FilmArray was housed in a central laboratory. Although not an RCT, it demonstrated reductions in antibiotic use in those testing positive for viruses with the FilmArray, although only when results were available within 4 hours, underscoring the importance of rapid results and suggesting possible further reductions if the platform was used as a POCT.[103]

A 2014 Cochrane review evaluating the use of rapid viral diagnostics for acute febrile respiratory illness in children in the emergency department concluded that there is currently insufficient evidence to support rapid viral testing to reduce antibiotic use in this setting. The authors have suggested an adequately powered trial with antibiotic use as the primary outcome measure.[134]

1.7.2 Directed antiviral agent use against influenza

The neuraminidase inhibitors (NAI) Oseltamivir and Zanamivir are licensed antivirals for the treatment and prevention of influenza and are recommend by Public Health England (PHE) for the treatment of hospitalised adults with suspected and confined influenza A and B.[135]

Influenza A and B viruses have a surface glycoprotein (neuraminidase) that cleaves the terminal sialic acid residue from various glyco-conjugates and destroys the receptors recognised by viral haemagglutinin. This activity is essential for the release of virus from infected cells, preventing viral aggregates occurring, and for viral spread throughout the respiratory tract. The neuraminidase inhibitors are sialic acid analogues that specifically inhibit influenza neuraminidases at the active enzyme site. There are four NAIs that are available: oseltamivir and zanamivir are available globally, peramivir is approved in Japan, South Korea, China and the Unites States of America, and laninamivir is approved solely in Japan.[136] Oseltamivir, given orally, and zanamivir, given intravenously (it can also be given by inhalation), do not appear to differ in

their treatment effectiveness in hospitalised adults with severe influenza virus infection.[137] Although all four NAIs are based on sialic acid, there are some structural differences that mean there is little cross-resistance between NAIs, for example, resistance against oseltamivir generally does not confer resistance to zanamivir.[138] Resistance to NAIs of currently circulating influenza strains is rare but does develop at times during treatment within patients. Notably, previously circulating influenza A H1N1, prior to the 2009 pandemic, was generally resistant to oseltamivir and that resistance mutation went from 1-2% to over 90% prevalence worldwide in about two years.[138]

Although there has been controversy regarding the evidence for the efficacy of NAIs from the original pharma-sponsored trials, there is now a large body of evidence from observational studies suggesting a significant reduction in mortality in hospitalised adults with confirmed influenza.[139,140] While the degree of benefit from NAIs is probably greatest when they are started with 48 hours of symptom onset, there is evidence in adults to suggest ongoing benefit when started beyond this time and up to 5 days after the onset of symptoms.[139,141] This is particularly pertinent as patients infected with influenza often present to hospital after at least 48 hours of symptom duration.[3] PHE guidelines recommend patients with suspected influenza are treated empirically with NAIs whilst awaiting the results of laboratory PCR.[135] This strategy leads to maximum benefit for patients who actually have influenza but unnecessary NAI exposure with the associated risk of side effects in patients who are subsequently found not to have influenza.

Following the 2015 publication of the independent meta-analysis of oseltamivir trials,[142] an editorial letter (published in *The Lancet*) suggested that, in view of the modest efficacy and moderate risk of nausea and vomiting with oseltamivir, the administration of this drug should ideally be directed with the use of diagnostic tests rather than used empirically.[143]

The use of a molecular point-of-care test has the potential to allow implementation of directed rather than empirical NAI treatment thus maximising clinical benefit for those with influenza infection and minimising unnecessary antiviral exposure and drug related adverse events in those without. Several studies in children and a single study in adults have suggested improved use of NAIs using rapid antigen testing for influenza versus routine clinical care although there have been no studies evaluating molecular platform POCTs with this outcome measure.[128-132,144]

Several observational studies have shown that rapid molecular laboratory testing for respiratory viruses in hospitalised adults has the potential to improve antiviral prescribing in both commencing and discontinuing neuraminidase inhibitors in patients with and without influenza respectively.[145,146]

In addition to NAIs, there are several promising novel and repurposed anti-influenza agents currently in late stages of clinical development including nitazoxanide, favipiravir (T-705) and targeted antibody therapy.[147–149]

Baloxavir is a selective inhibitor of influenza cap-dependent endonuclease. It has very recently been licenced for use in influenza virus infection in the US and Japan as a single dose oral treatment, in healthy adult outpatients with acute uncomplicated influenza. Baloxavir was without evident safety concerns, was superior to placebo in alleviating symptoms and was superior to both oseltamivir and placebo in reducing influenza viral load after one day. However, evidence of emerging resistance to baloxavir was found in nearly 10% of treated patients and studies of clinical outcomes are still yet to be published in hospitalised adults. A trial of baloxavir in adult outpatients with high risk of complications from influenza has just been completed, and a crucial trial of patients admitted to hospital with severe influenza is ongoing.[150,151]

Pimodivir is an inhibitor of the PB2 cap-binding subunit of influenza A viruses. It has been developed in both oral and intravenous solution formulations. It has little or no activity against influenza B virus. In some similarity to baloxavir, emerging viral resistance appears to occur in about 10% of healthy volunteers challenge studies. Diarrhoea occurs in about a quarter of all people treated with pimodivir. Two large scale, phase 3, randomised controlled trials of pimodivir treatment are in progress using twice daily dosing for five days in adolescents and adults with influenza A virus infection: one in hospitalised patients and the other in outpatients at high risk of complications.[151]

Similar hypothesised clinical benefits to neuraminidase inhibitors could be reasonably expected with a molecular POCT-directed therapy for these novel anti-influenza agents once their clinical effectiveness is established in hospitalised adults.

There are no clinical benefits of combining oseltamivir with amantadine and ribavirin (other antivirals with potential anti-influenza properties) over oseltamivir alone.[152] However, there is still interest in investigating combinations of NAIs with potential novel treatments, notably as NAIs have largely become the standard of care worldwide and therefore offering placebo influenza treatment over NAI treatment appears unethical for any control group.[153] There is currently near-universal resistance to the previously-used influenza A M2 inhibitors, amantadine and rimantadine, and therefore there is no clinical benefit in using these medicines in influenza treatment.[136]

1.7.3 Directed antiviral agent use against non-influenza respiratory viruses

1.7.3.1 Respiratory syncytial virus

There are currently no specific antiviral agents licensed for RSV infection. The broad-spectrum antiviral agent ribavirin is sometimes used in immunocompromised adults with severe RSV infection, but its use is limited by safety concerns and difficulties with administering the nebulised solution and there have been no randomised controlled trials to evaluate its efficacy.[154,155]

Several small molecule anti-RSV agents are in the late stages of clinical development including GS-5806 and ALS-8176 which have both shown promising results in challenge studies in healthy adults and are currently being trialled in hospitalised adults.[156,157]

1.7.3.2 Rhinovirus

There are currently no specific antiviral agents licensed for the prevention or treatment of rhinovirus infection. The rhinovirus capsid binding agent pleconaril showed promise in clinical trials of naturally occurring common colds but was rejected by the US FDA due to the relatively high frequency of side effects, drug interactions and concerns over resistance.[158,159] A single-centre randomised controlled trial of inhaled beta interferon has demonstrated reduced severity of rhinovirus induced asthma exacerbation in severe asthmatics and further trials are being considered with this treatment.[160]

1.7.3.3 Adenovirus

Cidofovir is an intravenous nucleoside analogue that inhibits viral DNA polymerase and is used in immunocompromised patients with disseminated and progressive adenovirus infection or pre-emptively in patients with persistent replication.[161,162] Its use is limited by toxicity, most notably nephrotoxicity. Brincidofovir is an oral lipid conjugate of cidofovir that increases its cellular uptake and is potentially more potent and less toxic. It has been used experimentally in a small number of immunocompromised patients with severe adenovirus disease and a larger number with asymptomatic adenovirus infection as a pre-emptive treatment, but larger-scale randomized controlled trials are lacking.[163,164]

1.7.3.4 Parainfluenza and human metapneumovirus

Ribavirin has been used in heavily immunocompromised patients with parainfluenza and hMPV infections, but the evidence base is weak.[165] DAS181, for parainfluenza virus infection, has undergone phase I trials and has been used in a few case reports.[166–169] It has been found to be generally safe and well tolerated. In addition, DAS181 may also have activity against hMPV.[170,171]

1.7.3.5 Conclusion of directed antiviral agent use against non-influenza respiratory viruses

While current antiviral treatment options for non-influenza respiratory viruses are extremely limited, there are a large number, and wide range, of promising antiviral medications undergoing clinical trials.

Table 1 summarises the range of clinically important non-influenza respiratory viruses and summarises antiviral agents in development.

The effective use of these novel antiviral agents, especially in hospitalised patients with severe disease where most patient benefit and cost effectiveness likely lies, requires prompt identification from a range of potential pathogens to allow virus-specific and virus-directed treatment. Multiplex, molecular point-of-care testing may offer such a solution.

Table 1: Clinically important non-influenza respiratory viruses and key antiviral agents currently used or in development

Antiviral	Mechanism	Target	Route	Phase
Ribavirin	Multiple	Broad	Inhaled, oral, parenteral	N/A
GS-5806 (Presatovir)	F-protein inhibitor	RSV	Oral	2b
ALS-8176 (Lumicitabine)	RNA polymerase inhibitor	RSV	Oral	2a
AK0529	F-protein inhibitor	RSV	Oral	2
ALN-RSV01 (Asvasiran)	Small interfering RNA	RSV	Inhaled	2b
ALX-0171	F-protein binding 'nanobody'	RSV	Inhaled	1/2a
T-705 (Favipiravir)	RNA polymerase inhibitor	Broad (influenza, RSV, rhinovirus)	Oral	N/A
DAS181 (Fludase)	Sialidase	Broad (influenza, PIV, hMPV)	Oral	2
Nitazoxanide	Multiple	Broad	Oral	3
Interferon-Beta	Enhanced innate antiviral response	Broad	Inhaled	2b
Pleconaril	Capsid binder	Rhinovirus	Oral	N/A
BTA798 (Vapendavir)	Capsid binder	Rhinovirus	Oral	2b
Cidofovir	DNA polymerase inhibitor	Adenovirus	Parenteral	N/A
Brincidofovir	Prodrug of cidofovir	Adenovirus	Oral	3

hMPV=human metapneumovirus; N/A=not available; PIV=parainfluenza virus; RSV=respiratory syncytial virus.

1.7.4 Infection Control

Respiratory viruses are highly infectious and cause nosocomial outbreaks and so testing and isolation of suspected cases is a central tenet of infection control practices in hospitals. Currently cases are isolated based on clinical suspicion with laboratory testing providing definitive results in 24-48 hours. This leads to patients without infection occupying valuable isolation facilities unnecessarily for several days and reducing patient flow through the hospital.[172]

Conversely, missed-diagnoses may result in a failure to isolate those that are infectious, potentially resulting in nosocomial and healthcare-worker infection. Although intuitively POCTs for respiratory viruses performed in emergency departments should improve isolation facility use and patient flow, there is a paucity of quality evidence for the effects of POCTs in this setting. A systematic review of published literature on the subject of POCTs for the diagnosis of infectious diseases concluded that although POCTs may have a role in infection control, the lack of good, consistent clinical data surrounding their use outside of the laboratory is a limiting factor in their implementation.[172] A single centre, non-randomised paediatric study from the UK suggested significant improvement in isolation facility use and patient flow with rapid antigen based POCT for RSV during the winter months.[173] The previously mentioned non-randomised pre- and post-intervention paediatric study using the FilmArray respiratory panel as a POCT demonstrated a reduction in the time spent in isolation facilities in patients tested with POCT versus standards laboratory PCR testing.[103]

Therefore, the impact of molecular POCT for respiratory viruses on isolation facility use compared with usual care can be measured in several ways including the proportion of all patients isolated, the proportion of patients with confirmed respiratory virus detection isolated, the time to isolation and the time to de-isolation. The impact on organisation level patient flow could be measured for all patients as isolation facilities may become vacant and therefore available to move other patients into more rapidly because of molecular POCT use. Similarly, if a molecular POCT strategy leads to shorter length of hospital stay or even reduces the number of admissions, available beds in ward areas may be created more rapidly; this could be measured by both the average wait time between admission decision to patient arrival in a bed, or even the impact on waiting times to be seen by a clinician in the emergency department.

Rapid molecular diagnostics that are comprehensive in their range of viruses may also allow safe ‘cohorting’ of a group of patients with the same respiratory virus infection in a designated ward area, rather than using multiple single occupancy isolation rooms, further promoting rational use of isolation facilities and patient flow through the hospital.[172] While cohorting did not occur in the study described in this thesis, it is a common infection control and hospital bed management

technique that could be included within outcome measures of trials of POCT in respiratory virus outbreak and pandemic situations in the future.

1.7.5 Other benefits

Several studies evaluating the use of antigen detection based POCTs for influenza in children have shown a decrease in the number of investigations performed on influenza positive patients compared with those tested with standard of care.[130–132,144,174] One of these also suggested a reduction in the duration of hospitalisation for those testing positive for influenza,[130] as did the previously mentioned study using the FilmArray in children.[103] Another study showed that lumbar puncture to rule out meningitis was common in children who subsequently were diagnosed with laboratory-confirmed influenza, and suggested that near-patient testing for influenza may result in clinicians being less inclined to perform lumbar punctures.[175] There can be significant syndromic overlap between influenza-like illness and meningitis, as both can present with fever and systemic symptoms, and diagnostic confusion can occur.[30,31]

The potential clinical benefits listed above could translate into an overall economic benefit for health care organisations. A study using decision analytic modelling to ascertain the most cost-effective testing strategy in children presenting to the emergency department with influenza-like illness suggested that rapid PCR using the FilmArray respiratory panel was superior to standard laboratory PCR or rapid antigen testing. However, the incremental costs per Quality Adjusted Life Year (QALY) were high and many questionable assumptions about the effects of diagnosing a respiratory virus infection on investigations, antibiotic and antiviral use were made.[176] Another study using health economic modelling evaluated the cost effectiveness of PCR based rapid diagnostics (Cepheid Xpert Flu assay) for the diagnosis of influenza in high risk adults presenting to the emergency department. They concluded that PCR based rapid testing was the most cost-effective strategy although this depended on the prevalence of influenza and again was based on strong assumptions of antiviral use and efficacy.[177] A modelling study of molecular POCT for influenza in elderly outpatients with ILI in Hong Kong appeared cost effective.[178]

1.8 Conclusion

The current global priority of replacing empirical antimicrobial use with pathogen directed therapy to help combat resistance, coupled with the recent development of rapid, accurate and easy-to-use molecular test platforms for respiratory viruses, sets the scene for developing a point-of-care testing strategy in patients presenting with acute respiratory illness. The potential benefits of such a strategy include a reduction in unnecessary antibiotic use, improved use of directed

antiviral therapy for influenza and improved use of isolation facilities in secondary care. There is a substantial evidence gap and high quality randomised controlled trials evaluating molecular POCTs in adults and using clinically relevant outcomes such as antibiotic use, directed antiviral use and infection control facility use are clearly warranted.

Chapter 2 ResPOC Trial Methods

2.1 Overview

The ResPOC (Respiratory virus Point-Of-Care testing) trial was a large, pragmatic, randomised controlled trial of routine syndromic molecular point-of-care testing for respiratory viruses, versus routine clinical care, in adults presenting to hospital with acute respiratory illness.

2.2 Study aims and objectives

This study aimed to prospectively evaluate the clinical impact of using a syndromic molecular point-of-care diagnostic test for respiratory viruses in adult patients presenting to secondary care with acute respiratory illness.

The primary objective of the study was to evaluate the impact of POCT on antibiotic use.

The secondary objectives were to evaluate the impact of POCT on respiratory virus detection, influenza antiviral use, isolation facility use, duration of hospitalisation, safety and also the turnaround time of results compared with standard laboratory-based PCR.

2.3 Study design

This was a single-centre, pragmatic, open-label, randomised controlled trial with parallel groups allocated 1:1 to the intervention point-of-care testing (POCT) and control (routine clinical care) arms. The framework was superiority. The study protocol adheres to the SPIRIT statement.[179]

2.4 Study setting

This study recruited patients from the Acute Medical Unit (AMU) and Emergency Department (ED), in Southampton General Hospital, part of the University Hospital Southampton NHS Foundation Trust.

2.5 Trial registration

This study was prospectively registered with ISRCTN, an international trials registry, number ISRCTN90211642, on 14th January 2015.

2.6 Ethics committee approval and amendments

Approval was obtained prior to study start from National Research Ethics Service Regional Ethics Committee North West–Preston (reference NW/14/1467). Two amendments to the protocol have been approved by the ethics committee, the first, to change the study from a pilot study into a full study and to amend an exclusion criterion, the second, to add a laboratory analysis plan for the samples collected (current protocol version 3.0, date 26th January 2016). All protocol modifications were communicated to investigators and to trial registries.

2.7 Participant eligibility criteria

2.7.1 Inclusion criteria

- Aged 18 years or over
- Has the capacity to give informed, written consent and is able and willing to adhere to the study procedures
- Is a patient in Southampton General Hospital's Acute Medical Unit (AMU) or Emergency Department (ED)
- Can be recruited to the study within a 24 hour period of first triage by ED staff OR within a 24 hour period of arrival on AMU (if admitted directly to AMU)
- Has an acute respiratory illness* and/or fever >37.5 °C
- Duration of illness less than or equal to 7 days

*An episode of acute respiratory illness was defined as an acute pulmonary illness (including pneumonia, bronchitis and influenza-like illness) or an acute exacerbation of a chronic respiratory illness (including exacerbation of COPD, asthma and bronchiectasis).

Provisional or suspected clinical diagnoses of acute respiratory illnesses were made by an AMU or ED clinician.

2.7.2 Exclusion criteria

- Patients not fulfilling all the inclusion criteria
- A palliative approach being taken by the treating clinicians

- Previously included in this study and re-presenting within the last 30 days after hospital discharge*
- Declines nasal/pharyngeal swabbing

Concurrent, prior or subsequent enrolment in an observational study is not necessarily an exclusion criterion; this is at the discretion of the chief investigator and the relevant regulatory authorities.

*In the first season of recruitment, previous inclusion in this study was an exclusion criterion, but this was amended for the second season to permit enrolment again if the participant had been discharged for more than 30 days, as acute respiratory illnesses are typically short episodes.

2.8 Trial processes

2.8.1 Overview

Patients were identified in the AMU and ED by research staff according to eligibility criteria and once written informed consent was obtained, they were immediately randomised to the intervention or control group. Those randomised to the intervention group had a nose and throat swab performed immediately by a study doctor or research nurse and this was then tested on the FilmArray machine. Results were available after approximately 1 hour and were immediately communicated to the clinical team. Clinical data were then collected retrospectively for both groups. There were no follow up visits for either group.

2.8.2 Screening, recruitment and consent

Potentially eligible patients in the ED and AMU of Southampton General Hospital were identified by research staff who would regularly review the comprehensive IT admissions systems in each area on a daily basis. Clinical staff in the ED and AMU also referred potential patient-participants directly to the study team.

Recruitment ran from January 2015 until April 2015 and from October 2015 to April 2016 in order to include the periods of peak influenza circulation for those seasons.

Potential participants were approached by a research doctor or research nurse, and the study was explained to the patient. The potential participant was given a participant information sheet (see appendices) and time to read it, ask questions and have them satisfactorily answered. Fully-

informed, written consent was required from the eligible patients. Consent was documented on consent forms (see appendices).

2.8.3 Sequence generation, randomisation and implementation

Once an eligible patient had been screened, and given fully informed, written consent they were enrolled and assigned a unique participant identification number consecutively. A study team member used a dedicated internet-based randomisation service (sealedenvelope.com, which uses random permuted blocks of varying sizes) to obtain a computer-generated randomisation code for the patient which assigned them to either the intervention or control group. Research staff implemented the allocation sequence and assigned the patients to the group based on the allocation code from sealedenvelope.com.

2.8.4 Intervention

2.8.4.1 For those randomised to the interventional arm

A nose and throat swab was taken by a study doctor or research nurse according to the Trust standard operating procedure (SOP) that was modified into a research-specific SOP (reproduced in Appendix C). Combined nose and throat swabs have been described as an effective approach to maximise PCR sensitivity for a large number of respiratory viruses while maintaining patient acceptability.[180–183] Swabs were placed directly into viral transport medium (Sigma Virocult®, MWE, UK). The sample was analysed on the FilmArray Respiratory Panel as per training delivered by the apparatus manufacturer. The machines' standard operating procedures are detailed in Appendix C so as to demonstrate how the machine was used. The machines were operated by a study doctor or research technician. Test results were then normally available in about an hour using the FilmArray Respiratory Panel. Example of a test result is given in Appendix D.

In the event of a run failure, the analysis run was repeated using the same sample. The FilmArray machines were located in or near the patient-care areas (AMU and ED). The results of the test were documented in the patient's case notes and in the event of a pathogen being detected, a doctor from the clinical team responsible for the patient was directly informed. The participant was also informed of the result on the same day.

2.8.4.2 For those randomised to the control group

These patients were managed by routine clinical care, as per current practice in this large teaching hospital in the United Kingdom, which was considered a justifiable comparator. Respiratory virus

testing using laboratory PCR was at the discretion of the responsible clinical team and where it occurred used laboratory-based multiplex PCR for respiratory viruses.

2.8.4.3 For both groups

A subgroup of participants was approached for venous blood sampling and additional nose/throat swabs to be stored for further study including immunological testing and viral sequencing. All samples were stored devoid of participant identifiable information to protect participant confidentiality.

2.8.5 POCT quality control and validation

In this trial, the FilmArray Respiratory Panel was deployed as a molecular point-of-care test.

Prior to commencing recruitment each winter season, each FilmArray machine system underwent a process of positive and negative control testing in line with manufacturer's instructions. Positive and negative control test runs were done in the same manner as an intervention group patient-participant test run with substitution of the nose and throat swab viral transport medium for either a positive control sample in viral transport medium or viral transport medium alone for the negative control. All detectable targets were tested at least once with positive control material of purified, intact virus and bacteria particles that had been chemically modified to render them non-infectious and refrigerator stable (ZeptoMetrix Corporation, Buffalo, New York, USA). Different positive control samples were combined into 'pools' during this positive testing process as recommended by manufacturer documentation. Positive control pools were made up in biological safety cabinets (class II) and away from the FilmArray units to prevent contamination of either the FilmArray unit or the positive controls. In addition, at least one negative control on each unit was run using viral transport medium alone at set up during unit manufacturer installation each season and after the positive controls were run, prior to recruitment commencement.

Negative controls of viral transport medium alone were run on each machine at least once per month. Both positive and negative controls could be run, additionally, at the discretion of the chief investigator.

2.8.6 Infection control considerations

As research staff were likely to be in frequent contact with patients with communicable diseases, notably respiratory virus infections, protection of staff was important. University Hospital

Southampton NHS Foundation Trust, like all NHS trusts, has an annual influenza vaccination campaign for all staff including research staff with the aim of protecting staff and patients.

During the acquisition of a nose and throat swab, personal protective equipment (PPE) was worn by research staff. Specifically, the staff member wore a surgical facemask, disposable plastic apron and disposable gloves, with handwashing before and after PPE use.

For interaction with any patients already in an isolation room (e.g. to a high suspicion of contagious disease) where staff use of PPE was mandatory regardless of clinical task, research team members followed the same PPE instructions that clinical staff followed.

During preparation of the FilmArray pouch for a test run, the operator's body and face was shielded from the sample and pouch by a transparent acrylic screen that still permits lateral access for hands and arms in order to prepare the pouch. In addition, PPE including surgical facemask, gloves and apron were worn. These measures both protect the operator from potential infectious diseases in the sample and the sample from contamination by the operator.

The FilmArray machines were regularly cleaned in line with the FilmArray manufacture's guidelines. The machines, area surrounding the machines, and the sample preparation surfaces were cleaned with clinell universal wipes (GAMA Healthcare, Watford, UK) after use.

2.9 Blinding

As this was a pragmatic trial of the clinical impact of using a diagnostic device no attempt at blinding trial participants, research staff or care providers was made. As the purpose of the study was to inform the clinical teams of the POCT results, clinicians and participants could not be blinded to which group a participant had been allocated to. Data analysts were blinded to group allocation.

2.10 Outcomes

2.10.1 Primary outcome

The primary outcome was the proportion of patients treated with antibiotics, measured retrospectively from case notes for the entire duration of hospitalisation or at 30 days, whichever was shortest.

2.10.2 Secondary outcomes

- Median duration of antibiotic use, days
- Proportion of patients receiving only a single (stat) dose of antibiotics
- Proportion of patients receiving <48 hours of antibiotics
- Proportion of patients receiving intravenous antibiotics
- Median duration of intravenous antibiotics, days
- Proportion of patients with influenza treated with influenza antivirals
- Proportion of influenza antiviral use occurring in patients with influenza
- Median time to influenza antiviral use, hours
- Median duration of influenza antivirals, days
- Median duration of hospital stay, days
- Proportion of patients admitted to a side room*
- Median duration of side room use, days*
- Median time to isolation or de-isolation, days*
- Median length of hospital stay, days
- Median turnaround time of respiratory viruses testing, hours
- Proportion of patients with viruses detected
- Proportionate mortality in hospital and at 30 days post randomisation
- Proportion admitted to intensive care or high dependency units
- Proportion re-presenting to hospital within 30 days
- Proportion re-admitted to hospital within 30 days
- Proportion with prolonged (>7 days) inpatient stay

* A side room is a single-patient room; the use of which is a surrogate measure for isolation for the purpose of infection prevention and control.

As both the first trial in this field and a pragmatic trial, having a wide range of clinically relevant secondary outcome measures was important to explore and quantify the breadth of potential patient benefits of the intervention.

Every attempt was made to recruit patients and deliver the results of POCT prior to antibiotic use however it was understood due to the usual processes of care in hospital that on some occasions antibiotics will have been administered before POCT is performed or before results are available to clinical teams. Therefore, we planned to undertake a *post hoc* analysis of only those patients where antibiotics were not administered prior to POCT results being available to clinicians.

In view of the deliberately broad inclusion criteria, heterogeneity of treatment effect among different clinical groups was anticipated and therefore subgroup analysis for the primary and certain key secondary outcome measures was planned based on clinical group. Results for antibiotic use (including any use, single dose, and use of less than 48 hours duration), duration of antibiotics, admission and length of hospitalisation were assessed separately for each clinical subgroup.

2.11 Sample size calculation

Sample size was based upon the primary outcome measure of proportion of patients treated with antibiotics. Previous studies have demonstrated that around 75% of patients hospitalised in the UK with acute respiratory illness are treated with antibiotics.[3] Two small studies in hospitalised adult patients with acute respiratory illness have suggested reductions in the proportion of patients treated with antibiotics of around 10–15% in those tested for respiratory viruses.[128,184] To detect a reduction in antibiotic use from 75 to 65% (i.e. 10% reduction) with a power of 0.8 and significance level of 0.05, 326 patients would be required in each group (based on $\chi 2$ test without continuity correction). Allowing for withdrawals in up to 10% of patients we aimed to recruit 360 patients to each group (720 patients in total).

2.12 Data collection, management and analysis

2.12.1 Data collection methods

Clinical and demographic data were collected at the time of enrolment by research staff from patient paper case notes and electronic medical records. Outcome data were collected retrospectively by research staff primarily from electronic medical records and electronic prescribing systems, some paper records, and electronic radiological and laboratory results systems. Final clinical diagnosis was based on clinical discharge coding and discharge summaries

along with radiographic reports. All source data were entered into a standardised paper case report form. Patients withdrawn from the study had no further data collected beyond the point of withdrawal. The use of multiple imputation was considered should missing data have exceeded 10% for the primary outcome; this was not required.

2.12.2 Data management

The study was conducted in accordance with the approved protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), relevant regulations and standard operating procedures. Data were evaluated for compliance with the protocol and accuracy in relation to source documents. Data from case report forms were entered into a secure bespoke database at the completion of the study followed by data lock. All data were anonymised: volunteer participant data were identified by a unique study number in the case report forms and database. A separate confidential file containing identifiable information is stored in a secured location in accordance with the Data Protection Act 1998.

2.12.3 Confidentiality

All data were anonymised to protect participant confidentiality: volunteer participant data were identified by a unique study number in the case report forms and database. Serious Adverse Events (SAEs) were reported in line with Good Clinical Practice (GCP) and regulatory requirements. All study staff were prospectively trained in GCP.

2.12.4 Access to data

The participant-anonymised final data set is wholly accessible to the chief investigator, co-investigators and independent statistician and may be made available to other parties on request only with the permission of the chief investigator. Only the investigators and sponsor's representatives (including potential monitors) have access to participant data, which are kept securely.

2.12.5 Dissemination policy

Authorship of manuscripts stemming from this protocol has and will follow the ICMJE recommendations, and CONSORT statement where appropriate, and there is no intent to use professional writers. There are no plans to make the dataset publicly available. Beyond the study team and regulatory oversight, the full protocol is only made available at the discretion of the

chief investigator. The data and samples collected are expected to form multiple publications, and these publications must acknowledge this trial and study team as appropriate.

2.12.6 Statistical methods

This was performed by a dedicated medical statistician from the University of Southampton independent from the study team. Patients tested with the molecular point-of-care test for respiratory viruses were compared with patients treated by routine clinical care using standard descriptive and comparative statistical methods using Prism version 6.0 (GraphPad Software, La Jolla, California, USA) and/or Stata version 13.1 (StataCorp; College Station, Texas, USA).

Owing to the very small proportion of patients who withdrew after randomisation, and the fact that withdrawals were predominantly due to breaches in inclusion criteria, the decision was made for analyses to be modified intention-to-treat.

Summaries of all baseline characteristics are presented using means and standard deviations, medians and interquartile ranges, or frequencies and percentages, as appropriate.

The primary outcome (antibiotic use) between groups (intervention and control) was initially compared using difference in proportions and unadjusted odds ratio. The effect of group on the primary outcome was further assessed using multiple logistic regression to control for the following covariates: demographics (age, sex), influenza vaccination status, duration of symptoms, receipt of antibiotics before presentation, comorbidity, temperature, C-reactive protein concentration, and clinical group. Duration of antibiotic use (recorded in hours; analysed and presented in days) was compared between groups using mean difference and unadjusted rate ratio. The effect of group was further assessed using the adjusted rate ratio from multiple negative binomial regression, controlling for the same covariates as for the multiple logistic regression. For other secondary outcomes, the intervention and control groups were compared using differences in proportions for binary data, and t tests and Mann-Whitney U tests for continuous data (e.g. turnaround time), as appropriate; the choice between the latter two tests was based on the distribution of the observed data and the sample size. Where 95% confidence intervals (95% CI) are presented, Stata version 13.1 defaults are used.

In view of the deliberately broad inclusion criteria, heterogeneity of treatment effect among different clinical groups *a priori* was anticipated and therefore pre-planned subgroup analysis was done for the primary and certain key secondary outcome measures on the basis of diagnostic group (e.g. exacerbation of asthma, exacerbation of COPD, pneumonia). Results are displayed for antibiotic use (including any use, single dose, and use for <48 hours duration), duration of

antibiotics, admission, and length of hospitalisation separately for each diagnostic subgroup. The interaction between clinical subgroups and group in both regression models described above was assessed. The original analysis plan was to include the interaction between clinical group and trial arm in the multiple logistic regression; however, the model was unstable, possibly due to the size of the subgroups. Instead, unadjusted comparisons of subgroups are presented. Owing to the nature of the analyses, and the many comparisons made, the results should be interpreted cautiously. All results presented relate to absolute differences in means or proportions.

2.13 Participant Safety and Monitoring

2.13.1 Harms

The risks of nose and throat swabs and additional blood tests being taken were assessed as minimal, and any outcomes, mild. No additional adverse events related to POCT for respiratory viruses were anticipated. However active monitoring and reporting of severe adverse events was undertaken. Serious adverse events (SAE) were defined here for this trial as:

• Death during admission or within 30 days of enrolment

• Admission to the intensive care unit

• Evidence of prolonged hospital stay

• New or persistent significant disability or incapacity

• Evidence of congenital anomaly or birth defect

As participants in ED are not yet hospitalised but have a reasonable likelihood of being admitted to hospital, patients enrolled in ED who were subsequently admitted to the hospital were not counted as having experienced a SAE. Participants who were already admitted to AMU are already hospitalised however, any adverse event leading to prolongation of their existing hospitalisation was counted as a SAE. All SAEs were reported to the sponsor in line with regulatory requirements.

2.13.2 Data review

The study was reviewed by the sponsor and felt to be of low risk on the grounds that it is not a clinical trial of an investigational medicinal product and the low likelihood of harms associated with the intervention. Therefore, the creation of a data monitoring committee was not felt necessary. No interim analysis of data was planned.

2.13.3 Monitoring

Monitoring is the responsibility of the sponsor. Data may be evaluated for compliance with the protocol and accuracy in relation to source documents at their discretion. Monitors have the ability to verify that the clinical trial is conducted, data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

2.14 Sponsor and sponsorship details

The sponsor is University Hospital Southampton NHS Foundation Trust, and sponsorship is overseen by the Research and Development (R&D) Department, Southampton General Hospital.

The sponsor's study reference and sponsorship number is RHM MED1217.

2.15 Funding and support

Funding and other support was obtained by the chief investigator from the following:

1. University of Southampton, Faculty of Medicine, Research Management Committee Pump Priming Grant.

2. University Hospital Southampton NHS Foundation Trust and NIHR Respiratory Biomedical Research Unit provided research nurses, clinical trials assistants and data managers to support this trial.

3. NIHR Southampton Wellcome Trust Clinical Research Facility, University Hospital Southampton NHS Foundation Trust, provided clinical research fellows to support this trial.

4. NIHR Clinical Research Network, Wessex provided clinical research fellows to support this trial.

The study sponsor, study funders and manufacturers of the FilmArray platform had no involvement in the conception, design or running of this study and have no involvement in the analysis of the data or writing of subsequent manuscripts for publication. Equipment and consumables were purchased from bioMérieux, UK.

2.16 Contributions of myself and others

I co-conceived the study and co-designed the study with Dr Tristan Clark. I wrote the study protocol, informed consent form, participant information sheet and letter to the general practitioner, informing them of the participant's enrolment. The trial protocol development

included a background literature search. I wrote the application in the Integrated Research Application System (IRAS) form, which includes the Research Ethics Committee (REC) submission and local Research and Development (R&D) office submission. For the amendments to the study, I wrote the protocol and participant information sheet changes and submitted the amendments to the REC and R&D and also wrote the annual and end of study reports for the REC. Throughout all of these tasks I was guided and supervised by Dr Tristan Clark, who was the chief (CI) and principal investigator (PI) and my supervisor.

I acknowledge that a successfully-run clinical trial requires a team approach. Overall, I recruited about one third of all the study participants. I am grateful to Dr Ahalya Malachira, Dr Lawrence Armstrong and research nurse Esther Nyimbili and senior research sister Sandy Aitken for recruiting participants and acquiring clinical specimens, alongside myself and Dr Rebecca Houghton for acquiring some of the virology laboratory data. Retrospective data collection and collation was done by myself and Drs Malachira and Armstrong, plus Dr Patrick Lillie and the CI. Key statistical work was primarily done by a medical statistician, Dr Sean Ewings, the CI, and myself.

Chapter 3 ResPOC Trial Results

3.1 Overview

This chapter reports the recruitment, participant characteristics, primary and secondary outcomes and key *post hoc* analyses of the ResPOC trial.

3.2 Recruitment

We recruited patients in two seasons: between January 15th 2015, and April 30th 2015, and between October 1st 2015, and April 30th 2016. We assessed 868 patients for eligibility and stopped the trial when 720 patients had been recruited. 362 patients were randomly assigned to receive POCT and 358 were randomly assigned to receive routine clinical care (control). One patient assigned to POCT declined to be swabbed and so did not receive the intervention. One patient in the POCT group and four in the control group were withdrawn due to protocol violations (Figure 5).

Therefore, 360 patients in the POCT group and 354 patients in the routine clinical care group were analysed in the modified intention-to-treat analysis (Figure 5).

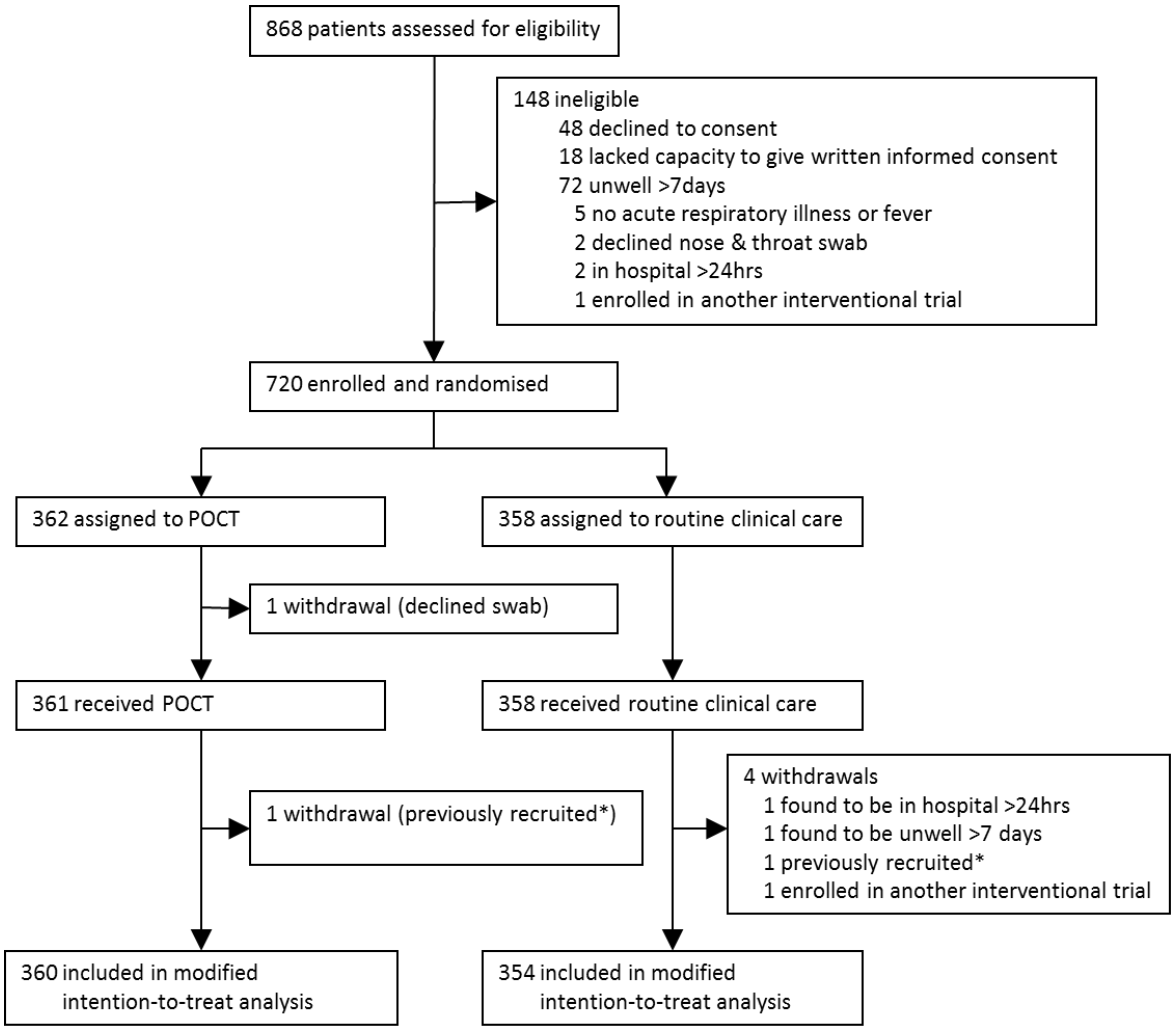


Figure 5: Trial profile

POCT= point-of-care testing. *Previous recruitment was an exclusion criterion during the first season (January 2015 to April 2015); this was changed for the second season (October 2015 to April 2016) to permit inclusion for patients presenting >30 days after hospital discharge.

3.3 Baseline demographics and clinical characteristics

Baseline demographics and clinical characteristics appeared similar between groups (Table 2).

Table 2: Baseline characteristics of participants

	POCT (n=360)	Control (n=354)
Age (years)	63 (41-75)	62 (44-74)
Sex		
Female	183 (51%)	185 (52%)
Male	177 (49%)	169 (48%)
Ethnic origin		
White British	337 (94%)	331 (94%)
Other	23 (6%)	23 (6%)
Current smoker		
Yes	92 (26%)	89 (25%)
No	268 (74%)	265 (75%)
Influenza vaccine*		
Yes	206 (57%)	208 (59%)
No	151 (42%)	143 (40%)
Duration of symptoms (days)	4 (2-6)	4 (3-5)
Antibiotics within 14 days†		
Yes	90 (25%)	91 (26%)
No	270 (75%)	263 (74%)
Antivirals within 14 days†		
Yes	0	0
No	360 (100%)	354 (100%)
Comorbidity		
Cardiovascular disease	132 (37%)	133 (38%)
Respiratory disease	213 (59%)	206 (58%)
Renal disease	20 (6%)	22 (6%)
Liver disease	7 (2%)	2 (1%)
Diabetes	48 (13%)	64 (18%)
Immunocompromised	18 (5%)	21 (6%)
Cancer	23 (6%)	25 (7%)
Observations		
Temperature (°C)	36.9 (36.4-37.7)	37.0 (36.4-37.8)
Temperature ≥38°C	64 (18%)	78 (22%)
Pulse rate (bpm)	100 (85-110)	100 (84-110)
Respiratory rate (bpm)	23 (19-28)	22 (18-26)
O_2 saturations (%)	96 (94-98)	95 (93-97)
Supplementary O_2	96 (27%)	76 (21%)
BP (mmHg)		
Systolic	130 (118-149)	133 (120-152)
Diastolic	72 (63-81)	72 (64-83)
Laboratory and radiology		
CRP (mg/L)	39.5 (12-127)	43.5 (13-99)
White cell count (x10^9 per L)	10.8 (8.1-14.8)	10.4 (8.0-14.0)
Neutrophils (x10^9 per L)	8.4 (5.7-11.0)	7.9 (5.5-11.1)
Chest X-ray done		
Yes	346 (96%)	340 (96%)

No	14 (4%)	14 (4%)
Final diagnosis		
Asthma	62 (17%)	57 (16%)
IECOPD	81 (23%)	83 (23%)
Pneumonia	94 (26%)	98 (28%)
Influenza-like illness/NPLRTI	76 (21%)	69 (19%)
Other‡	47 (13%)	47 (13%)
Location of recruitment		
Emergency department	134 (37%)	147 (42%)
Acute medical unit	226 (63%)	207 (58%)

Data are n (%) or median (IQR). POCT=point-of-care testing. O_2=oxygen. BP=blood pressure. CRP=C-reactive protein. IECOPD=infective exacerbation of COPD. NPLRTI=non-pneumonic lower respiratory tract infection. *Received vaccine for the current influenza season. †Received within 14 days before presentation to hospital. ‡Table 24 (in the appendices) contains the breakdown of individual clinical diagnoses.

3.4 Virus testing and turnaround time

All patients in the POCT group were tested for respiratory viruses compared with 158 (45%) of 354 patients in the control group. More patients in the POCT group had a respiratory virus detected than in the control group. The mean turnaround time (time from the decision to test a patient to the result being available to clinicians) for respiratory virus testing was substantially lower in the POCT groups (Table 3). The positive and negative agreement between the POCT results and laboratory PCR (for patients in the POCT group in whom both were done) are given in Table 16 .

Table 3: Patients tested for viruses, rate of detection, and turnaround time

	POCT (n=360)	Control (n=354)	Difference (95% CI)	Odds ratio (95% CI)	Number needed to test (95% CI)	p value
Patients tested for viruses	360 (100%)	158 (45%)	55.4% (50.1 to 60.0)	..	..	<0.0001
Patients with any virus detected	161 (45%)	52 (15%)	30.0% (23.3 to 36.8)	4.70 (3.28 to 6.74)	4 (2.8 to 4.2)	<0.0001
Influenza A or B	61 (17%)	37 (10%)	6.5% (1.5 to 11.5)	1.75 (1.13 to 2.71)	16 (9 to 68)	0.0124
Rhinovirus or enterovirus (unspecified)*	55 (15%)	..	..	..	..	..
Coronavirus*	18 (5%)	..	..	..	..	..
Human metapneumovirus	14 (4%)	5 (1%)	2.5% (0.1 to 4.8)	..	..	0.060
Parainfluenza	11 (3%)	2 (<1%)	2.5% (0.6 to 4.4)	..	..	0.0214
RSV	9 (3%)	6 (2%)	0.8% (−1.3 to 2.9)	..	..	0.60
Adenovirus	1 (<1%)	2 (<1%)	−0.3% (−1.2 to 0.7	.. ..	..	0.62
Viral co-detection	8 (2%)	0	2.2% (0.7 to 3.7)	..	..	0.0075
Turnaround time (hours)	2.3 (1.4)†	37.1 (21.5)	−34.7 (−38.1 to −31.4)			<0.0001

Data are n (%) or mean (SD). POCT=point-of-care testing. RSV=respiratory syncytial virus. *Not tested for by laboratory PCR. †Assessed in 356 patients.

3.5 Primary outcome

For the primary outcome, 301 (84%) of 360 patients in the POCT group received antibiotics during their admission compared with 294 (83%) of 354 patients in the control group (difference 0.6% in favour of the control group, 95% CI –4.9 to 6.0; p=0.84; Table 4). Multiple logistic regression analysis on the primary outcome altered the direction of the difference but did not change the broader interpretation (Table 25, in the appendices, has full results from the multiple logistic regression model).

Table 4: Comparison of antibiotic use

	POCT (n=360)	Control (n=354)	Risk Difference (95% CI)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Number needed to test (95% CI)	p value
All antibiotics							
Antibiotics given	301 (84%)	294 (83%)	0.6% (-4.9 to 6.0)	1.04 (0.70 to 1.54)	0.99 (0.57 to 1.70)	..	0.96*
Single dose only	31/301 (10%)	10/294 (3%)	6.9% (2.9 to 11.0)	3.26 (1.59 to 6.68)	..	15 (9 to 35)†	0.0010
Given for <48 hours	50/301 (17%)	26/294 (9%)	7.8% (2.5 to 13.1)	2.05 (1.40 to 3.39)	..	13 (8 to 41)‡	0.0047
Duration (days)	7.2 (5.1)	7.7 (4.9)	0.4 (-1.2 to 0.4)ʃ	0.95 (0.85 to 1.05)¶	0.91 (0.80 to 1.04)‖	..	0.17*
IV antibiotics							
IV antibiotics given	196 (54)	183 (52%)	2.7% (-4.6 to 10.0)	1.15 (0.83 to 1.50)	..	..	0.46
Single dose only	50/196 (26%)	37/183 (20%)	5.3% (-3.1 to 14.0)	1.35 (0.84 to 2.19)	..	..	0.22
Given for <48 hours	106/196 (54%)	100/183 (55%)	-0.5% (-11.0 to 9.5)	0.98 (0.65 to 1.46)	..	..	0.91
Duration (days)	3.1 (4.6)	2.9 (3.7)	0.3 (-0.6 to 1.1)ʃ	1.09 (0.86 to 1.40)¶	..	..	0.48

Data are n (%) or mean (SD). POCT=point-of-care testing. IV, intravenous. *Applies to adjusted effect sizes. †Number needed to test to change a standard course to a single dose. ‡Number needed to test to change a standard course to a brief course. ʃMean difference. ¶Unadjusted rate ratio. ‖Adjusted rate ratio.

3.6 Duration of antibiotics

Mean duration of antibiotics did not differ between groups (difference −0.4, −1.2 to 0.4; p=0.32; Table 4), and multiple negative binomial regression analysis did not significantly alter the interpretation of the results (Table 26, in the appendices, has full results from the multiple negative binomial regression model).

3.7 Other antibiotic outcomes

Among patients given antibiotics, a greater proportion of patients in the POCT group received only a single dose of antibiotics than in the control group (Table 4). Similarly, a greater proportion of patients in the POCT group received a brief course (<48 hours) of antibiotics than in the control group. The proportion of patients treated with intravenous antibiotics and their duration did not differ between the groups.

3.8 *Post hoc* analysis based on the primary outcome

Owing to hospital processes of care, many recruited patients had antibiotics prescribed very early in the course of their assessment and often before they could be randomly allocated or before the POCT results were available to the clinical teams for the POCT group. We therefore did a *post hoc* analysis on patients in whom antibiotics had not been prescribed before randomisation and before POCT results were available to the clinical teams. In this subgroup, antibiotics were prescribed in 61 (51%) of 120 patients in the POCT group compared with 107 (64%) of 167 in the control group (difference −13.7%, 95% CI −25.2 to −2.2; p=0.022; number needed to test to prevent one patient being treated with antibiotics is eight; Table 27 and Table 28 in the appendices).

3.9 *Post hoc* analysis based of antibiotic duration

Post hoc analysis of antibiotic duration in the intervention group by POCT result showed that patients with positive results received shorter courses of antibiotics (mean 6.2 days [SD 4.8]) than did patients with negative results (8.0 days [5.3]; difference −1.7 days, 95% CI −2.9 to −0.6; p=0.0033); Table 29, in the appendices). The distribution of antibiotic duration according to POCT results is shown in Figure 6.

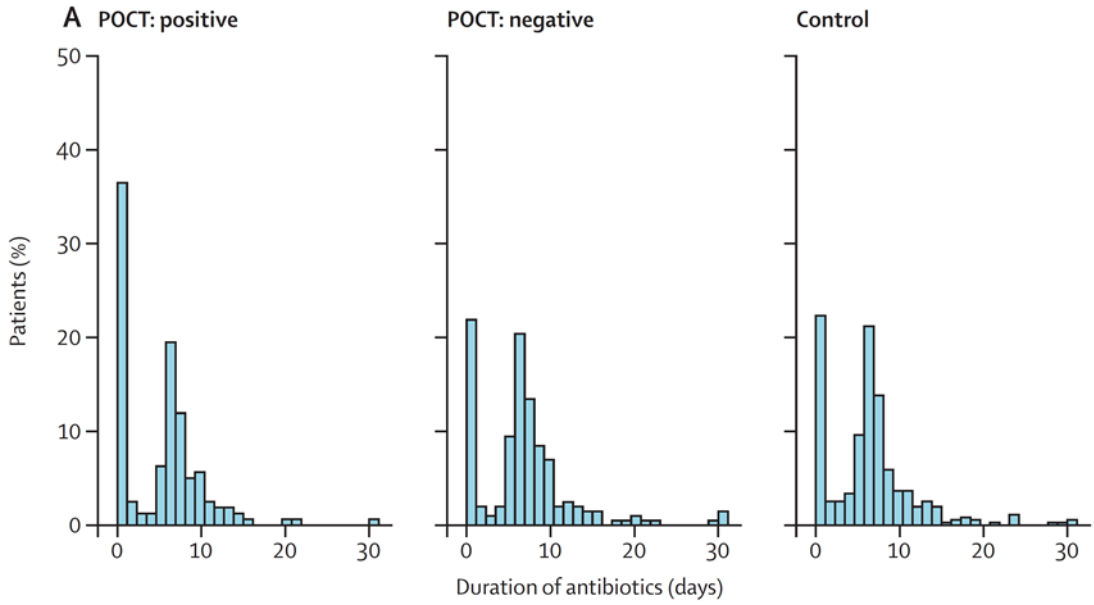


Figure 6: Distribution of the duration of antibiotics

Distributions are shown for patients with positive POCT results, patients with negative POCT results, and patients in the control group. POCT=point-of-care testing.

3.10 Length of hospital stay

Most patients presenting to secondary care were hospitalised in both groups (Table 5). Mean hospital length of stay was shorter in the POCT group than in the control group. The proportion of patients with a prolonged inpatient stay (≥7 days) did not differ between groups.

Table 5: Length of hospital stay

	POCT (n=360)	Control (n=354)	Difference (95% CI)	Odds ratio (95% CI)	p value
Admitted	332 (92%)	327 (92%)	-0.2% (-4.1 to 3.8)	0.98 (0.56 to 1.70)	0.94
Length of hospital stay (days)*	5.7 (6.3)	6.8 (7.7)	-1.1 (-2.2 to -0.3)	..	0.0443
Prolonged inpatient stay†	81/327 (25%)	86/311 (28%)	-2.9% (-9.7 to 3.9)	0.86 (0.61 to 1.23)	0.42

Data are n (%) or mean (SD). POCT=point-of-care testing. *Adjusted for in-hospital mortality. †Defined as ≥7 days (adjusted for in-hospital mortality).

3.11 *Post hoc* analysis of hospital length of stay

Post hoc analysis of hospital length of stay in the intervention group by POCT result showed that patients with positive results had a shorter duration of hospitalisation (4.7 days [4.6]) than did patients with negative results (6.5 days [7.2]; difference −1.7 days, 95% CI −3.0 to −0.4; p=0.0085; Table 29, in the appendices). The distributions of length of hospital stay according to POCT results are shown in Figure 7.

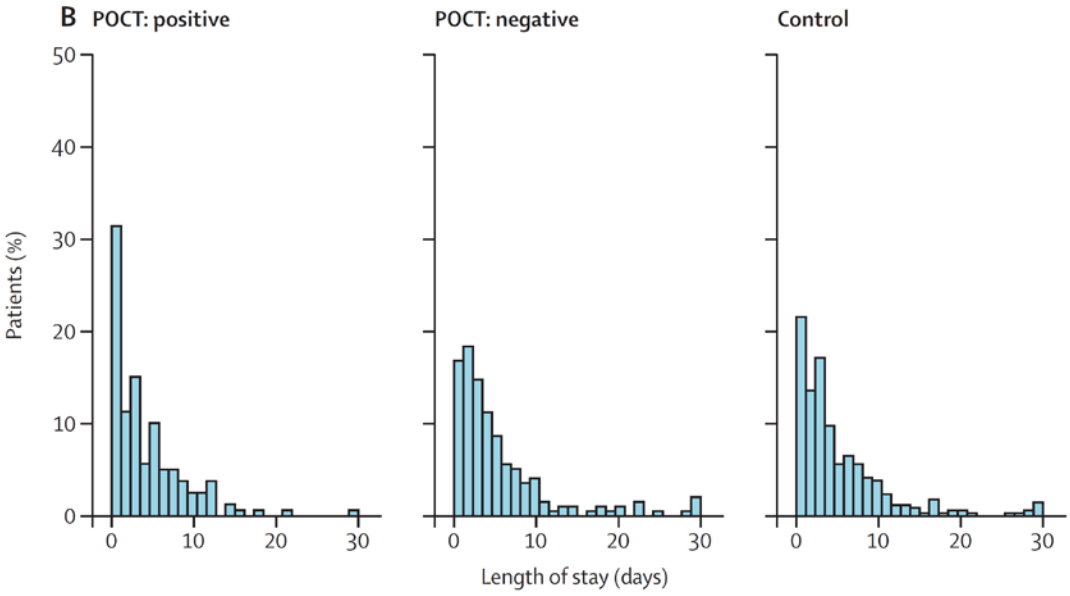


Figure 7: Distribution of the length of hospital stay

Distributions are shown for patients with positive POCT results, patients with negative POCT results, and patients in the control group. POCT=point-of-care testing.

3.12 Pre-specified clinical subgroup analysis

We explored potentially different treatment effects in pre-specified clinical subgroups (Table 6). In the exacerbation of asthma and COPD subgroups, mean duration of antibiotics was lower for patients in the POCT group than in the control group. Non-significant differences were observed in the other subgroups. For patients with asthma exacerbation treated with antibiotics, a greater proportion of patients in the POCT group than in the control group received only a single dose of antibiotics. For patients with infective exacerbation of COPD treated with antibiotics, a greater proportion of patients in the POCT group than in the control group received less than 48 hours of antibiotics (for distributions of antibiotic duration for asthma and COPD subgroups see Figure 14 and Figure 15 in the appendices). For patients with infective exacerbation of COPD, the mean length of hospital stay was lower in the POCT group than in the control group (for distribution of

length of stay in this clinical subgroup, see Figure 16 in the appendices); mean length of hospital stay did not differ within other subgroups.

Table 6: Antibiotic use and length of stay for exacerbation of asthma and exacerbation of COPD clinical subgroups

	POCT (n=360)	Control (n=354)	Difference (95% CI)	Odds ratio (95% CI)	p value
Asthma exacerbation	62 (17%)	57 (16%)	..	..	..
Antibiotics given	43/62 (69%)	36/57 (63%)	6.2% (-10 to 23)	1.32 (0.62 to 2.83)	0.56
Single dose only	14/43 (33%)	3/36 (8%)	24.2% (6.1 to 40.1)	5.31 (1.38 to 20.41)	0.0125
Given for <48 hours	18/43 (42%)	4/36 (11%)	30.8% (11.2 to 47.0)	5.76 (1.73 to 19.20)	0.0026
Duration of antibiotics (days)	3.9 (3.4)	5.3 (2.3)	-1.4 (-2.7 to -0.1)	..	0.0382
Length of hospital stay (days)	3.4 (3.3)	3.9 (3.5)	-0.5 (-1.8 to 0.9)	..	0.49
COPD exacerbation	81 (23%)	83 (23%)	..	..	..
Antibiotics given	75/81 (93%)	75/83 (90%)	2.2% (-6.9 to 11.4)	1.33 (0.44 to 4.03)	0.78
Single dose only	7/75 (9%)	3/75 (4%)	5.3% (-3.2 to 14.4)	2.47 (0.61 to 9.95)	0.33
Given for <48 hours	11/75 (15%)	3/75 (4%)	10.7% (1.2 to 20.7)	4.13 (1.10 to 15.50)	0.0462
Duration of antibiotics (days)	6.1 (3.2)	8.0 (5.0)	-1.9 (-3.2 to -0.5)	..	0.0078
Length of hospital stay (days)	4.5 (3.6)	6.3 (6.2)	-1.8 (-3.4 to -0.2)	..	0.0276
Asthma or COPD exacerbation	143 (40%)	140 (40%)	..	..	..
Antibiotics given	118/143 (83%)	111/140 (79%)	3.2% (-6.0 to 12.4)	1.23 (0.68 to 2.24)	0.55
Single dose only	21/118 (18%)	6/111 (5%)	12.4 (4.1 to 20.8)	3.79 (1.47 to 9.78)	0.0041
Given for <48 hours	29/118 (25%)	7/111 (6%)	18.3% (9.0 to 27.4)	4.84 (2.02 to 11.59)	0.0002
Duration of antibiotics (days)	5.3 (3.4)	7.1 (4.5)	-1.8 (-2.8 to -1.8)	..	0.0008
Length of hospital stay (days)	4.0 (3.5)	5.4 (5.5)	-1.4 (-2.5 to -0.2)	..	0.0186

Data are n/N (%) or mean (SD). POCT= point-of-care testing. IECOPD= infective exacerbation of COPD.

3.13 Neuraminidase inhibitor treatment

No difference was observed in the number of patients treated with neuraminidase inhibitors (Table 7). A greater proportion of patients treated with neuraminidase inhibitors in the POCT group had confirmed influenza infection than in the control group. In addition, patients treated empirically with neuraminidase inhibitors who then tested negative for influenza received shorter courses of neuraminidase inhibitors in the POCT group compared with the control group. UK Public Health England guidelines recommend neuraminidase inhibitor treatment for all hospitalised adults with influenza.[135] A greater proportion of hospitalised patients with confirmed influenza in the POCT group were treated with neuraminidase inhibitors than in the control group. The mean time to starting neuraminidase inhibitor treatment in these patients did not differ between groups. All neuraminidase inhibitor treatment was with oseltamivir.

Table 7: Neuraminidase inhibitor use

	POCT (n=360)	Control (n=354)	Difference (95% CI)	Odds ratio (95% CI)	Number needed to test (95% CI)	p value
Neuraminidase inhibitor used (total)	66 (18%)	51 (14%)	3.9% (-1.5 to 9.4)	1.33 (0.89 to 1.99)	..	0.16
Used in influenza-positive patients	54/66 (82%)	24/51 (47%)	34.7% (17.5 to 52.0)	5.06 (2.20 to 11.65)	3 (1.9 to 5.5)	0.0001
Used in influenza-negative patients	12/66 (18%)	27/51 (53%)	..	..	..	..
Influenza-positive patients treated with neuraminidase inhibitor*	52/57 (91%)	24/37 (65%)	26.4% (9.6 to 43.2)	5.63 (1.80 to 17.60)	4 (2.3 to 10.7)	0.0026
Duration of neuraminidase inhibitor use in influenza-negative patients (doses)†	2.0 (2.6)	6.1 (4.1)	-4.1 (-6.3 to -1.9)	..	..	0.0006
Time to first dose of neuraminidase inhibitor (hours)*	8.8 (15.3)	21.0 (28.7)	-12.2 (-24.9 to 0.5)	..	..	0.0597

Data are n (%) or mean (SD). POCT=point-of-care testing. *For hospitalised influenza-positive patients only. †Oseltamivir is given twice daily.

3.14 Isolation facility use

Data on isolation facility use were only available for the second year of recruitment (winter of 2015–16) owing to the introduction of a new hospital information system that allowed hospital side room use to be accurately tracked in real time (to the nearest whole day). Overall isolation facility use did not differ between the groups (Table 8). However, a greater proportion of patients in the POCT group were isolated for confirmed respiratory virus infection compared with the control group. The proportion of influenza-positive patients admitted to hospital and staying for at least 6 hours who were isolated did not differ significantly between groups. Mean time to isolation for influenza-positive patients not empirically isolated at presentation was significantly shorter in the POCT group than in the control group, as was mean time to de-isolation for patents initially isolated with suspected influenza but subsequently testing negative.

Table 8: Hospital isolation facility use

	POCT (n=191)‡	Control (n=194)‡	Difference (95% CI)	Odds ratio (95% CI)	Number needed to test (95% CI)	p value
All patients isolated	63/191 (33%)	49/194 (25%)	7.7% (−1.3 to 16.8)	1.45 (0.94 to 2.27)	..	0.12
Isolated with confirmed respiratory virus infection[ʃ]	32/191 (17%)	17/194 (9%)	8.0% (1.3 to 14.7)	2.10 (1.12 to 3.92)	13 (6.8 to 73.2)	0.0217
Influenza-positive patients isolated*	20/27 (74%)	13/23 (57%)	17.6% (-8.8 to 43.9)	2.20 (0.67 to 7.24)	..	0.24
Time to isolation (days)¶	0.5 (0.5)	1.0 (0.4)	-0.5 (-0.9 to -0.2)	..	..	0.0071
Time to de-isolation (days)‖	1.0 (0.0)	3.1 (2.2)	-2.1 (-3.6 to -0.7)	..	..	0.0057

Data are n (%) or mean (SD). POCT=point-of-care testing. ‡Side room (isolation room) data only available for the second season of the study. [ʃ]Includes influenza and respiratory syncytial virus. *For hospitalised influenza-positive patients only. ¶For patients not empirically isolated at admission, but subsequently found to be influenza positive. ‖For patients isolated empirically on admission for suspected influenza infection, but subsequently found to be influenza negative.

3.15 Adverse event outcomes

We found no differences between the groups in overall rates of adverse event outcomes, or in individual rates of high dependency or intensive care unit admissions during hospitalisation, re-presentation (without readmission), or readmission to hospital within 30 days of discharge, or 30-day mortality (Table 9). Every SAE was classified as being unrelated to the study.

Table 9: Adverse outcomes

	POCT (n=360)	Control (n=354)	Difference (95% CI)	Odds ratio (95% CI)	p value
Any adverse outcome (total)	77 (21%)	88 (25%)	-3.5% (-9.7 to 2.7)	0.82 (0.6 to 1.2)	0.29
High dependency unit admission	6 (2%)	3 (1%)	0.8% (-1.2 to 2.8)	1.98 (0.5 to 8.0)	0.33
Intensive care unit admission	11 (3%)	7 (2%)	1.1% (-1.2 to 3.4)	1.56 (0.6 to 4.1)	0.36
Died within 30 days	9 (3%)	16 (5%)	-2.0% (-4.7 to 0.6)	0.54 (0.3 to 1.2)	0.15
Re-presented within 30 days*	49 (14%)	49 (14%)	0.2% (-4.8 to 5.2)	0.98 (0.6 to 1.5)	1.00
Readmitted within 30 days	45 (13%)	55 (16%)	-3.0% (-8.3 to 2.1)	0.78 (0.5 to 1.2)	0.28

Data are n (%). POCT=point-of-care testing. *Re-presenting to hospital but not admitted.

3.16 *Post hoc* analysis of lumbar punctures done

Five (1.4%) of 360 patients underwent lumbar puncture during admission in the POCT group as opposed to 10 (2.8%) of 354 patients in the control group (difference -1.4%, 95% CI -0.39 to 0.79%, p=0.20). Timings of lumbar punctures relative to respiratory virus testing or results, where done, were not recorded.

3.17 *Post hoc* analysis of bacterial detection

3.17.1 Bacterial detection via conventional microbiological methods

289 (40%) of 714 of patients in the ResPOC trial had blood cultures taken. The positivity rate was low: 17 (5.9%) of the 289 patients who had blood cultures taken. Sputum cultures were taken less frequently than blood cultures (99 (13.9%) of 714 patients) but had a higher diagnostic yield (31 (31.3%) of 99 patients). Legionella urinary antigen and pneumococcal urinary antigen were infrequently tested (6.6% and 1.0% respectively) and no patients were found to be positive for these tests. Table 10 describes bacterial pathogen detection in ResPOC trial patients.

Table 10: Bacterial pathogen detection in ResPOC trial participants

	POCT (n=360)	Control (n=354)	Total (n=714)
Blood cultures			
Positive*	9 (2.5%)	8 (2.3%)	17 (2.4%)
Patients who had at least one test done	143 (39.7%)	146 (41.2%)	289 (40.5%)
Positivity rate	9/143 (6.3%)	8/146 (5.5%)	17/289 (5.9%)
Sputum cultures			
Positive†	9 (2.5%)	22 (6.2%)	31 (4.3%)
Patients who had at least one test done	44 (12.2%)	55 (15.5%)	99 (13.9%)
Positivity rate	9/44 (20.5%)	22/55 (40.0%)	31/99 (31.3%)
Legionella urinary antigen			
Positive	0	0	0
Patients who had at least one test done	24 (6.7%)	23 (6.5%)	47 (6.6%)
Positivity rate	0/24	0/23	0/47
Pneumococcal urinary antigen¶			
Positive	0	0	0
Patients who had at least one test done	3 (0.8%)	4 (1.1%)	7 (1.0%)
Positivity rate	0/3	0/4	0/7

*non-contaminants; †non-'oral flora' but includes yeasts.

¶ UHS has a policy of not doing pneumococcal antigen tests except at the discretion of a microbiologist.

POCT= point-of-care test.

Where a patient had more than one of a particular test done, the patient is only counted once.

3.17.2 Bacterial results from the POCT

In addition to respiratory viruses, the FilmArray Respiratory Panel detects *Mycoplasma pneumoniae*, *Bordetella pertussis* and *Chlamydophila pneumoniae*.

In the POCT group, nine (2.5%) of 360 patients had *Mycoplasma pneumoniae* detected by FilmArray respiratory panel (Table 11). Both the POCT group and the control group had serology testing requested at the discretion of the responsible clinical team. There were no serological diagnoses of *M. pneumoniae* in either group, except one of the *M. pneumoniae*-POCT- positive

patients had positive convalescent serology (described in Table 11 and Table 12, the latter also suggests limited use of serology in acute diagnosis). Testing for *M. pneumoniae* by serology was generally very infrequent: only 3.9% of all trial patients had either acute or convalescent *M. pneumoniae* serology tested. Three of the nine patients with *M. pneumoniae* detection also had a virus co-detected by POCT.

Seven (77.8%) of the nine patients with *M. pneumoniae* detected by POCT had a clinical diagnosis of pneumonia. Therefore, this group was compared to patients with pneumonia who did not have *M. pneumoniae* detected by POCT (Table 13). Three (42.9%) of the seven patients with mycoplasma detected by POCT and pneumonia were on macrolide monotherapy after their POCT result compared with none in the mycoplasma-negative group (p=0.0003). Two (28.6%) of seven patients with mycoplasma detected by POCT had a macrolide started after their mycoplasma POCT result compared with 17.2% of patients without mycoplasma detected, but the difference was not significant. Of the two patients with *M. pneumoniae* detected by POCT who did not have pneumonia, one had an exacerbation of COPD, the other an exacerbation of asthma. All macrolide use was clarithromycin.

There were no detections in either the POCT group or the control group of *Bordetella pertussis* or *Chlamydophila pneumoniae*.

Table 11: *Mycoplasma pneumoniae* detection in ResPOC trial participants

	POCT (n=360)	Control (n=354)	Total (n=714)
Positive by molecular POCT	9 (2.5%)	NA	NA
Positive by acute serology	0	0	0
Positive by convalescent serology	1∫	0	0
Patients who were tested by serology*	16 (4.4%)	12 (3.4%)	28 (3.9%)
Positivity rate (any method)	9/360 (2.5%)	0/12	NA

N/A=not applicable; POCT=point-of-care test; *either acute or convalescent, or both, serology. ∫ The single patient positive for convalescent mycoplasma serology was also positive by molecular POCT.

Table 12: *Mycoplasma pneumoniae* serology testing in *M. pneumoniae* POCT-positive patients

	(n=9)
No serology tested	5
Negative acute serology, no convalescent serology tested	3
Negative acute serology, convalescent serology positive	1

Table 13: Demographics and Outcomes of *Mycoplasma pneumoniae*-positive with pneumonia compared with *M. pneumoniae*-negative patients with pneumonia

	***M. pneumoniae* POCT positive with pneumonia (n=7)**	***M. pneumoniae* POCT negative with pneumonia (n=87)**	**Difference**	**Odds Ratio (95% CI)**	**p value**
Demographics					
(Co)infected with a respiratory virus	2 (28.6%)	28 (32.2%)	3.6%	0.8 (0.2 to 4.4)	1
Age (mean), years	41.3	62.5	21.2		0.008
Male sex	3 (42.9%)	41 (47.1%)	4.2%	0.8 (0.2 to3.3)	1
Mean number of antibiotic agents received prior to POCT	1.71	1.48	-0.23		0.582
Already on a regimen containing macrolide when tested	4 (57.1%)	27 (31.0%)	-26.1%	3.0 (0.7 to 12.2)	0.213
Outcomes					
On macrolide monotherapy after POCT result	3 (42.9%)*	0	-42.9%	N/A	0.0003
Macrolide started after POCT result	2 (28.6%)	15 (17.2%)	-11.4%	1.9 (0.4 to 10.7)	0.606

*One of these patients had a single dose of amoxicillin given 42 minutes after POCT result communicated.

7 (77.8%) out of 9 patients positive for *M.pneumoniae* detected by POCT had pneumonia.

All macrolide use was clarithromycin. POCT=point-of-care test.

3.18 *Post hoc* analysis of patients with influenza in the POCT group

61 patients in the POCT group had influenza detected, of which 29 (47.5%) had ILI/LRTI and 14 (23.0%) had pneumonia (Table 14).

Table 14: Influenza-positive patients by clinical subgroup, in POCT group

	Number of patients with influenza detected (n=61)	
Pneumonia	14	(23.0%)
Exacerbation of Asthma	6	(9.8%)
Exacerbation of COPD	8	(13.1%)
ILI/LRTI	29	(47.5%)
Other*	4	(6.6%)

*3 out of the 4 patients with an 'Other' diagnosis had an exacerbation of another chronic respiratory condition.

To see if there were any differences in influenza-positive patients which might be used to predict patients who have pneumonia as opposed to another diagnosis, further analysis of influenza-positive patients with and without pneumonia was done (Table 15). Influenza-positive patients with pneumonia were more likely to have cardiovascular comorbidity than influenza-positive patients without pneumonia (8 (57.1%) of 14 vs 10 (21.3%) of 47, p=0.018); there were trends towards significance for age and CRP level (both higher in pneumonia), however the small number of patients limited further analysis or interpretation.

Table 15: Demographics and clinical characteristics of patients with influenza in the POCT group, with and without pneumonia

	Pneumonia (n=14)		No pneumonia (n=47)		Difference (95%CI)		p value
Demographics							
Age	59	(56 to 75)	47	(36.5 to 65)	12	(0 to 23)	0.060
Male sex	7	(50.0%)	22	(46.8%)	3.2%	(-10.5% to 16.7%)	1.00
White British ethnicity	13	(92.9%)	43	(91.5%)	1.4%	(-6.5% to 9.4%)	1.00
Smoker	5	(35.7%)	11	(23.4%)	12.3%	(-0.4% to 24.4%)	0.490
Influenza vaccination*	6	(42.9%)	18	(38.3%)	4.6%	(-8.8% to 18.9%)	0.765
Comorbidity							
Cardiovascular	8	(57.1%)	10	(21.3%)	35.9%	(22.5% to 47.3%)	0.018
Respiratory	3	(21.4%)	22	(46.8%)	-25.4%	(-37.3% to 12.3%)	0.125
Renal	0	(0)	3	(6.4%)	-6.4%	(-13.0% to 1.4%)	1.00
Liver	0	(0)	0	(0)	0		
Diabetes	3	(21.4%)	3	(6.4%)	15.0%	(5.5% to 24.6%)	0.128
Cancer	2	(14.3%)	0	(0)	14.3%	(7.6% to 22.6%)	0.050
Immunosuppression	0	(0)	1	(2.1%)	-2.1%	(-7.2% to 1.9%)	1.00
Observations							
Pulse rate (beats per min)	105	(90 to 119)	100	(89 to 110)	5	(-9 to 20)	0.426
Supplemental oxygen use	6	(42.9%)	15	(31.9%)	10.9%	(-2.4% to 23.8%)	0.528
Temperature (°C)	37.6	(36.7 to 38.2)	37.2	(36.7 to 37.6)	0.4	(-0.3 to 0.9)	0.379
Temperature ≥38°C	5	(35.7%)	9	(19.1%)	16.6%	(4.3% to 28.3%)	0.277
C reactive protein (mg/L)	68.5	(60 to 216)	39	(14 to 115)	29.5	(-1 to 101)	0.053

Data are n (%) or median (IQR). *Received vaccine for the current influenza season.

3.19 Concordance of the FilmArray Respiratory Panel with laboratory RT-PCR testing

110 (30.6%) of 360 patients in the POCT group had samples sent to the laboratory at the discretion of the treating team, which allow comparison of the results between the FilmArray Respiratory Panel and laboratory RT-PCR (Table 16). The overall positive agreement percentage was 93% and the overall negative agreement percentage was 96%. The overall positive agreement percentage is brought down from 100% by two discordant influenza A results and one discordant influenza B result.

Table 16: Percentage positive and negative agreement between FilmArray respiratory panel (POCT) and Laboratory PCR results for patients in the intervention group who received both tests

	Number detected by FilmArray/ Laboratory PCR*				Positive % agreement [a/(a+c)] (95%CI)	Negative % agreement [d/(b+d)] (95%CI)
	+ /+ (a)	+ / - (b)	- / + (c)	- / - (d)		
Influenza A	19	2	2	87	90 (70 to 99)	98 (92 to 100)
Influenza B	11	1	1	97	92 (61 to 100)	99 (94 to 100)
RSV	2	0	0	108	100 (16 to 100)	100 (97 to 100)
Parainfluenza 3	2	0	0	108	100 (16 to 100)	100 (97 to 100)
hMPV	3	0	0	107	100 (30 to 100)	100 (97 to 100)
Adenovirus	1	0	0	109	100 (30 to100)	100 (97 to 100)
Rhino/enterovirus	NA	12	NA	NA		
Coronavirus	NA	5	NA	NA		
Total	38	3	3	66	93 (80 to 98)	96 (88 to 99)

n=110. PCR=polymerase chain reaction. 95% CI=95% confidence interval. RSV=respiratory syncytial virus. hMPV=human metapneumovirus. NA=not applicable; rhino/enterovirus and coronavirus were not targets on the laboratory PCR panel. *Includes all viruses detected by laboratory PCR with Ct value of ≤38 of 45 cycles.

Chapter 4 Discussion

4.1 Overview

This large, pragmatic, randomised controlled trial is the first, to our knowledge, to report on the effect of routine molecular POCT for viruses, on a broad range of clinical outcomes including antibiotic use, length of hospital stay, influenza antiviral use, isolation facility use, and safety.

4.2 Antibiotic use

The results showed that a routine molecular POCT strategy in adults presenting to secondary care with acute respiratory illness led to a higher detection rate of viruses and a faster turnaround time for results compared with laboratory PCR but did not reduce the proportion of patients treated with antibiotics or the overall duration of antibiotics compared with routine clinical care (i.e. the primary outcome was not met).

However, it did lead to an increased proportion of patients receiving single doses and brief (<48 hours) courses of antibiotics. The reason that the increase in single doses and brief courses did not translate into an overall reduction in the duration of antibiotics is likely to relate to the uniformly high use of prolonged antibiotics in certain clinical groups, especially patients with pneumonia (mean duration of around 9 days), which was not affected by POCT. Our subgroup analyses suggest that the increase in single doses and brief courses of antibiotics occurred mainly in patients with exacerbation of airways disease (asthma and COPD), and in these groups POCT was associated with a significant reduction in antibiotic duration.

Our analysis also suggests that this reduction in antibiotic use occurs mainly in those patients testing positive for respiratory viruses and that a positive test reduces antibiotic duration by leading clinicians to stop antibiotics earlier, after a single dose or a brief course of one to two days, rather than completing a standard five to seven-day course.

Although premature discontinuation of an antibiotic course has previously been regarded as inadvisable owing to concerns over generating resistance, evidence suggests that early discontinuation is safe from this perspective and is in fact associated with a reduced risk of drug resistance.[185]

Around 200 000 patients are hospitalised with exacerbation of asthma and COPD combined each year in the UK,[186,187] and more than two thirds of these patients are treated with antibiotics; therefore, being conservative regarding the effect size, a reduction in antibiotic duration of

around 1 day per patient treated would equate to a total reduction of around 150 000 antibiotic days per year. This reduction would contribute substantially to the antimicrobial reduction targets set by National Health Service (NHS) organisations to address the threat of antimicrobial resistance. Although this trial is the first large randomised controlled trial of molecular POCT for respiratory viruses examining antibiotic use in detail, other smaller studies have suggested the potential of this strategy to reduce antibiotic use.[188]

4.3 Length of hospital stay

In addition to the changes in antibiotic use, our study shows that POCT might be associated with a reduction in hospital length of stay, and subgroup analyses suggest that this reduction was also principally in patients with exacerbation of airways disease. Again, our data suggest that this result was due to earlier discharge in patients testing positive for respiratory viruses in the POCT group. Notably, duration of hospitalisation in the COPD control group is consistent with that quoted in a large, contemporaneous UK study.[189] The reduction in length of stay for patients with exacerbation of airways disease was just over one day, which would equate to around 200 000 bed days saved per year across the NHS with an associated cost saving of around £80 million per year.[190]

4.4 Virus detection and neuraminidase inhibitor use

Routine POCT for respiratory viruses was associated with an increased rate of detection of influenza cases and an improvement in appropriate antiviral use. Although only patients with clinically suspected infection were tested in the control group, the lower detection rate compared with the POCT group suggests that many cases of influenza were missed and remained undiagnosed in this group. This result is unsurprising as physician-diagnosed influenza is well known to be an insensitive method of case detection, even during periods of high influenza activity.[191,1192] In view of the potential consequences including nosocomial spread and the unrealised opportunity to benefit from neuraminidase inhibitor treatment in undiagnosed influenza, these data suggest that influenza testing should be done routinely in patients hospitalised with acute respiratory illness during periods of influenza circulation. Neuraminidase inhibitor treatment is recommended by Public Health England for all patients hospitalised with influenza,[135] and although treatment is recommended irrespective of the duration of illness, neuraminidase inhibitors are likely to be most effective when administered earlier in the course of infection.[139,141] Our study shows that POCT for respiratory viruses leads to an increased proportion of influenza-positive patients correctly receiving treatment with neuraminidase inhibitors and

suggests a reduced time to administration of the first dose. Additionally, most neuraminidase inhibitor use was directed towards influenza-positive patients in the POCT group, whereas most use was empirical in the control group and led to many influenza-negative patients receiving neuraminidase inhibitors unnecessarily.

Neuraminidase inhibitor use in influenza-negative patients was also prolonged in the control group, presumably due to the long turnaround time of laboratory PCR compared with POCT. This unnecessary neuraminidase inhibitor use exposes patients to the side-effects of neuraminidase inhibitors without any chance of associated benefit. The improvements in neuraminidase inhibitor use seen in this study are consistent with the findings of a previous non-randomised study of hospitalised adults in which similar differences in the turnaround time between rapid testing and laboratory PCR were also noted.[193]

4.5 Isolation facility use

Hospital side rooms, used for isolating potentially infectious patients, are a limited resource in most UK hospitals and, reassuringly, the use of POCT for respiratory viruses did not lead to a significant overall increase in side room use despite the increased detection rate of respiratory viruses.

However, side room isolation for confirmed respiratory virus infection was more common in the POCT group than in the control group. This result reflects the high rate of directed use of side rooms for patients with confirmed influenza and other viruses compared with the empirical use of side rooms in the control group, many of whom subsequently tested negative and were de-isolated. POCT was also associated with other improvements in side room use including reduced time from admission to isolation with confirmed influenza and reduced time to de-isolation in patients isolated with suspected influenza but subsequently testing negative. Rapid and appropriate assignment of hospital side rooms for patients with respiratory virus infection is hugely important to reduce the risk of nosocomial transmission to other vulnerable hospitalised patients and to improve the flow of patients through acute areas within the hospital.

4.6 Conclusion for key outcomes

A molecular POCT result for respiratory viruses can be expected to directly influence patient management in several ways. A positive result can identify the need for isolation facility use or neuraminidase inhibitor use in the case of influenza. Although the detection of a virus does not rule out the possibility of a bacterial infection or a benefit from antibiotics, a positive result might

also allow the premature discontinuation of precautionary antibiotics in patients with exacerbation of airways disease, if not required on the basis of other criteria such as severity of illness. If negative, a POCT result can prevent or shorten the unnecessary use of isolation facilities and neuraminidase inhibitors.

4.7 Study strengths

The strengths of our study include the large number of patients recruited, the setting of a typical large acute hospital, and its pragmatic design with broad inclusion criteria representing typical patients admitted to UK secondary care, simple intervention, and comparison to routine clinical care. Our study also took place over two winter seasons with very different patterns of influenza and other respiratory virus activity. These factors suggest that the findings of this study are likely to be generalisable to other similar UK and international centres.

4.8 Limitations of the study

Although a randomised clinical trial such as this represents high-quality evidence, care must be taken when applying the findings a wider sense.[194] One relevant example in this study would be that by excluding those who cannot consent to be part of the trial, such as those with delirium, dementia or severe respiratory failure requiring intensive care intervention, the results and conclusions might not apply to these specific patient groups.

Our study has the weakness of being a single-centre study, and additionally was not powered specifically to detect differences in the subgroups. No attempt was made at blinding anyone in the study other than the analysts. Because the purpose of the study was to inform the clinical teams of the POCT results, they could not be blinded to which group a participant had been randomised. Patients and those collecting data could have been blinded with the use of a sham swab; however, we felt that the risk of bias due to non-blinding was very low.

Because the study took place during winter months when respiratory virus infections are more common, the findings cannot be readily extrapolated outside of this period.

The UK has a comparatively low number of critical care beds per capita compared to other countries in Europe.[195] Therefore, patient access to these beds may be different in different countries, meaning that the findings related to admission to high-dependency and intensive care units between the POCT and control group may not easily be extrapolated to other countries.

In this trial, POCT was done by research staff rather than clinicians and so uncertainties remain about how such a test could be delivered routinely. Several models of delivery are potentially possible and include training clinicians or nursing staff to do the test (with the attendant consumption of their time) or the development of a POCT hub within acute areas, staffed by dedicated technicians and linked to a centralised laboratory.

Our findings should ideally be replicated in further studies before a definitive conclusion can be made.

The presence of our interventional trial may have changed the working practice of the trial environment (i.e. in AMU and ED), resulting in more frequent laboratory PCR tests over time. While this would likely result in more frequent influenza and other respiratory virus diagnoses, the time taken to come to these diagnoses (about 30 hours in this study) mean that the impact on patient care would not make a significant difference to the results presented here otherwise. Similarly, the empirical use of NAIs may have increased as clinicians became more familiar with prescribing them. However, this would likely have led to even more appropriate discontinuation of NAI use in the POCT group.

4.9 Primary outcome consideration

In retrospect, the choice of primary outcome measure was not ideal to assess the effect of POCT on antibiotic use, because the processes of care for patients with acute respiratory illness presenting to hospital lead to patients being started on antibiotics very early in the course of their assessment and often before the results of POCT could be made available. Therefore, the results of the POCT were not able to influence the primary outcome in a large proportion of patients. The *a priori* secondary outcome measures of duration of antibiotic use and proportion of patients treated with single doses or brief courses of antibiotics are arguably more relevant to standard clinical management in this group—antibiotics are started very early in most patients with acute respiratory illness but might subsequently be continued or discontinued based on test results and clinical course. The *post hoc* analysis of patients who had not yet been given antibiotics when POCT results were available arguably examines a slightly different population than does the primary outcome: patients in whom clinicians did not feel it necessary to start antibiotics very early on. However, the reduction in antibiotic use in the POCT group compared with the control group in this subgroup gives further credibility to the antibiotic reductions seen in the main study and suggests that POCT at an even earlier point might reduce unnecessary antibiotic use further.

Based on the positive impact of clinical outcomes, future studies in this field may benefit from having a primary outcome based on neuraminidase inhibitor use, or isolation facility use. A future

study of molecular POCT for respiratory viruses in adults hospitalised with acute respiratory illness using a primary outcome of mortality at 30-days would be extremely persuasive should there be a difference in favour of POCT. The 30-day mortality in the POCT group was around half of the control group (3% vs. 5%, p=0.15) which although not a significant difference may show a trend towards a difference. However, a study adequately powered to show a significant difference in mortality would likely have to be very large. As neuraminidase inhibitor treatment in hospitalised adults is associated with reduced mortality in observational studies,[139,140,196] the driving force behind any difference in mortality would most likely be driven by an increase in the proportion of detections of influenza infection by POCT leading to subsequent pathogen-directed rapid commencement of neuraminidase inhibitor treatment in these patients.

4.10 Adverse events

Showing that the intervention of molecular POCT and the resultant change in practice, including early discontinuation of antibiotics, does not lead to an increase in patient harm, was vital. The adverse event outcome measures chosen were based on the internationally-agreed key serious adverse events but give an augmented view of life-threatening events and also re-presentation to hospital, which would not count as readmission. There were small, non-significant rises in admissions to the high-dependency unit and intensive care unit in the POCT group and these differences are likely to be down to chance. If there was a genuine rise in admissions to a critical care unit in those receiving POCT, this could be explained by critical care physicians viewing these patients as having a potentially reversible and treatable cause of their serious illness (i.e. viral infection) and therefore being more willing to take on these patients.

This study was not powered to judge adverse outcomes, therefore caution should be taken when interpreting these data.

4.11 Lumbar puncture

The number of patients who received lumbar punctures in this study is too small to make any firm conclusions, however, only half the number of lumbar punctures were done in the POCT group compared with the routine clinical care group. Larger datasets may confirm the potential for virus-positive results from molecular point-of-care testing for respiratory viruses to prevent diagnostic confusion with meningitis and therefore prevent unnecessary lumbar punctures in patients presenting with an influenza-like illness with other features such as neck stiffness, headache and photophobia. The safety of such an intervention in this context must be evaluated to adequately capture any missed diagnoses of clinically significant meningitis.

4.12 Bacterial detection

4.12.1 Bacterial detection via conventional methods

Similar to previous studies, detection in clinical practice of bacterial pathogens by conventional methods such as blood and sputum culture was infrequent among all patients.[3,197] Some bacterial pathogen tests were done infrequently, and even in patients who were tested, the yield was low. Sputum cultures were taken less frequently than blood cultures but had a higher positive yield of microbiological diagnosis. Although positive test results may be useful in management, the relative rarity of such positive tests must call into question the cost effectiveness and value of doing such tests. The low rate of detection of bacteria in this patient cohort is too small to generate meaningful novel analyses of outcomes.

4.12.2 *Mycoplasma pneumoniae* detection as part of the POCT

The British Thoracic Society pneumonia guidelines recommend PCR testing of respiratory tract samples for *Mycoplasma pneumoniae* in at-risk clinical groups including most hospitalised adults. However, serology rather than PCR remains commonplace in UK practice including in Southampton despite the guidelines acknowledging "considerable caution" being required in serology interpretation.[198] Use of a PCR POCT with potential for *Mycoplasma pneumoniae* detection has the potential to alter antibiotic therapy from broad spectrum cover including a beta-lactam to solely a macrolide or respiratory quinolone effective against mycoplasma. In addition, although many adults hospitalised with pneumonia will receive appropriate antibiotics that would manage an atypical pneumonia pathogen, those who would not have done so could now benefit from a POCT including mycoplasma detection, and unnecessary beta-lactam antibiotics could be stopped. Patients in the ResPOC trial had clarithromycin started and were switched to clarithromycin monotherapy after a mycoplasma POCT detection (Table 13). The number of patients with mycoplasma detected in the ResPOC study is too small from which to make any firm conclusions. However, the benefits of POCT ascribed to respiratory virus detection, including early detection of epidemics, which mycoplasma causes about every four years, may also be true for mycoplasma detection by molecular POCT.

There were no detections of *Bordetella pertussis* or *Chlamydophila pneumoniae* in the ResPOC trial. Concerns have been raised over the FilmArray Respiratory Panel's poor sensitivity of *B. pertussis* detection.[199] The newer version of the Respiratory Panel has an additional new target of *Bordetella parapertussis*.[104] Despite the initial sensitivity criticism, the use of the FilmArray Respiratory Panel to detect pertussis in children has already been demonstrated in children in

China, therefore, there is the potential for clinical outcome benefits of a molecular POCT that detects for bacteria causing pertussis.[200]

4.13 Patients with influenza

It would be advantageous to clinicians to be able to predict which patients with influenza detected by POCT would need antibiotics for pneumonia, and those who would not necessarily need antibiotics. However, in the *post* hoc analysis, for patients with influenza detected in the POCT group there was essentially no factor that could distinguish clearly between patients who had pneumonia and who did not have pneumonia. A study that evaluated the impact on antibiotic use of rapid molecular testing for respiratory viruses plus a biomarker for bacterial infection is discussed later. The analysis of patients with pneumonia is limited by relatively small numbers in both groups. It is also limited by the clinical heterogeneity of patients who did not have pneumonia.

4.14 FilmArray Respiratory Panel Concordance with Laboratory RT-PCR

The concordance of the FilmArray Respiratory Panel compared with laboratory RT-PCR testing in this study was not as good as in most other published studies, which suggest a sensitivity of over 95% for key virus targets.[91,92,100-102]

Comparison of this study with other studies of the FilmArray system compared to laboratory PCR must be done with caution. The key factor in the comparatively low positive percentage agreement is the lower number of patients: just three patients with discordant results between the FilmArray and RT-PCR laboratory testing brought the positive percentage agreement down from 100% to 93%.

Several possibilities may explain both the positive and negative suboptimal discordance. The nose and throat swabs taken in the POCT group were acquired by research staff trained in correct sample acquisition technique (see Appendix C for the standard operating procedure) compared with the laboratory samples that were taken by ward staff who would not have received the same training. In addition, the POCT samples were nose and throat swabs, which is a more sensitive technique, compared to the throat swabs sent to the laboratory for RT-PCR testing. Study staff also had to wear personal protective equipment which would have limited any contamination from staff member to the specimen, whereas ward staff practice was unrecorded.

Importantly, the research group had no influence on when the samples sent to the laboratory were acquired in comparison to when the POCT samples were acquired. Therefore, samples sent

to the laboratory could have been taken very early in the patient journey and so had a greater chance of detecting a virus in a patient with a low viral load.

The limits of detection of the FilmArray Respiratory Panel for different viruses have been published.[96] However, this was a single dataset produced by the manufacturer. It is likely that RT-PCR testing in a laboratory has lower limits of detection than the FilmArray platform, meaning the RT-PCR is likely to detect viruses at a higher frequency. The clinical significance of these viruses on the cusp of detection at low viral loads is currently undetermined.

It is possible that some of the situations where the FilmArray did not detect a virus, but the laboratory RT-PCR test did detect a virus, that the patient was towards the end of their infection and thus had a low viral load. In these cases, secondary bacterial infection or viral-induced airways inflammation may have been responsible for the patient's hospitalisation rather than direct virus infection itself.

The FilmArray Respiratory Panel detects a higher number of respiratory viruses than our hospital RT-PCR laboratory panel; non-pandemic coronaviruses would not have been detected by laboratory testing whereas the FilmArray system provided potentially important positive results for many patients.

4.15 Other investigations

As the length of hospital stay was shorter in the POCT group, it is conceivable that there were fewer medical investigations and interventions in the POCT-virus-positive group than the POCT-virus-negative group and control group based on diagnostic uncertainty in the latter groups. This may translate into another component that influences a health economic analysis in favour of molecular POCT in this setting.

4.16 The potential impact of novel antivirals

Multiple novel antivirals for a variety of respiratory viruses are in late stage development and some of these may be licenced within the next few years, including baloxavir for influenza, which was licenced in the USA in October 2018.[150,151] Pathogen-directed use of these antiviral medications is key to maximising patient benefit and cost effectiveness of these new agents. Routine multiplex molecular POCT for respiratory viruses may result in similar benefits for novel antivirals as those seen with neuraminidase inhibitors for influenza. As such, molecular POCT may result in these novel treatments being used more frequently in pathogen-positive patients, limit their use in patients who do not have the relevant viruses, and promote very rapid initiation of

appropriate, specific treatment. These effects, notably promoting a short time to treatment initiation, may result in improved patient outcomes, which is consistent with the patient benefits seen with neuraminidase inhibitors where greater benefit is seen with rapid instigation of treatment.[139-141]

4.17 The ResPOC trial in the context of another point-of-care FilmArray-based study in hospitalised adults

There has been only one other syndromic molecular point-of-care testing randomised controlled trial in hospitalised adults.[201] The results were published after the ResPOC study was published. This was a quasi-randomised study in adults presenting to a large London-based hospital trust with influenza-like illness or upper respiratory tract infection (with or without lower respiratory tract infection). The quasi-randomised trial design (based on even or odd days of the month) led to a skewed 3:2 ratio of intervention to control patient recruitment. The intervention group received FilmArray Respiratory Panel testing as a molecular point-of-care test and the control group all had laboratory testing.

This study found no association between POCT and length of hospital stay, or most antibiotic-related outcomes, but this was likely due to a long delay between patients presenting to the admissions unit and the initiation of the study procedures. Despite this, POCT in this study still resulted in faster initiation of neuraminidase inhibitors in influenza-positive patients than in the control group. While there are similarities with ResPOC in terms of rapid and appropriate antiviral prescribing, the delay in patient recruitment in this study may have meant POCT results did not influence decisions and treatment plan in a timely manner.

The total number of participants was smaller than in the ResPOC study and the patient population somewhat different; notably the impact of POCT in ResPOC was greatest in terms antibiotic and length of stay in patients with exacerbations of airways disease whereas this study focused on adults with influenza-like-illness and upper respiratory tract infection.

This study does highlight that the new technology itself is insufficient to improve patient outcomes unless accompanied by the correct systems to garner the benefits to the patient. This study suggests that at least some of the patient benefits of POCT are time-dependent.

4.18 The ResPOC trial in the context of another FilmArray-based study in hospitalised adults

A small US randomised controlled trial was published in 2015 that assessed the feasibility of using procalcitonin measurement algorithms plus FilmArray Respiratory Panel results to direct antibiotic use in hospitalised adults with non-pneumonic lower respiratory tract infection.[188] The FilmArray Respiratory Panel was not used as a point-of-care test but was based in the laboratory instead. No significant difference was found in the proportion of patients who received antibiotics. However, where physicians were adherent to the algorithm, patients received shorter courses of antibiotics without any indication of increased patient harm. A follow-up report examined factors associated with optimal algorithm adherence but not all factors were necessarily amenable to change or readily resolvable by physician training.[202] Previous observational studies examining the impact of combined procalcitonin and laboratory PCR in adults hospitalised with suspected respiratory infection also showed no significant reduction in antibiotic use.[203]

The ResPOC study was larger than this RCT, had more broad inclusion criteria, and was a pragmatic study that featured no treatment-algorithms or other biomarker-related data. Similarities include that the primary outcomes were related to proportion of antibiotics and that no significant difference was found but that there were positive outcomes in other antibiotic related outcomes.

There is a conflicting evidence of the real-world benefit of the use of procalcitonin in hospitalised adults with acute respiratory tract infection in terms of guiding treatment. A 2018 Cochrane systematic review, also published in *The Lancet Infectious Diseases*, concluded that, in this patient group, procalcitonin reduced antibiotic use and side-effects and improved survival.[204,205] Contrasting this, a subsequent large multi-centre RCT published in the *New England Journal of Medicine* concluded that antibiotic use was not lessened in adults presenting to hospital with acute respiratory illness by procalcitonin use.[206] The Cochrane review's RCTs were predominantly based on studies with strict adherence to the procalcitonin algorithm for antibiotics whereas the *New England Journal of Medicine*'s RCT was more pragmatic and likely represents a real-world implementation of procalcitonin testing.

4.19 Molecular POCT in a future pandemic

During the early phase of the 2009 influenza pandemic, it was discovered that rapid antigen tests for influenza, which were already known to have poor sensitivity, also failed to adequately detect

the newly circulating swine-origin virus, and false-negative tests may have contributed to additional influenza transmission.[207,208]

Molecular point-of-care tests, especially PCR-based platforms, are less likely to fail to detect a novel influenza A virus. This is because they have primers that detect highly conserved regions of the influenza virus genome, and some may have multiple influenza A primers as the FilmArray Respiratory Panel does, some of which should still detect the virus even if some parts of the genome are altered by antigenic shift in a novel virus.[96] Molecular platforms are therefore still likely to be able to return a result of untypable influenza A with a novel strain. This untypable result may even alert molecular POCT operators to expedite laboratory and reference laboratory RT-PCR results.

This demonstrates how in a pandemic caused by a novel influenza virus, molecular point-of-care testing can still potentially detect the presence of a novel virus, and even be improved during a pandemic. The diagnostic accuracy of molecular POCT for influenza for drifted and divergent strains is therefore likely to be preserved but is currently unknown.

Molecular point-of-care tests can be updated with novel primers to detect newly circulating strains and refine existing detection and sensitivity. The FilmArray Respiratory Panel improved the sensitivity of adenovirus detection with an upgraded version and the FilmArray Respiratory Panel 2, released after the start of the ResPOC study, has improved sensitivity for a range of viruses.[100,104] In these cases the hardware platform stayed the same, but the testing consumables themselves along with the software were updated. Similarly, in a potential pandemic, one key strength of implementation of molecular point-of-care testing for a novel pathogen is that the hardware of the testing unit remains unchanged. The consumables including the pouch are retained too, with the only change being the addition of new primers for the pandemic pathogen, and, in some cases a software update might be required. The US Centers for Disease Control and Prevention developed, validated and started distributing a PCR assay for the novel H1N1 influenza virus during the 2009 pandemic in under two weeks from recognition of the novel virus, demonstrating how rapid the development of an updated molecular POCT might be.[208]

The benefits shown in this study from molecular POCT of rapid and appropriate NAI administration and isolation facility rationalisation are likely to be applicable in an influenza pandemic setting. However, as the proportion of patients tested by laboratory PCR for respiratory viruses would likely be greatly increased, virus detection would likely be increased, and a pandemic setting alters the healthcare environment, direct extrapolation of the clinical impacts of molecular POCT is unwise; however, it is conceivable that some benefits are increased, some lessened.

Pandemic plans generally use influenza-like illness criteria for triage and directing supply of antiviral medication.[30,31] Influenza-like illness criteria are highly inaccurate for the correct diagnosis of influenza,[30,31,209] and so molecular POCT-directed treatment with antivirals, presuming that they are active against divergent strains of influenza, could potentially be of national importance. However, issues of molecular POCT cost and high patient numbers could render a routine testing strategy unfeasible.

The FilmArray platform was used in multiple locations during the West African Ebola virus epidemic. BioFire had developed the BioThreat and BioThreat-E panels, which includes Ebola virus detection, before the pandemic started; these panels retain the ability to generate a result in about an hour. Sensitivity was very good compared to laboratory PCR even under field conditions, and the platform has been used in small hospitals and under austere conditions.[210,211]

As molecular platforms have the capability to detect multiple pathogens, there is also the potential to rule out or diagnose infections other than the pandemic pathogen. As these molecular platforms have limited hands-on time, healthcare and laboratory staff may have less exposure to hazardous samples reducing risk to staff members.

Molecular testing can also be linked to automatically report results to centralised facilities to enhance surveillance capabilities within a pandemic. There is already a cloud-based autonomous surveillance network for respiratory pathogens using results from FilmArray systems.[212]

Therefore, molecular point-of-care testing during a pandemic caused by a respiratory virus or other pathogen such as the Ebola virus has huge potential to rapidly diagnose and improve patient care even in resource-limited settings.

4.20 The role of laboratory PCR where POCT is introduced

4.20.1 Benefits to different sized hospitals

The ResPOC study was conducted in a large teaching hospital with a large regional laboratory on the hospital site that processed and gave the results for the laboratory RT-PCR for respiratory viruses.

Some hospitals, notably smaller or more isolated hospitals which lack the specialised equipment and personnel for PCR, rely on respiratory specimens being couriered to centralised laboratories. This delay could increase the turnaround time beyond the 30 hours seen in the ResPOC study and lessen any clinical benefits of doing a laboratory test for respiratory viruses. In these settings, molecular POCT for respiratory viruses has the capacity to make even more of a difference to

patient outcomes. With the excellent sensitivity of multiplex PCR-based platforms such as the FilmArray, introducing molecular POCT to these hospitals may mean that sending samples on to a centralised laboratory for RT-PCR would be of minimal added value to patients and the hospital.

4.20.2 Infection surveillance

There is still a need for centralised, large-scale and widespread respiratory virus surveillance for the benefit of public health, vaccination programmes, and early detection of pandemics. Centralised laboratory RT-PCR testing for respiratory viruses allows surveillance data to be easily collected and distributed on a national and international scale.[37,38,53] Early detection of a novel, rapidly spreading virus by laboratory methods was of benefit in response to the 2009 pandemic.[207,208] However, as molecular platforms tend to be digital platforms that can be added to a network, data from a molecular POCT platform could feasibly be linked to a local or regional laboratory and national reporting systems. There is already a system, BioFire Syndromic Trends, whereby anonymised patient test results from FilmArray machines are sent directly to a cloud-based database. This system autonomously exports, aggregates and analyses these results from US laboratories and summaries of these data are displayed in near real time on a public website.[212] The use of the FilmArray platform in this way is applicable to FilmArray units deployed as molecular POCT and so demonstrates the capability of molecular point-of-care systems to contribute to infectious disease public health surveillance in the same manner as RT-PCR in laboratories.

4.20.3 Neuraminidase inhibitor resistance testing

Rapid molecular testing platforms currently lack any neuraminidase inhibitor resistance gene detection whereas this is commonly tested for in laboratory RT-PCR testing. This is important for individual patient treatment and for regional surveillance of antiviral resistance. While resistance to NAIs is currently rare, H1N1 resistance to oseltamivir spread very quickly prior to the pandemic.[138] Therefore, laboratory RT-PCR testing is still needed in this surveillance and treatment role. However, some molecular platforms including FilmArray have resistance gene PCR-based testing for bacteria, meaning that development of virus resistance gene testing for molecular POCT platforms is feasible.[213]

4.20.4 Range of viruses detected

It is worth noting that the FilmArray panel detects more targets than the hospital laboratory's RT-PCR panel. This can broadly be thought of as a positive aspect of the FilmArray panel, although

clinical support to clinicians unfamiliar with some of the lesser known viruses may be needed to maximise clinical benefits.

4.21 Challenges to implementation of routine molecular POCT

4.21.1 Models of POCT delivery

In the ResPOC study, the POCT was operated exclusively by study staff. There are two key models for integrating routine molecular POCT for respiratory viruses into standard care: one of front-line clinical staff as POCT operators, the other, laboratory-trained staff operating the POCT platform.

Doctors, nurses and healthcare assistants operate POCTs such as dipstick urinalysis and urine pregnancy tests frequently in acute care settings. They may also operate influenza rapid antigen-based POCT. In one UK interventional study using the FilmArray platform as a POCT in hospitalised adults (discussed in 4.15), ward staff were trained to use the FilmArray in an AMU setting. However, over two-thirds of tests were done by study staff, which may reflect a reluctance to operate the POCT or unwillingness to add to their own workload.[193,195] Regardless of the reasons, the lack of use by ward staff resulted in delays in using the POCT, curtailing the potential benefits of rapid detection. When the POCT was operated by the ward staff, they performed it without incident.

Establishing and maintaining POCT operator competencies, particularly in ED and AMU where junior doctors rotate posts so frequently, is an area of concern already long-established with other point-of-care systems.[214] Similarly, untrained operator use of POCT raises quality assurance concerns.[215] As systems evolve over time, the simplicity of use should improve, reducing these concerns.

One criticism of POCT is that even with an established framework of POCT operator competencies, POCT operators are likely to be healthcare professionals untrained in laboratory sciences. This means that the analytical phase may be prone to error, as opposed to laboratory testing where the risk of error in the analytical phase is far lower.[216] However, the integrated nature of the molecular platforms suitable for deployment as POCT means that the analytical phase is integrated into the test kit, removing the risk of error by the operator.[96]

An alternative model to front-line staff delivering molecular POCT would be to have laboratory-trained staff operating the POCT. This has several advantages. Having staff trained in laboratory sciences may improve infection control measures surrounding the use of the POCT by bringing in practices common to a laboratory setting. It may mean that quality control measures are easier to

organise and manage as the laboratory RT-PCR positive and negative control procedures can be copied and adapted for the POCT. Having dedicated, experienced staff may reduce the risk of operator errors. Deploying laboratory staff forward in clinical areas may improve communication and understanding between the laboratory and acute care areas. Having laboratory-trained staff operating the POCT may mean that when unusual or questionable results occur, prompt confirmatory testing can be arranged as internal communication between laboratory staff members may result in faster outcomes than clinician to laboratory communication. With the laboratory taking responsibility for the hardware rather than an acute care area, any hardware faults may be dealt with by staff more experienced with scientific equipment and their suppliers, resulting in shorter periods of time without a POCT service. As other POCT systems, including blood glucose testing machines and blood gas analysis machines, are often managed by the laboratory service, some laboratories already have personnel dedicated to the provision of POCT.[217]

Developing the model of laboratory staff operating POCT further, the creation of a POCT hub or POCT laboratory within clinical areas could offer further advantages. The FilmArray molecular platform has tests for gastrointestinal pathogens from stool, and also meningitis and encephalitis pathogens from cerebrospinal fluid; both tests have potential to be deployed as POCT.[218,219] Therefore a POCT hub or laboratory situated in a clinical area has the potential to run several different infection tests, even from a single molecular platform.

One disadvantage of having laboratory-trained staff members operate the POCT is that the acute care area directly looking after patients risks losing control of the POCT process, to a system where speed of generating results may be less of a priority. In addition, laboratory managers may view the POCT systems as less of a priority than laboratory-based systems, and so when resources and staff members are in short supply, POCT services may be the first to be cut-back.

New systems and workflow processes in acute clinical areas take time to implement and time for the benefits to clinical care to come to fruition. One observational study of implementing POCT for haematology and biochemistry tests introduced into emergency care concluded a POCT strategy improved patient care, however, the authors noted, “a longer time is seemingly needed to adopt a new working process in the ED, and to establish its full benefit.”[220] Thus any planning and evaluation of implementing POCT for respiratory viruses in adults presenting to hospital, must not underestimate the time these changes may make.

There are uncertainties around the correct model of implementation and real-world effectiveness data post implementation will be key to evaluating and improving a point-of-care testing service for infectious diseases.

4.21.2 Infrastructure and cost

Regardless of the model of POCT delivery, a physical space is required in or near patient care areas for the POCT hardware and consumables, along with suitable space to prepare the testing kit. Given the competing demands upon a busy acute medical unit or emergency department, acquiring and developing a suitable space for the POCT system may be difficult. The space for POCT must be within or close to patient areas, or the point-of-care benefits over laboratory processing are lost. The space must have sufficient electrical outlets for the molecular POCT. To minimise the infection control concerns, the space must be able to be decontaminated easily.

The cost to hospitals of new point-of-care test hardware, set-up, consumables and training may be substantial. Hospital budgets are typically compartmentalised, so that the cost savings from shorter patient admission duration in an acute medical unit, would not be passed on to the microbiology laboratory which may be responsible for the expenditure required to deploy molecular POCT hardware and consumables.[221]

4.21.3 Quality Control

In the ResPOC trial, positive control panels, purchased from a specialist company, and negative controls were run on the FilmArray units at baseline and periodically during the study. There were no instances of false positives or false negatives found with the positive and negative controls in the study, which may reflect the robust nature of the machines. Nevertheless, when deployed as POCT any system must undergo appropriate validation and quality control testing as part of maintaining confidence and the reliability of the platforms and tests. While each FilmArray pouch has two internal controls, dedicated testing of all pathogen targets must be done.[96]

Where the model of implementation of molecular POCT is delivered by a laboratory rather than the clinical service, the current quality control systems including positive and negative control testing in place for laboratory RT-PCR for respiratory viruses should broadly be applicable and adaptable for PCR testing at the point-of-care. With the centralisation of laboratory services, some smaller hospitals may not have these processes in place, however, adaptation of their centralised laboratory's protocols may be possible.

The United Kingdom National External Quality Assessment Service (UK NEQAS) is the body that independently assesses microbiology laboratories processes and capabilities in the UK. While molecular testing for some pathogens is assessed by UK NEQAS, there are no assessment schemes for any infection-related molecular POCT. Therefore, it is likely that UK NEQAS will need to develop a new assessment process for molecular POCT.

Where a clinical service, rather than a laboratory, is responsible for implementing molecular POCT for respiratory viruses, templates for quality control processes may be adapted from other clinically-based POCT systems such as glucose monitoring, anticoagulant testing and point-of-care ultrasound systems. One limitation of a clinical service implementing molecular POCT for respiratory viruses rather than laboratory-trained staff would be that there is potentially more effort involved in creating quality control purposes.

4.21.4 Results delivery

In the ResPOC trial, virus-positive results were delivered directly to the clinicians responsible for the patient. If the model of POCT implementation was based on having dedicated POCT staff, then a similar model of results delivery could be implemented also (i.e. POCT staff member directly informs the requesting clinician). Where clinicians are responsible for operating the POCT, the clinicians operating the test is are likely to collect the result themselves and therefore be in a position to act directly upon the results.

One challenge of results delivery from POCT for respiratory viruses is that for optimal patient management and outcomes, several clinical team members need to receive and act upon the results. The clinician looking after the patient is likely to prescribe a neuraminidase inhibitor if influenza is detected by the POCT or discontinue neuraminidase inhibitor treatment if prescribed empirically and influenza is not detected. Depending on a positive or negative POCT result, a clinician may consider prescribing or discontinuing antibiotics. The POCT result also must be incorporated into the medical record (including integration into the laboratory information management system) so that clinicians looking after the patient beyond immediate patient management have the result; this includes the POCT results being accessible for the medical consultant post-take ward round. In the emergency department or acute medical unit, the nurse-in-charge, flow co-ordinator, or bed manager is responsible for the allocation of side rooms (i.e. isolation rooms); therefore, for appropriate isolation of virus-positive and for appropriate de-escalation of virus-negative patients this key decision maker must be informed of the POCT results in a timely manner. Similarly, the infection control team also need to be informed of POCT results to optimise infection control measures in the hospital.

One key aspect of results delivery is incorporating the POCT results into patients' medical records so that they can be accessed as needed by any healthcare professional. Any results delivery process must integrate into a potential variety of medical records systems, from paper to electronic. As respiratory virus POCT results are an investigation result, it would be consistent to include the POCT results into the hospital's system for reporting investigation results, to be

available alongside haematology and biochemistry test results and other microbiology test results including laboratory respiratory virus RT-PCR results. As most molecular platforms are digitally-based and already report electronically to a results system when-laboratory based, integration of POCT-deployed platforms into a hospital's computer network and results reporting software may be feasible without significant technical difficulty.

4.21.5 Infection control

There are two key infection prevention and control issues relating to implementing molecular POCT for respiratory viruses into an acute, hospital-based care setting.

The first key issue is ensuring the testing environment and machines remain uncontaminated by the preparation and testing process to limit the risk of pathogen spread and risk to staff and patients. The FilmArray machine comes with instructions on shielding the operator from direct contact with the viral transport media. In addition, each test pouch is heat-sealed by the machine, with one benefit of this being the containment of potential pathogens. This heat-sealing, plus having all reagents and reaction confined to the testing pouch, also limits the likelihood of contamination from and of the machine itself.[96]

The second key issue relating to infection prevention and control is the timely communication of results to infection prevention and control staff members. This could be facilitated by the POCT result being integrated with the laboratory information management system, which the infection control team would already rely on for their important pathogen results. Detection of a pathogen is important for ensuring prompt patient isolation and implementation of personal protective equipment for staff members and visitors, and a negative result is important for de-escalation of such measures.[172] Infection control nurses and other key decision makers need to be made aware of these results to assist and advise in appropriate patient care.

4.21.6 Interpretation of results

One of the strengths of the ResPOC study was that the actions of healthcare professionals on receiving the POCT results were not protocol-driven; therefore, it was a pragmatic study applicable to 'real-world' clinical care. However, to optimise the introduction of molecular POCT for respiratory viruses into routine care, some additional clinical support to healthcare professionals to interpret the results may be required to maximise benefit to patient outcomes. In the ResPOC study, for example, the FilmArray respiratory panel had the capacity to detect a wider range of respiratory viruses than the hospital laboratory's RT-PCR panel, consequently when a coronavirus was detected by POCT, staff members were unfamiliar with this virus and there was

the potential for inappropriate clinical action including failure to de-escalate antibiotic prescribing or inappropriate antiviral use, as well as undue uncertainty and concern to patient and staff.

One strategy to assist in POCT result interpretation would be to have infection specialists based in the emergency department and acute medical unit (i.e. the integration of infection specialist services within emergency care). The paucity of clinical infection specialists in the UK and worldwide, along with increasing infection demands, means that having such specialists consistently present in these frontline areas is not feasible for most hospitals. However, given widespread concern over inappropriate antimicrobial prescribing in ED and AMU, and the potential to improve this prescribing by implementation of molecular POCT for respiratory viruses as shown in the ResPOC study, antimicrobial stewardship intervention is required to fully realise the potential clinical impact of such rapid diagnostic tests. Educational programmes, antimicrobial use audits and feedback, and tools including antimicrobial prescribing guides on paper and smartphones have all been proposed to support frontline clinicians in using the results from rapid diagnostic tests and optimising patient care.[203,222]

Given the recent controversy over oseltamivir (Tamiflu) trials data, UK hospital clinician confidence in neuraminidase inhibitor treatment for influenza virus infection may have wavered, despite large-scale observational data suggesting improved clinical outcomes with treatment.[139,223] Timely detection of influenza by molecular POCT may fail to translate into optimised patient outcomes if clinicians are unwilling to prescribe neuraminidase inhibitors to infected patients.

4.22 Conclusion

Routine molecular POCT for respiratory viruses in adults presenting to secondary care with acute respiratory illness improved the turnaround time of results and the detection rate of respiratory viruses but did not reduce the proportion of patients treated with antibiotics or the overall duration of antibiotic use. However, routine molecular POCT was associated with an increased proportion of patients receiving single doses or brief courses of antibiotics, reduced length of hospital stay, improved use of neuraminidase inhibitors for influenza, improved use of hospital isolation facilities, and appeared to be safe. If these findings are reproduced in further studies and are associated with health economic benefit, routine molecular POCT for viruses should be introduced into diagnostic pathways for acute respiratory illness in adults presenting to hospital during the winter months. There are some uncertainties around the correct model in implementation and therefore there is a need to collect real-world effectiveness data post implementation.

Chapter 5 Turnaround time and clinical outcomes

5.1 Overview

Lower turnaround time (<1.6 hours) for molecular point-of-care testing for respiratory viruses in adults hospitalised with acute respiratory illness is associated with improved clinical outcomes compared to longer turnaround time (>1.6 hours). As very rapid turnaround times lead to better outcomes and are unlikely to be achieved with laboratory-based testing, respiratory virus diagnostics should be performed at the point-of-care.

5.2 Introduction

Although the evidence presented in the ResPOC trial would suggest that rapid molecular testing needs to be performed within clinical areas for these improved clinical outcomes, it has been suggested that rapid molecular test platforms used within centralised laboratories might also be associated with these clinical benefits, although the turnaround times are likely to be much longer.[103,193]

5.3 Aim

In this follow-on study, the aim was to evaluate the impact of POCT TAT on clinical outcomes with a view to determining how rapid molecular testing for respiratory viruses should be best implemented in clinical practice.

5.4 Methods

5.4.1 Design

A *post hoc* analysis was performed to explore the impact of the TAT of POCT on clinical outcomes having previously shown significant differences between the POCT group and control group for overall LOS and antibiotic use. TAT is defined as the time from a patient being recruited to the results being communicated to clinicians. As our previous study demonstrated that the improved outcomes seen with POCT occurs only in patients testing positive for viruses (with those testing negative having similar outcome to control patients) we restricted our analysis to those patients testing positive for viruses by POCT. We examined the association between POCT TAT, LOS and antibiotic use and assessed the effect of a TAT of less than or greater than 1.6 hours (the median).

5.4.2 Statistical analysis

Statistical analyses were done using Prism version 7.0 (Graphpad software; La Jolla, CA, USA) and Stata version 13.1 (StataCorp; College Station, TX, USA). Correlation was assessed using Spearman's rank correlation coefficient (rs). We compared LOS and antibiotic use between groups using median differences and the Mann-Whitney U test and differences in proportions using Chi squared or Fisher's exact test, as appropriate. ROC curves were generated to determine the optimal cut off for TAT. We performed a subgroup analysis of patients positive for influenza A or B and for patients positive for rhinovirus.

5.4.3 My contribution to this analysis

My contribution to the design, execution and analysis of the ResPOC trial has been outlined previously. For this *post hoc* analysis using ResPOC trial data, I participated in the design and interpretation.

5.5 Results

Of the 720 patents recruited in the parent randomised controlled trial, 360 allocated to the intervention (POCT) group and 354 allocated to the control (routine clinical care) group were included in the original analysis. TAT for POCT results varied from 1.1 to 6.4 hours with a median [IQR] of 1.6 [1.3 to 3.1] hours compared to a median of 29.8 [24.7 to 45.8] hours for laboratory PCR, in the control group. Of the 360 patients tested for respiratory viruses by POCT, 153 (43%) were positive. Human rhinovirus (55 [36%] of 153) and Influenza A and B (53 [35%] of 153) were the most commonly detected viruses.

For patients testing positive for viruses by POCT (n=153), the median [IQR] TAT was 1.6 hours [1.3 to 3.0] and therefore this time was chosen as cut-off for this study. 16 (10%) of 153 patients were discharged directly from the emergency department (ED). For patients admitted to hospital (n=137), TAT was positively correlated with length of hospital stay (rs = 0.24 [95% CI 0.07 to 0.39]; p=0.0051) and duration of antibiotics (rs = 0.22 [95% CI 0.05 to 0.38; p=0.0096]. All findings are described in Table 17.

There was no difference in the proportions discharged directly from ED with a TAT of ≤1.6 hours vs >1.6 hours, 8 (10%) of 77 vs 8 (10%) of 76, Odds ratio 0.99 (95%CI 0.3 to 2.8), p=1.0. For those admitted (n=137), the median [IQR] length of hospital stay was 2.3 [1.0 to 4.0] days for TAT of ≤1.6 hours vs 5.1 [2.4 to 8.3] days for TAT of ≥1.6 hours, difference of 2.8 (95%CI 1.0 to 3.5) days, p<0.0001. This difference in LOS was due to a higher proportion of patients with a TAT of ≤1.6

hours being discharged within 24 hours of admission, 18 (26%) of 69 vs 9 (13%) of 68 , Odds ratio 2.3 (95%CI 1.0 to 5.4), p=0.058 [number needed to test = 8] or within 48 hours of admission, 34 (49%) of 69 vs 15 (22%) of 68, Odds ratio 3.4 (95%CI 1.6 to 7.0), p=0.0012 [number needed to test =4].

A smaller proportion of patients with a TAT of ≤1.6 hours vs >1.6 hours were treated with antibiotics, 55 (80%) of 69 vs 63 (93%) of 68, Odds ratio 0.3 (95%CI 0.1 to 0.9), p=0.029 [number needed to test=8]. The median [IQR] duration of antibiotics was 2.9 [0.1 to 6.9] days for a TAT of ≤1.6 days vs 6.5 [2.4 to 8.5] days for a TAT of >1.6 days, a difference of 2.3 (95%CI 0 to 2.8) days; p=0.0097. This was due to a higher proportion of patients with a TAT ≤1.6 hours receiving <24 hour and <48 hours of antibiotics, 29 (42%) of 69 vs 16 (23%) of 68, Odds ratio of 2.3 (95%CI 1.1 to 5.0), p= 0.021 [number needed to test = 5] and 32 (46%) 69 of vs 17 (25%) of 68, Odds ratio 2.6 (95%CI 1.3 to 5.4); p=0.012 [number needed to test = 5], compared to those with a TAT of >1.6 hours for POCT testing.

ROC curve analysis showed that a TAT cut off of <1.6 hours had optimal sensitivity and specificity for association with early discharge, 48% (95%CI 32 to 56) and 77% (95%CI 65 to 87%), AUC of 0.68, p=0.0002, and early discontinuation of antibiotics, 45% (95%CI 33 to 57) and 74% (95%CI 23 to 84), AUC of 0.61, p=0.021.

49 (92%) of 53 influenza positive patients were admitted to hospital. Excluding viral co-infections, 49 (96%) of 51 rhinovirus positive patients were admitted to hospital. Subgroup analysis for hospitalised influenza and rhinovirus positive patients showed that rapid TAT (<1.6 hours) was associated with shorter length of stay and antibiotic duration for influenza positive patients but not for rhinovirus positive patients, although the numbers in the individual groups were small.

Table 17: Diagnostic group and outcomes by turnaround time, for patients testing positive for viruses

		Turnaround time for POCT		Difference (95%CI)	Odds ratio (95%CI)	p value
		≤1.6 hours	>1.6 hours			
	n	**77**	**76**			
Diagnosis (all patients)						
Exacerbation Asthma/COPD	153	32 (42)	30 (39)	-	1.1 (0.6 to 2.1)	0.87
CAP	153	13 (17)	17 (22)	-	0.7 (0.3 to 1.6)	0.42
ILI /NPLRTI	153	24 (31)	22 (29)	-	1.1 (0.6 to 2.2)	0.86
Other	153	8 (10)	7 (9)	-	1.1 (0.4 to 3.0)	0.99
Severity (all patients)						
Pulse rate (bpm)	153	105 [90 to 120]	100 [88 to 110]	-5.5 (-12 to 0)	-	0.055
Respiratory rate (bpm)	153	25 [20 to 28]	20 [18 to 26]	-5 (-5 to -1)	-	**0.0012**
Systolic BP (mmHg)	153	134 [118 to 153]	132 [117 to 150]	-2 (-9 to 6)	-	0.74
Saturations (%)	153	96 [93 to 98]	96 [93 to 98]	0 (-1 to 1)	-	0.76
CRP (mg/L)	153	37 [16 to 93]	61 [12 to 129]	24 (-10 to 24)	-	0.61
WCC ($x10^9$/L)	153	10.8 [7.6 to 15.2]	10.2 [7.9 to 13.2]	-0.6 (-2 to 0.9)	-	0.47

Outcomes (all patients)						
Discharged from ED	153	8 (10)	8 (10)	-	1.0 (0.3 to 2.8)	0.99
Admitted	153	69 (90)	68 (90)	-	1.0 (0.4 to 2.9)	0.99
Length of hospital stay (days)	137	2.3 [1.0 to 4.0]	5.1 [2.4 to 8.3]	2.8 (1.0 to 3.5)	-	**<0.0001**
Discharged within 24 hours	137	18 (26)	9 (13)	-	2.3 (1.0 to 5.4)	0.058
Discharged within 48 hours	137	34 (49)	15 (22)	-	3.4 (1.6 to 7.0)	**0.0012**
Treated with antibiotics	137	55 (80)	63 (93)	-	0.3 (0.1 to 0.9)	**0.029**
Duration of antibiotics, days	137	2.9 [0.1 to 6.9]	6.5 [2.4 to 8.5]	2.3 (0 to 2.8)	-	**0.0097**
Treated with <24 hours antibiotics	137	29 (42)	16 (23)	-	2.3 (1.1 to 5.0)	**0.021**
Treated with <48 hours antibiotics	137	32 (46)	17 (25)	-	2.6 (1.3 to 5.4)	**0.012**
Influenza positive only		23	26			
Length of hospital stay (days)	49	2.0 [0.9 to 3.7]	5.1 [1.8 to 7.1]	3.1 (0.3 to 4.2)	-	**0.023**
Discharged within 24 hours	49	7 (30)	4 (15)	-	2.4 (0.6 to 8.2)	0.31
Discharged within 48 hours	49	12 (52)	7 (27)	-	3.0 (0.9 to 10.0)	0.086
Treated with antibiotics	49	18 (78)	25 (96)	-	0.1 (0.1 to 1.3)	0.086
Duration of antibiotics, days	49	1.1 [0.1 to 6.9]	7.0 [3.8 to 8.9]	5.9 (0.3 to 6.1)	-	**0.0048**
Treated with <24 hours antibiotics	49	11 (42)	6 (23)	-	3.1 (0.8 to 9.5)	0.082

Treated with <48 hours antibiotics	49	12 (52)	6 (23)	-	3.6 (1.0 to 11.2)	**0.043**
Treated with NAIs	49	23 (100)	24 (92)	-	4.4 (0.2 to 97)	0.34
Rhinovirus positive only		29	17			
Length of hospital stay (days)	46	2.4 [1.0 to 4.5]	2.6 [1.0 to 8.5]	0.2 (-1.0 to 2.3)	-	0.44
Discharged within 24 hours	46	7 (24)	4 (24)	-	1.0 (0.3 to 3.6)	1.0
Discharged within 48 hours	46	13 (45)	5 (29)	-	2.0 (0.6 to 6.6)	0.36
Treated with antibiotics	46	23 (79)	15 (88)	-	0.5 (0.1 to 2.6)	0.69
Duration of antibiotics, days	46	6.0 [0.1 to 7.5]	6.4 [3.1 to 8.6]	0.4 (-0.7 to 5.4)	-	0.44
Treated with <24 hours antibiotics	46	11 (38)	4 (22)	-	2.1 (0.6 to 7.0)	0.34
Treated with <48 hours antibiotics	46	12 (41)	4 (22)	-	2.2 (0.6 to 7.6)	0.34
Safety (all patients)						
ICU admission*	137	1(1)	3(4)	-	0.3 (0.1 to 2.2)	0.37
Death*	153	0 (0)	0 (0)	-	-	1.0
Re-presentation to ED*	153	6 (8)	11 (14)	-	0.5 (0.2 to 1.5)	0.21
Readmission*	138	4 (6)	5 (7)	-	0.8 (0.2 to 2.7)	0.72

POCT=point-of-care testing. CI=confidence interval. CAP= community acquired pneumonia. ILI=influenza-like illness. NPLRTI=non-pneumonic lower respiratory tract infection. BP=blood pressure. CRP=C-reactive protein. WCC=white cell count. NAI=neuraminidase inhibitors. ED=

emergency department. ICU=intensive care unit. *Measured for 30 days post enrolment. Due to the large size of the table, significant values are highlighted in bold for clarity.

5.6 Discussion

This study shows that even with the rapid turnaround times for results seen with molecular POCT compared to centralised laboratory PCR testing, TAT for results remains an important determinant of clinical outcome for respiratory virus testing. Very rapid turnaround times are associated with higher rates of early discharge and early discontinuation of antibiotics compared to longer TATs in adults with acute respiratory illness. This suggests that there is a brief and early 'window period' for the results of respiratory virus testing to alter patient management after admission to hospital. Although the TAT of laboratory PCR testing is variable across different institutions, and may be a short as several hours in some centres, a very short TAT of under 2 hours is unlikely to be achievable within centralised laboratories and so rapid molecular viral diagnostics should be performed in clinical areas at the point-of-care in order to realise these clinical benefits.

Although this study is a *post hoc* analysis, its strengths include the randomised nature of the parent study, the large cohort of patients studied and its pragmatic nature. In addition, our findings are consistent with observational studies using rapid molecular diagnostics for respiratory viruses and showing improvements in clinical outcome, dependent on short TATs.[103,193] Although it is likely to be generalisable to other centres, we cannot rule out that the changes seen are dependent on the processes of care in UK hospitals.

Other limitations of this *post hoc* analysis include potential bias and cofounders which may affect the results of this study. There may be an unconscious selection bias affecting whether a recruited patient had a rapid or slow POCT turnaround time depending on the priority assigned to that patient's results by the facilitating research staff. For example, a POCT test and result that might affect a patient's care more may be prioritised over a patient whose POCT result would be less likely to impact clinical outcomes. However, diagnosis and severity of illness markers appear broadly similar between both the <1.6 hours and the >1.6 hours groups, and if a difference could be argued, the disease severity (and potential for worse outcomes) was worse in the <1.6 hours group (Table 17).

The cost effectiveness of a routine molecular POCT testing strategy for respiratory viruses in hospitalised adults is currently unknown. As length of hospital stay is the key determinant of cost for patients hospitalised with ARI, the increase in early discharge with POCT strongly suggests that even a modestly more expensive diagnostic strategy is likely to be cost saving compared to routine clinical care. It is currently uncertain as to how molecular POCT for respiratory viruses could be implemented within the NHS and other health systems. Potential models include training

clinical staff to perform the testing or the development of dedicated point-of-care testing laboratories within or close to acute areas.

In conclusion, POCT with a TAT of <1.6 hours was associated with higher rates of early hospital discharge and early discontinuation of antibiotics, compared to longer TATs. As these very rapid TATs are unlikely to be achievable with centralised laboratory testing, viral diagnostics should be performed at the point-of-care and models for the implementation of this need strategy to be explored.

Chapter 6 Survey of influenza testing and neuraminidase inhibitor use in suspected influenza

6.1 Overview

UK Public Health England (PHE) guidelines recommend the liberal use of neuraminidase inhibitors (NAIs) in hospitalised adults with suspected influenza and are aligned with international guidelines.[135] NAI use is recommended to start as early as possible and empirical use is recommended whilst awaiting laboratory results. Current UK hospital physician knowledge, attitudes and practises regarding the use of NAIs, and levels of adherence to guideline recommendations are not known.

An online, cross-sectional questionnaire-based survey of self-reported prescribing practice using clinical scenarios was distributed to secondary care physicians involved in the assessment of adults presenting to hospital with suspected influenza. The primary outcome measure was adherence to PHE guidelines.

There were 237 respondents to the survey. 157 (67%) of 233 respondents reported awareness of PHE guidelines. Adherence to treatment guidelines in the clinical scenarios ranged from 56% (95% CI 49-63%) to 72% (95% CI 66-79%) with considerable variability between specialities (p=0.0008). Not treating suspected cases was common as was withholding of NAIs whilst awaiting laboratory results, despite the acknowledgment of prolonged turnaround times. 73 of 220 (33%) respondents reported that concerns about NAI efficacy influenced their prescribing.

Adherence to national guidelines for the treatment of influenza is sub-optimal. Lack of guideline awareness and concerns over the effectiveness of NAIs are contributing factors. This study highlights a disparity between public health policy and clinical practice and suggests that strategies that promote diagnostic testing and adherence to treatment guidelines are required.

6.2 Introduction

The effectiveness of neuraminidase inhibitors (NAIs) for the treatment of influenza has been the source of much debate over recent years.[223] The original placebo controlled trials of NAIs were performed mainly in otherwise healthy patients with uncomplicated influenza and showed a modest reduction in the duration of illness symptoms and viral shedding.[142,224] These largely community-based trials contributed to a Cochrane review process that suggested pharmaceutical

firms withheld publishing data from some trials, and when trial data was released to the Cochrane group, they concluded that there were multiple weaknesses in the methods and data collection of the trials.[223,224] Hospitalised adults with already complicated disease represent a priority for NAI treatment but no placebo controlled trials have been performed in this group. There is now a large body of data from observational studies which have consistently shown clinical benefits of NAIs in this group, including reduction in mortality.[139,140,196,225-229] Consistent with this evidence, PHE guidelines recommend the use of NAI treatment for hospitalised adults and are strongly aligned with other national and international guidelines,[135,230,231] and supported by a recent UK Department of Health commissioned review.[232] As early NAI treatment is associated with better outcomes,[139,140] and the turnaround time of conventional PCR laboratory testing for influenza is generally 1-2 days, empirical use is recommend at the point of presentation, whilst awaiting results.

Internationally there is great variation in the use of NAIs for influenza, including for hospitalised adults.[233,234] Current knowledge, attitudes and prescribing practises regarding the use of NAIs and adherence to PHE guidelines among front-line UK physicians are not known.

6.3 Aim

The aim of this study was to explore the current knowledge, attitudes and prescribing practises regarding NAIs by UK-based front-line physicians involved in the initial assessment of adults presenting to secondary care with suspected influenza by means of a cross-sectional survey of practice.

6.4 Methods

6.4.1 Design

We performed an online, cross-sectional questionnaire-based survey of self-reported practice using the web-based survey tool SurveyMonkey. UK physicians involved in the initial assessment of adults presenting to hospital with suspected influenza, and therefore involved in the decision to use NAIs, were invited to complete the questionnaire. Entry into a prize draw was offered as an incentive to complete the questionnaire. Following conceptualisation and a scoping exercise the questionnaire was designed by a panel and piloted internally at University Hospital Southampton Foundation NHS Trust. The questionnaire format and questions were subsequently adapted to improve validity and reliability. It was then emailed out with an introductory/explanatory letter to potential participants via the following participating specialist societies; the British Infection

Association, the British Thoracic Society, the British Geriatrics Society and the Society of Acute Medicine. The Royal College of Emergency Physicians was approached but declined to take part. To increase the coverage and response rate the introductory letter and questionnaire were also emailed out to physicians via the R&D departments at NHS trusts. The names of the 13 participating trusts are listed in Table 18. The survey was sent out a further two times at monthly intervals via the specialist societies and participating NHS trusts, to maximise response rate.

Table 18: List of Survey Participating Hospital Trusts

1. West Suffolk NHS Foundation Trust
2. The Shrewsbury and Telford Hospital NHS Trust
3. Salford Royal NHS Foundation Trust
4. Colchester Hospital University NHS Foundation Trust
5. Dorset County Hospital NHS Foundation Trust
6. Hull and East Yorkshire Hospitals NHS Foundation Trust
7. Torbay and South Devon NHS Foundation Trust
8. North Bristol NHS Foundation Trust
9. North Tees and Hartlepool Hospitals NHS Foundation Trust
10. University Hospital Bristol NHS Foundation Trust
11. Medway NHS Foundation Trust
12. East Lancashire Hospitals NHS Trust
13. University Hospital Southampton NHS Foundation Trust

The survey used a combination of closed question statements and open ended free-text comments. The first question confirmed the appropriateness of the respondent to be completing the questionnaire. The questionnaire contained questions relating to demographic data, local testing methods for influenza and turnaround time for results, knowledge of national treatment guidelines and a relevant Cochrane review, and factors affecting physician's decisions to use NAIs. It was made clear that the clinical scenarios all took place during periods of peak influenza transmission. There were 4 separate clinical scenarios representing the four categories of patients considered for NAI treatment in national and international guidelines: (1) uncomplicated influenza with no risk factors for subsequent complicated disease, presenting within 48 hours of symptom onset; (2) uncomplicated influenza with risk factors for subsequent complicated disease,

presenting within 48 hours of symptom onset; (3) already complicated disease presenting within 48 hours of symptom onset; (4) complicated (life threatening) disease presenting after 48 hours of symptom duration. Respondents were asked to pick responses from a list of options with the choice to add free text comments to justify their answers or provide additional details. In addition, there was provision for free text comments at the end of the survey for respondents to mention any other relevant factors or opinions. Responses were anonymised. The full survey questionnaire is reproduced in the Appendix E.

6.4.2 Ethics approval

As this was a web-based questionnaire-based survey of practise among physicians, specific written informed consent from participants was not deemed necessary - consent being implied by the decision to take part in the survey (a process made clear on the SurveyMonkey webpage). Ethical approval was granted by University of Southampton Faculty of Medicine Ethics Committee on the 25th of September 2015 (ID: 17321).

6.4.3 Study registration

This study was prospectively registered with an international trials database (ISRCTN18249297).

6.4.4 Statistical methods

Anonymised data were entered into a dedicated database and cleaned. Analysis was conducted using Prism version 6.0 (GraphPad Software Inc; La Jolla, California) and Stata version 13.1 (StataCorp, College Station, Texas). The primary outcome measure was adherence to PHE guidelines as measured by the proportion of respondents selecting the appropriate responses for each scenario. Baseline characteristics were summarised using appropriate descriptive statistics. The level of respondent adherence to PHE guidelines for each scenario was calculated as an overall proportion and then for individual specialities. Results for all four Scenarios were pooled to give a combined estimate of guideline adherence for all respondents and then calculated for the individual specialities and compared across them (Chi squared test). Where 95% confidence intervals are presented, the Copper-Pearson ‘Exact’ method is used. Sample size was calculated based on an estimate of the population of UK adult physicians (consultant and registrar level) involved in the initial assessment of patients presenting to hospital with suspected influenza, of approximately 8,000. Using a confidence level of 95% and a maximum acceptable margin of error of 7%, 192 respondents were needed to estimate adherence to PHE guidelines for the scenarios.

6.4.5 Qualitative methods

Free-text comments from the scenarios and other sections were sought to yield detailed views on the use of NAIs and to explore potential reasons underlying the responses in the clinical scenarios. Free-text comments were repeatedly studied independently by two researchers who then inductively identified emerging themes and collated responses within them. Responses could be entered into more than one theme.

6.4.6 My contribution to this study

I contributed to the conception and study design, wrote the study protocol, entered the questionnaire into the online survey platform, acquired the data from the online survey platform, and participated in the data analysis. I am grateful to all the clinicians who responded to this survey, and to my supervisor and research group colleagues.

6.5 Results

6.5.1 Respondents

Between 20th July 2015 and 1st Feb 2017, 237 respondents completed the survey. It was not possible to calculate the response rate as the speciality societies and R&D departments who distributed the survey were unable to give details on the numbers of eligible physicians. Based on data from the Royal Colleges of Physicians, the Royal College of Emergency Medicine, the Royal College of Pathologists, the General Medical Council, and Health Education England on UK consultant and trainee physician numbers, there are between 7,500 and 8,000 consultants and specialist registrars in the relevant specialities within the UK, giving a sampling rate of approximately 3%.

226 (95%) of 237 respondents reported being regularly involved in the assessment and management of patients with suspected influenza. Consultants and specialist registrars made up 163 (69%) and 60 (25%) of 237 respondents, respectively. The respondents were from the following specialities: respiratory medicine, 53 (22%), microbiology, 50 (21%), infectious diseases, 40 (17%), geriatric medicine, 28 (12%), emergency medicine, 28 (12%), acute medicine, 23 (10%) and other specialities, 15 (6%). 157 (67%) of 233 respondents reported being aware of the current PHE guidelines. Baseline characteristics and awareness of guidelines are summarised in Table 19.

Table 19: Baseline characteristics of respondents, n=237

Variable	n	%
Primary place of work		
Tertiary referral centre	101	42.6%
District general	125	52.7%
Other	11	4.6%
Speciality		
Acute Medicine	23	9.7%
Emergency Medicine	28	11.8%
Geriatric Medicine	28	11.8%
Infectious Diseases	40	16.9%
Microbiology	50	21.1%
Respiratory Medicine	53	22.4%
Other**	15	6.3%
Grade		
Consultant	163	68.8%
Associate Specialist	2	0.8%
Specialist Registrar	60	25.3%
Trust grade	10	4.2%
Other	2	0.8%
Duration Qualified		
≤10 years	57	24.1%
11 to 20 years	97	40.9%
21 to 30 years	60	25.3%
31 to 40 years	21	8.9%
>40 years	2	0.8%
Document awareness*		
PHE Influenza treatment guidelines	157	67.4%
Cochrane Review (2014)	109	46.8%

PHE=Public Health England. *Assessed in 233 respondents.

**Responses included: virology, public health, general medicine, diabetes, rheumatology, stroke medicine and respiratory high dependency.

6.5.2 Turnaround time of laboratory PCR for influenza

180 (77%) of 233 respondents provided estimates of the turnaround time for laboratory influenza testing at their institution. The majority of respondents (96 [53%] of 180) reported a turnaround time of 24-72 hours, shown in Figure 8.

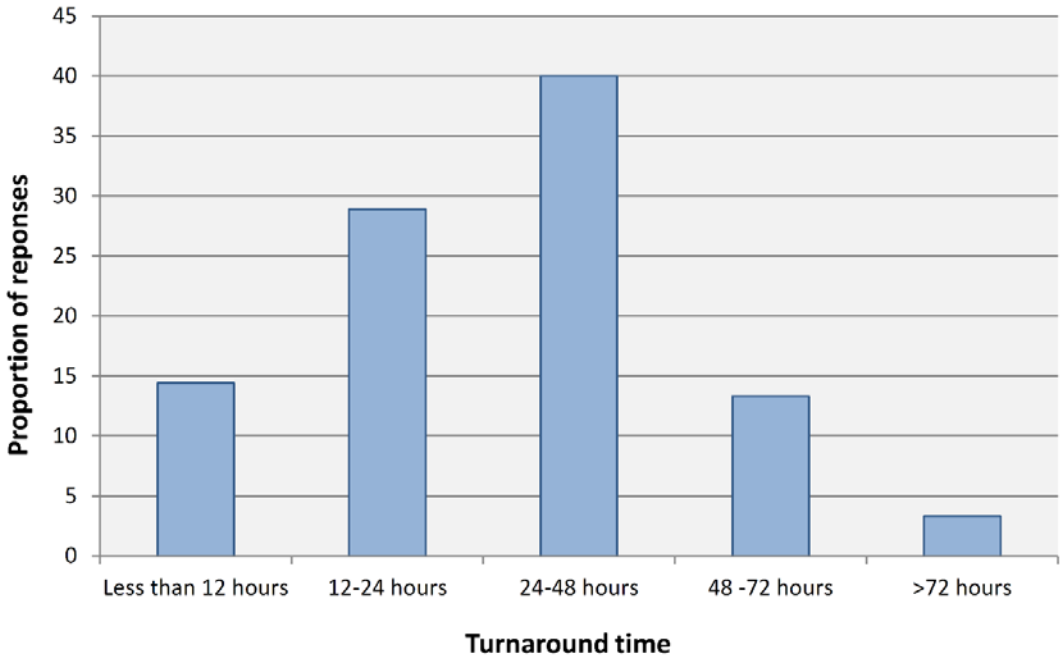


Figure 8: Reported turnaround time (hours) for laboratory influenza PCR testing at the respondents' institutions (n=180)

6.5.3 Adherence to PHE guidelines

Adherence to PHE guidelines in the 4 scenarios ranged from 56% (95% CI 49 to 63%) to 72% (95% CI 66 to 79%). For scenario 1 PHE guidelines do not recommend treatment with NAIs. 140 (63%) of 222 respondents reported that they would not treat with NAIs, 75 (34%) reported that they would treat with NAIs and 6 (3%) reported 'other' practice. For scenario 2 PHE guidelines recommend treating with NAIs empirically whilst awaiting results. 123 (56%) of 220 respondents reported that they would treat empirically with NAIs, 53 (24%) of 220 reported that they would withhold NAIs whilst awaiting the results of PCR testing, 35 (16%) of 220 reported that they would not treat with NAIs and 9 (4%) reported 'other' practice. For scenario 3 PHE guidelines recommend treating with NAIs empirically whilst awaiting results. 157 (72%) of 216 respondents reported that they would treat empirically with NAIs, 33 (15%) reported that they would withhold NAIs whilst awaiting the results of PCR testing, 20 (9%) reported that they would not treat with NAIs and 10 (4%) reported other practice. For scenario 4 PHE guidelines recommend treating with NAIs empirically whilst awaiting results. 140 (66%) of 210 respondents reported that they would treat empirically with NAIs, 29 (14%) reported that they would withhold NAIs whilst awaiting the results of PCR testing, 35 (17%) reported that they would not treat with NAIs and 6 (3%) reported other practice. Across all scenarios 7% to 35% reported that they would not test for influenza.

When combining the results from all 4 scenarios, adherence to PHE guidelines was 64% (95%CI 61 to 68%) overall and varied significantly by speciality (p=0.0008). Among individual specialities adherence was highest amongst infection specialists (Microbiologists 74% [95%CI 67 to 80%] and Infectious Diseases physicians 72% [95%CI 64 to 79%]) and lowest for Emergency physicians (56% [95%CI 46 to 67%]). Adherence to PHE guidelines, overall and by specialty, for the 4 scenarios and combined results for all scenarios are shown in the subsequent figures.

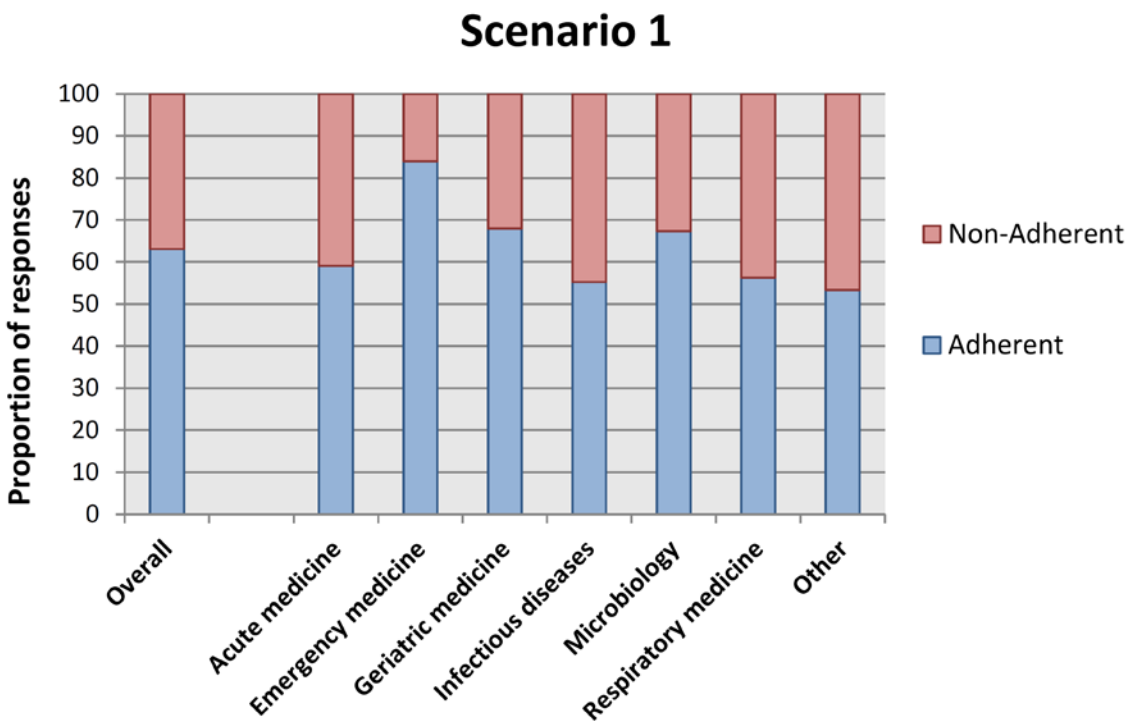


Figure 9: Scenario 1 (n=222); Adherence to PHE guidelines for the clinical scenario, overall and by speciality

Uncomplicated influenza in an adult without risk factors for developing complications, presenting within 48 hours of symptom onset.

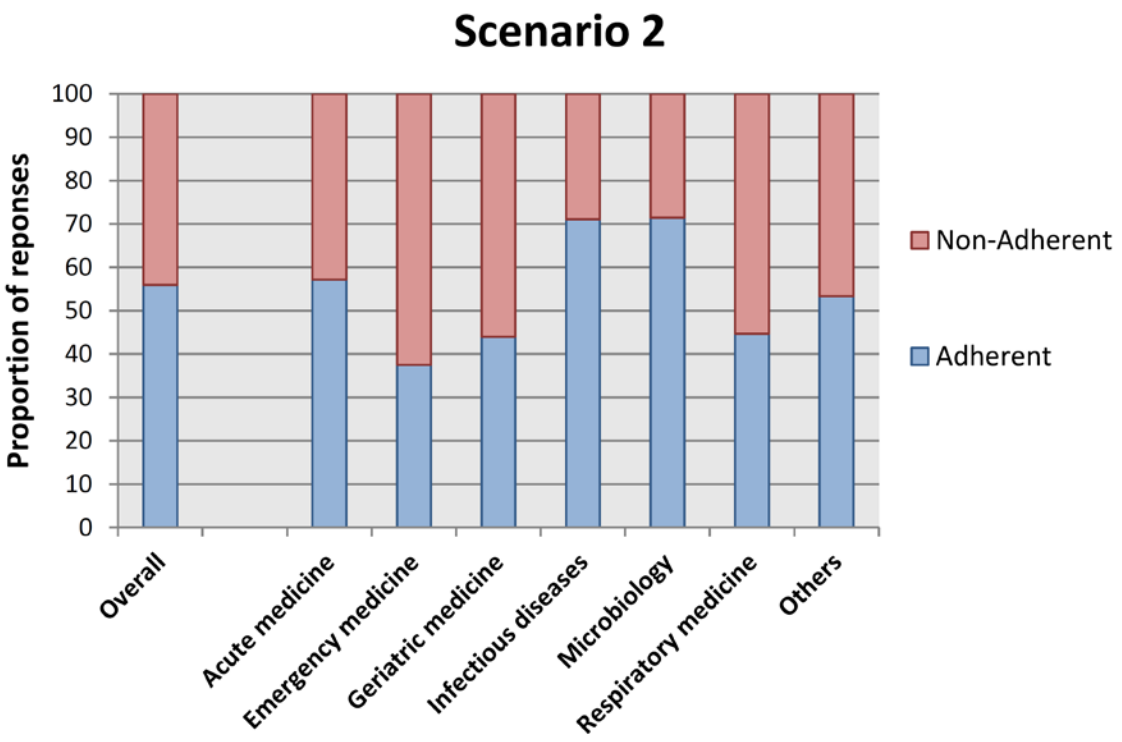


Figure 10: Scenario 2 (n=220); Adherence to PHE guidelines for the clinical scenario, overall and by speciality

Uncomplicated influenza in an adult with risk factors for developing complications, presenting within 48 hours of symptom onset.

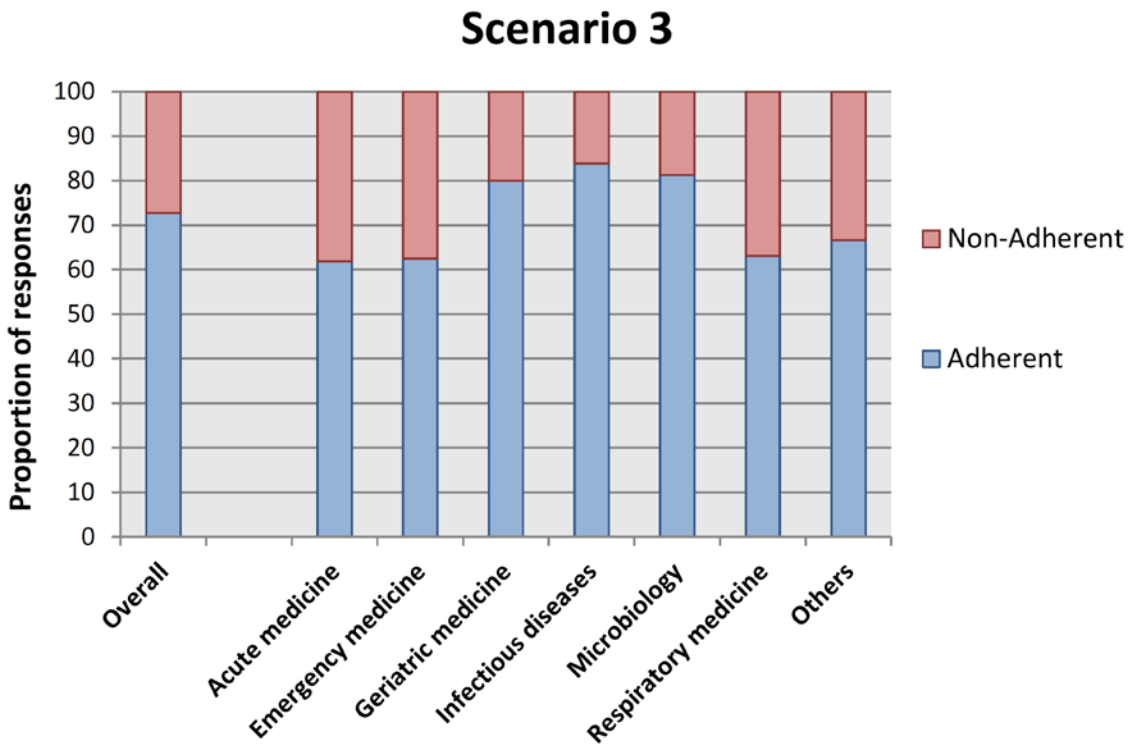


Figure 11: Scenario 3 (n=216); Adherence to PHE guidelines for the clinical scenario, overall and by speciality

Already complicated influenza in an adult, presenting within 48 hours of symptom onset.

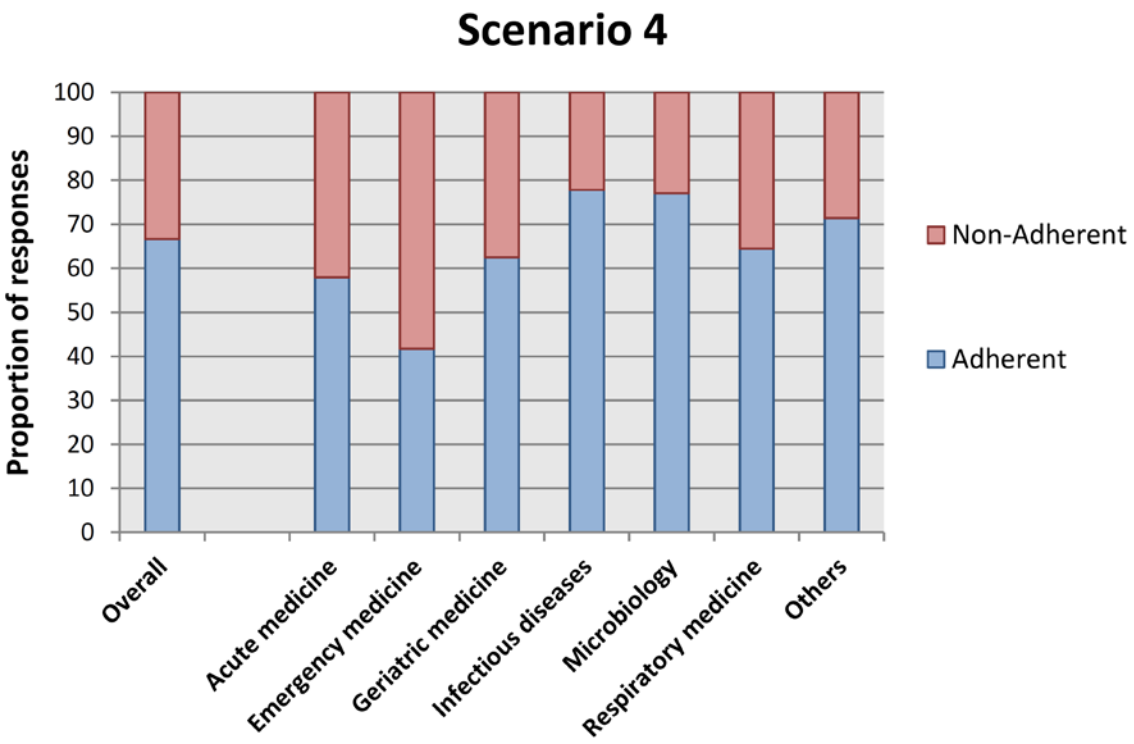


Figure 12: Scenario 4 (n=210); Adherence to PHE guidelines for the clinical scenario, overall and by speciality.

Critical illness due to influenza, in an adult presenting after 48 hours of symptom onset.

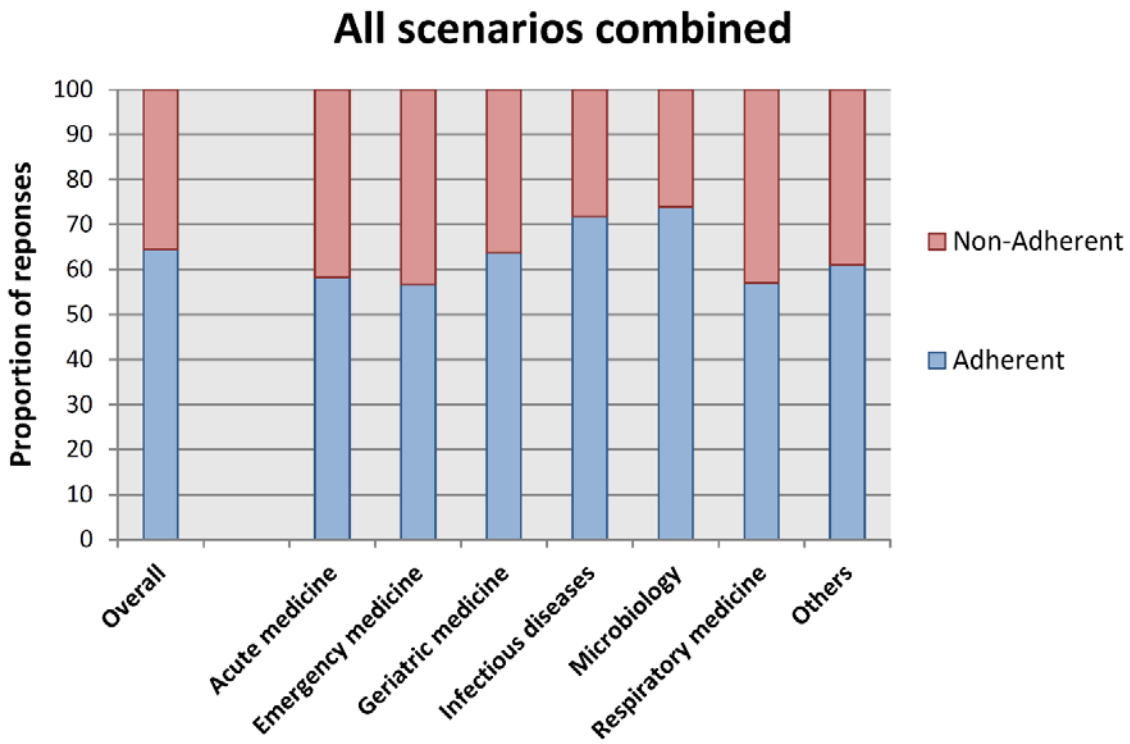


Figure 13: Combined adherence to PHE guidelines for all scenarios, overall and by speciality.

6.5.4 Factors influencing use of neuraminidase inhibitors

220 (93%) of 237 respondents provided responses regarding factors that influenced their use of NAIs. 73 of 220 (33%) respondents reported that concerns about NAI efficacy influenced their decisions on NAI prescribing. 41 (19%) and 29 (13%) of 220 reported that concerns over the side effects and concerns over NAI resistance respectively, influenced their prescribing decisions. 36 (16%) of 220 reported that a history of influenza vaccination influenced their NAI prescribing decisions. Factors reported to influence a clinician's use of NAIs in suspected influenza are shown in Table 20.

The most common themes to emerge from the free-text comments with the scenarios and other sections were: the lack of routine use of NAIs for suspected influenza in participant's institutions and the perceived lack of effectiveness of NAIs. Additional themes included perceived discouragement of liberal influenza testing and empirical NAI use due to hospital infection control policies, and the perception that pneumonia was not caused by influenza viruses. Themes and examples of individual responses are given in Table 21.

Table 20: Reported factors influencing respondents' decisions to prescribe neuraminidase inhibitors to patient with suspected influenza (respondents could select multiple factors from the list)

Factor	(n=220)	%
Duration of illness	120	54.5%
Presence of risk factors for complicated disease*	195	88.6%
Presence of already complicated or severe diseases (e.g. pneumonia or exacerbation of airways disease)	176	80.0%
Concerns over the efficacy of oseltamivir	73	33.2%
Concerns over the side effects or oseltamivir	41	18.6%
Concerns over resistance to oseltamivir	29	13.2%
A history of influenza vaccination for the current season	36	16.4%

*Age >65, chronic cardiovascular, respiratory, renal, liver or neurological diseases, diabetes mellitus or immune suppression.

Table 21: Themes and individual responses from free-text comments

Themes	Example comments
1. Culture of non-routine use of NAIs in institution	'We don't currently use NAIs' 'I have never prescribed NAIs' 'I do not prescribe NAIs for suspected influenza' 'We hardly ever use NAIs here'
2. Perceived lack of effectiveness of NAIs	'I am unconvinced of any real benefit with oseltamivir' 'This guy is ill so he'd get Tamiflu even though it'll do nothing' 'I still think oseltamivir is rubbish despite the meta-analysis paper last year' 'I don't think oseltamivir makes enough difference to dish it out prior to the results, which I will have within 24 hrs' 'NAIs are widely perceived to be ineffective' 'I'm not sure flu drugs make any difference, I'd like lots more good evidence that they actually work' 'I don't trust the drug companies to be honest about Tamiflu' 'There is substantial doubt in my mind that oseltamivir is definitely beneficial in most patients with influenza and I rarely use it on those grounds''
3. Perception that pneumonia is not caused by influenza viruses	'I would probably assume this is just community acquired pneumonia and not use NAIs' 'Sounds like community acquired pneumonia and I would treat as such, Tamiflu adds nothing'
4. Concerns over infection controls policies	'In the Medical Admissions Unit we have problems explaining to infection control staff and bed managers that patients on Tamiflu do not have confirmed influenza and so do not need isolation' 'The panic that ensues when we test and need to isolate causes far more harm than not giving the Tamiflu 'placebo' to patients'

6.6 Discussion

This study demonstrates overall sub-optimal adherence to national guidelines for the treatment of influenza with NAIs and considerable variation between physician specialities. This includes not using NAIs in patients presenting to hospital with suspected influenza, not testing patients with suspected influenza and the practice of withholding NAI treatment whilst awaiting laboratory results. It suggests that a lack of knowledge of guidelines and ongoing concerns over the effectiveness of NAIs are major contributing factors to this practice. Our study also reveals other potential contributing factors in the under-utilisation of influenza testing and NAIs use such as hospital infection control policies and misperceptions about the causes of pneumonia.

This study is the first, to our knowledge, to examine in detail current UK physician NAI prescribing practices and to explore their underlying knowledge, beliefs and attitudes, and therefore fills an important knowledge gap. The ongoing concerns over the effectiveness of NAIs for influenza reported by respondents are likely to originate from the considerable media attention surrounding the Cochrane review authors' publicised concerns over the evidence base for NAIs and the lack of data transparency from industry-sponsored studies.[223,224] These concerns related to the original placebo controlled studies of NAIs which were largely conducted in healthy people, and have now been addressed by subsequent independent meta-analyses.[142] These studies are of questionable relevance to the use of NAIs in hospitalised adults who represent the group at highest risk of poor outcomes and have the most to benefit from an effective antiviral treatment. Although no placebo controlled trials have ever been performed in this group, the results of multiple separate observational studies have consistently suggested improved clinical outcomes with NAI treatment.[196, 225-229] More recently a large, well-controlled meta-analysis using patient level data from nearly 30,000 hospitalised patients with pandemic H1N1 influenza A demonstrated a reduction in mortality in adults treated with NAIs, which extended beyond 48 hours of symptom duration in critically ill patients.[139] It is highly unlikely that placebo controlled trials will ever be performed in this patient group due to the obvious ethical constraints and so management recommendations for severe influenza will continue to derive from observational studies. As evidence continues to accumulate for the benefit of NAIs in hospitalised adults it becomes increasingly ethically questionable to deny treatment in these patients, either through the lack of routine influenza testing or by withholding or delaying treatment from those with positive tests.

The practice of withholding of NAIs until laboratory results are available despite the reported acknowledgement of the prolonged turnaround time of laboratory PCR testing is concerning,

particularly for critically ill patients where the risks of poor outcome are highest. Multiple studies have shown that earlier treatment with NAIs is associated with superior clinical outcomes,[140,196,235] and treatment at the point of initial assessment is clearly desirable. This practice may derive from concerns over the side effects of oseltamivir highlighted by the Cochrane review authors, however these are typically mild and unlikely to be pertinent in the hospitalised cohort, especially among the critically ill. The concerns reported by respondents over the generation of resistance are also largely unjustified since empirically treating patients who subsequently test negative for influenza cannot credibly generate resistance, and the development of resistance in those who do have influenza and are treated has been rare with current influenza strains. A history of influenza vaccination should not influence empirical NAI use as influenza vaccine effectiveness is only moderate in adults with comorbidity and is even lower in the elderly, and so many patients hospitalised with influenza will have a history of vaccination.

Another potential reason for physicians failing to perform influenza testing and treating empirically with NAIs revealed by this survey was the issue of hospital infection control policies mandating isolation in patients tested for influenza or prescribed NAIs, whilst awaiting definitive results. Single-occupancy rooms are a limited resource in most UK hospitals and so isolating large numbers of patients with suspected influenza can lead to bed management problems and impairment of patient flow through acute areas. Physicians may therefore feel discouraged from testing patients with suspected influenza and treating empirically with NAIs. The consequences of failing to diagnose hospitalised patients with influenza are serious for both patients and hospitals and nosocomial outbreaks of influenza lead to multiple ward closures and avoidable patient deaths every year.

One potential solution to these problems may be the introduction into hospitals of routine molecular point-of-care testing (POCT), in adults presenting with acute respiratory illness, for influenza and other respiratory viruses that is performed at the point of hospitalisation. The ResPOC trial, evaluated routine POCT in hospitalised adults and described in this thesis, demonstrated improved adherence to PHE guidelines for the treatment of influenza and more rapid administration of NAIs, compared to standard clinical care.

The strengths of this survey include the sampling of a broad range of physician specialists involved in the initial assessment of patient presenting with suspected influenza from across the entire UK. Collecting qualitative data allowed us to explore possible underlying reasons behind the poor adherence rate for PHE guideline so that potential interventions can be directed appropriately. Although we were unable to calculate the response rate for our study, it is likely to be low, and therefore we cannot exclude the possibility of non-response bias. The number of respondents

does however represent an adequate sample size for the estimated population of UK physicians involved in the initial assessment of patients presenting to hospital with suspected influenza, and so our conclusions regarding the overall poor adherence to PHE guidelines are likely to be valid and reproducible. Survey responses obviously represent self-reported practice which may not accurately represent actual practice and so the findings of the study should ideally be corroborated by examining laboratory and pharmacy data collected on laboratory testing and NAI use in hospitals. Of note the use of NAI in hospitalised adults with confirmed influenza was 65% in the control group of the aforementioned randomised controlled trial of routine molecular POCT for respiratory viruses, which is consistent with the results in this survey. We did not seek responses from intensive care unit (ICU) physicians because patients presenting to hospitals with suspected influenza in the UK are initially assessed by other physician groups with subsequent referral to ICU if required. The practices and opinions of ICU staff should be explored in subsequent studies. We included two scenarios involving patients with uncomplicated disease, one with indications for NAI treatment and one without. It could be argued that most patients presenting to hospital would be expected to have already complicated disease such as exacerbation of airways disease or pneumonia. In reality however, many patients with uncomplicated disease will present to secondary care and some of these will in fact go on to be hospitalised, often due to diagnostic uncertainty, caused by the slow turnaround time of diagnostic testing for influenza.

In conclusion this study demonstrates sub-optimal adherence to current PHE guidelines for the treatment of suspected influenza among front-line UK physicians, with considerable variability across specialities. Lack of confidence in the effectiveness of NAIs seems to be a major contributing factor in this. Acknowledging and highlighting this discrepancy between public health policy and clinical practice is an important first step in improving the care of patients with influenza. As there are unlikely to ever be definitive trials evaluating the efficacy of NAIs in hospitalised adults, and a wealth of observational studies now supports their use in this patient group, efforts should now focus on improving physician knowledge and adherence to current management guidelines.

Chapter 7 Pneumonia diagnoses in ResPOC trial participants

7.1 Overview

Using data from the ResPOC trial of adults hospitalised with acute respiratory illness, we examined the reliability of pneumonia diagnosis on discharge documentation. 50 (28.2%) of 177 patients with a pneumonia diagnosis had no radiological evidence of pneumonia. 67 (34.9%) of 192 patients with clinico-radiological evidence of pneumonia did not have a diagnosis of pneumonia listed; 'COPD exacerbation' or 'lower respiratory tract infection' was often listed instead. These patients more frequently had a respiratory comorbidity and lower oxygen saturations, and increased CRP and temperature at presentation. Pneumonia diagnoses misclassification on discharge documentation may have clinical, financial, and research data implications.

7.2 Introduction

A recent study British Thoracic Society (BTS) study identified a cohort of hospitalised adult patients diagnosed and coded as having community-acquired pneumonia (CAP) who did not have radiological evidence of pneumonia, and these patients had differing clinical characteristics.[236] However, the magnitude of this misattribution of diagnosis was not calculable and in addition, the counter entity (i.e. patients with clinico-radiological evidence of CAP who are not correctly recorded as having CAP), was not studied. The dataset generated by the ResPOC trial, as a large pragmatic study of adults presenting to hospital with acute respiratory illness, has the potential to address both these evidence gaps.

7.3 Aims

The aims of this *post hoc* study were to use the ResPOC trial data to find the proportion of patients diagnosed with pneumonia but who had no radiological evidence of community-acquired pneumonia, and to identify if there are a group of patients with clinico-radiological evidence of community-acquired pneumonia who were not recorded as having pneumonia, and describe them.

7.4 Methods

7.4.1 Design

The ResPOC trial enrolled adults with acute respiratory illness presenting to the emergency department or acute medical unit of a large teaching hospital in the UK during winter months. Patients were ≥18 years old, had acute respiratory illness and/or fever of ≤7 days duration, and were enrolled within 24 hours of presentation to hospital. The trial was prospectively registered on a trials database (ISRCTN90211642).

We used the BTS definition of CAP in hospitalised adults: "symptoms and signs consistent with an acute lower respiratory tract infection associated with new radiographic shadowing for which there is no other explanation."[237] The Infectious Diseases Society of America / American Thoracic Society consensus guidelines definition is, in essence, the same.[238] Trial participants were classified as having CAP by their admission chest radiograph and/or first computed tomography (CT) scan where performed. In patients who had a CT scan, CT scan reports superseded chest radiograph reports. Subsequent chest radiographs or CT scans were not reviewed. All imaging was reported by radiologists not associated with the study. Discharge summary data were analysed from hospital electronic records. A discharge summary may have multiple diagnoses listed and a diagnoses list that included the word 'pneumonia' was considered as pneumonia for this study, excluding hospital-acquired pneumonia.

The ResPOC trial analysis described elsewhere in this thesis used the BTS definition to classify patients as having pneumonia, rather than their discharge summary diagnosis.

7.4.2 Statistical analysis

Statistical analyses were done with Prism version 7.03 (GraphPad Software, La Jolla, CA, USA). Groups were compared using differences in proportions for binary data (using Chi-square test or Fisher's exact test as appropriate), and Mann-Whitney U tests for continuous data.

7.4.3 My contribution to this analysis

I developed this line of inquiry, led the data collection, and did the analysis and interpretation, all under my supervisor's guidance.

7.5 Results

The ResPOC trial included 714 patients in the modified intention-to-treat analysis. 177 patients had a diagnosis of CAP listed on their discharge summary, of which 50 (28.2%) had no radiological evidence of pneumonia.

192 of 714 patients had clinico-radiological evidence of CAP. 67 (34.9%) of the 192 patients with pneumonia did not have a diagnosis of pneumonia recorded on their discharge summary. Of these patients, 24 (35.8%) of 67 patients had 'COPD exacerbation' listed as a diagnosis and 20 (29.9%) of 67 had 'lower respiratory tract infection' or 'LRTI' listed. 14 (20.9%) of 67 patients with pneumonia had no acute respiratory diagnosis recorded (Table 22).

Table 22: Pneumonia diagnosis in the ResPOC trial

	n	total	percentage
Patients with pneumonia listed on discharge summary	177	714	24.8%
Patients with clinico-radiological evidence of pneumonia	192	714	26.9%
Patients with pneumonia on discharge summary and clinico-radiological evidence	125	714	17.5%
Patients with clinico-radiological evidence of pneumonia without pneumonia recorded on their discharge summary	67	192	34.9%
Patients with pneumonia recorded on their discharge summary but no radiological evidence of pneumonia	50	177	28.2%
Patients with clinico-radiological evidence of pneumonia without pneumonia recorded on discharge summary:			
with asthma exacerbation listed as a discharge diagnosis*	9	67	13.4%
with COPD exacerbation listed as a discharge diagnosis*	24	67	35.8%
with bronchiectasis exacerbation listed as a discharge diagnosis*	5	67	7.5%
with lower respiratory tract infection or 'LRTI' listed as a discharge diagnosis*	20	67	29.9%
with ILD listed as a discharge diagnosis*	1	67	1.5%
with no respiratory diagnosis recorded	14	67	20.9%

*patients may have more than one respiratory diagnosis listed on their discharge summary. ILD=interstitial lung disease.

Patients with pneumonia where pneumonia was not listed as a discharge diagnosis more frequently had an underlying respiratory comorbidity (64.2% vs 38.4%; p<0.001), and a lower median temperature (37.0 vs 37.5, p=0.032), a lower median CRP level (66 vs 109.5, p=0.017), and lower O2 saturations (94% vs 95%; p=0.032) at presentation, compared with patients correctly recorded as having pneumonia. (Patient characteristics are shown in Table 23).

Table 23: Comparison of demographics and clinical characteristics of patients with clinico-radiological evidence of pneumonia, where pneumonia was not documented on the discharge summary, and where pneumonia was listed on the discharge summary

	Pneumonia not on discharge summary (n=67)	Pneumonia on discharge summary (n=125)	Difference (95% CI)	p value
Age, years	68 (53-76.5)	65 (44-77)	3 (-4 to 8)	0.553
Female	42 (62.7%)	62 (49.6%)	13.1% (-1.7 to 26.8)	0.096
Current Smoker	18 (26.9%)	26 (20.8%)	6.1% (-6.0 to 19.3)	0.370
Received influenza vaccine*	42 (62.7%)	74 (59.2%)	3.5% (-11.0 to 17.2)	0.757
Ethnicity				
White British	64 (95.5%)	119 (95.2%)	0.3% (-8.0 to 6.4)	1.0
Comorbidities				
Pregnant	1 (1.5%)	2 (1.6%)	-0.1% (-4.3 to 6.5)	1.0
Cardiovascular disease	32 (47.8%)	51 (40.8%)	7.0% (-7.5 to 21.3)	0.364
Respiratory disease	43 (64.2%)	48 (38.4%)	25.8% (11.0 to 39.0)	**<0.001**
Renal disease	5 (7.5%)	9 (7.2%)	0.3% (-7.0 to 9.7)	1.0
Liver disease	0	1 (0.8%)	-0.8% (-4.4 to 4.7)	1.0

Diabetes Mellitus	11 (16.4%)	18 (14.4%)	2.0% (-8.8 to 13.8)	0.833
Cancer	6 (9.0%)	10 (8.0%)	1.0% (-6.8 to 10.9)	0.791
Immunocompromised	3 (4.5%)	4 (3.2%)	1.3% (-5.0 to 9.4)	0.697
Clinical features on admission				
Duration of symptoms, days	4 (3-5)	3 (3-5.25)	1 (0 to 1)	0.658
Pulse rate, beats/min	100 (90-120)	100 (85-110)	0 (-5 to 8)	0.600
Respiratory rate, breaths/min	24 (20-28)	22 (18.5-28.5)	2 (-2 to 2)	0.532
Oxygen saturations, %	94 (91.5-96)	95 (93-97)	-1 (-2 to 0)	**0.032**
Use of supplementary oxygen	19 (28.4%)	44 (35.2%)	-6.8% (-19.6 to 7.3)	0.420
Temperature, °C	37.0 (36.4-37.8)	37.5 (36.6-38.3)	-0.5 (-0.7 to 0)	**0.032**
C-Reactive Protein, mg/L	66 (25.8-156.5)	109.5 (56.5-205.5)	-43.5 (-59 to 5)	**0.017**
CURB65 score†				
0	28 (41.8%)	64 (51.2%)	-9.4% (-23.4 to 5.3)	0.229
1	32 (47.8%)	39 (31.2%)	16.6% (2.2 to 30.5)	**0.028**
2	7 (10.4%)	18 (14.4%)	-4.0% (-12.9 to 6.9)	0.506
3	0	4 (3.2%)	-3.2% (-7.9 to 2.6)	0.300
4	0	0	0.0%	1.0
Median CURB65	1	0	1 (0 to 0)	0.628

Adverse events				
ICU admission	4 (6.0%)	9 (7.2%)	-1.2% (-8.2 to 7.8)	1.0
RHDU admission	2 (3.0%)	4 (3.2%)	-0.2% (-5.4 to 7.3)	1.0
30-day mortality	4 (6.0%)	8 (6.4%)	-0.4% (-7.2 to 8.5)	1.0
Readmitted‡	4 (6.0%)	9 (7.2%)	-1.2% (-8.2 to 7.8)	1.0
Re-presented (but not admitted)‡	13 (19.4%)	14 (11.2%)	8.2% (-2.0 to 20.1)	0.132
Grade of doctor signing discharge summary‖				
Consultant	2 (3.0%)	3 (2.4%)	0.6% (-4.3 to 8.0)	1.0
Registrar	1 (1.5%)	5 (4.0%)	-2.5% (-7.7 to 4.4)	0.667
FY2 or SHO	28 (41.8%)	59 (47.2%)	-5.4% (-19.5 to 9.3)	0.544
FY1	29 (43.3%)	54 (43.2%)	0.1% (-14.1 to 14.6)	1.0

Data are n (%), or median (IQR), or stated otherwise. *Received vaccine for current influenza season.

†For CURB65 scores 0 to 3 combined p=0.082. ‡Within 30 days. ‖For overall Grade of doctor signing discharge summary p=0.388.

RHDU= respiratory high dependency unit; ICU= intensive care unit. FY1=Foundation Year 1 doctor; FY2=Foundation Year 2 doctor; SHO=Senior House Officer (includes Core/Specialty Trainee Year 1 and Year 2 doctors). Figures in **bold** are those with p<0.05.

7.6 Discussion

We identified that around a third of patients diagnosed as having community-acquired pneumonia did not in fact have radiological evidence of pneumonia. This adds to previous research by providing an estimate of the prevalence of this misdiagnosis.[236]

We also found that around a third of patients with clinico-radiological evidence of CAP did not have pneumonia recorded as a diagnosis on their discharge summary. These patients were frequently recorded as having a 'COPD exacerbation' or 'lower respiratory tract infection' and around a fifth of patients had no respiratory diagnosis on their discharge summary. These patients more frequently had underlying respiratory disease which may have complicated the clinical picture and led to an error in diagnosis. Similarly, having a lower CRP or temperature may have falsely suggested to clinicians that these patients did not have pneumonia.

Patients who had pneumonia that was not recorded as a diagnosis at discharge had different clinical characteristics. Therefore, it is possible that they may have different clinical outcomes. It is common practice to follow up patients with pneumonia as unresolved symptoms or persistent radiological changes may indicate malignancy,[239] and a previous study has suggested a higher prevalence of malignancies in similar incorrectly coded patients.[240] As the majority of hospitalised patients treated for COPD exacerbations and other acute respiratory illnesses receive antibiotics, most patients with undiagnosed pneumonia are still likely to have received appropriate antimicrobial treatment.[3]

This study highlights the limitations of electronic medical records due to incorrect data input by clinicians. Poor quality pneumonia data may contribute to invalid conclusions in disease prevalence research and vaccine effectiveness studies, an area already burdened by imprecise data.[241] Where hospital diagnosis coding data are misleading, hospitals may receive incorrect reimbursements for patient hospitalisations in both nationalised healthcare and insurance-based systems.

Limitations of this study include that it is a single-centre study and that patients lacking capacity to consent through cognitive impairment or severe illness were excluded. However, as the study had broad inclusion criteria and set in a typical large teaching hospital in the UK, the findings are likely to be applicable to patients in similar hospitals nationally and internationally. Recent studies have demonstrated that chest radiographs are imperfect in the diagnosis or exclusion of pneumonia in adults hospitalised with acute respiratory illness and methods of improving pneumonia diagnoses including routine CT scans and biomarkers have been considered.[242,243]

However, chest radiography is the imaging modality currently recommended by guidelines internationally to define pneumonia in hospitalised patients,[237,238] and routine CT scans to diagnose pneumonia are not justifiable with current technology and healthcare resources.[242,243]

A larger study is required to corroborate these findings and assess if misdiagnosis has an impact on clinical outcomes. Interventions to highlight senior physicians' opinions or radiologists' reports on chest imaging to junior physicians who typically write discharge summaries may improve the reliability of the recorded diagnoses.

This study is based on data generated in the ResPOC trial, which evaluated molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness. Previous studies have shown that laboratory-confirmed influenza and other respiratory virus infection is infrequently recorded as a diagnosis at discharge instead, with presenting complaints or deleterious events more likely to be recorded instead.[22] The rapid turnaround time of molecular POCT means that respiratory virus testing results may be available to senior decision-making clinicians at the 'front-door' of the hospital. Therefore, it is possible that POCT is associated with an increased proportion of respiratory virus infections being accurately recorded on discharge documentation. The ResPOC trial data are unsuitable to test this hypothesis for this but successor trials are likely to capture this information and be able to answer this evidence gap.

In conclusion, we found that around a third of patients with clinico-radiological evidence of community-acquired pneumonia did not have pneumonia recorded on their discharge summary. We also found that around a third of patients classified as having pneumonia on their discharge summary had no radiological evidence of pneumonia. Patients with a diagnosis of pneumonia missed from their discharge summary had different clinical characteristics compared with patients with a correct pneumonia diagnosis. The misclassification of pneumonia diagnosis on discharge documentation may have clinical, financial and research implications. Interventions are needed to improve the reliability of hospital discharge data.

Chapter 8 Future work

8.1 Skills acquired during this candidature and skills to obtain in the future

I have learned the skills, processes and patience to set up and run a clinical trial from conception, through protocol and document development, ethics committee application, patient randomisation and enrolment, data entry into case report forms and databases, statistical analysis, to writing up a study manuscript for publication. I have developed a knowledge of the regulatory and legal frameworks underpinning studies involving human participants. I have learned how to use the trial data to answer further important research questions. I have also developed skills in developing questionnaire-based research findings and use of qualitative data.

Developing my research skillset for the future could come from involvement in different types of research, notably laboratory-based research or large dataset observational or implementation studies, alongside more experience in clinical trials using different methods. The future work discussed in this chapter reflect some potential skills development.

8.2 Directly stemming from this work

To confirm the findings of the single centre ResPOC trial and to assess the generalisable nature of this POCT strategy to other hospitals and nationally, a multi-centre trial should be considered. Cluster randomisation of either geographical area or hospital site could be considered in order to alleviate any study-effect of increased respiratory virus PCR testing sent to the laboratory. Considering the findings, a trial adequately powered to examine other key clinical and health economic outcome measures is warranted. The FluPOC trial (ISRCTN17197293) is now open to recruitment, led from Southampton. This is a large, pragmatic, multi-centre, randomised controlled trial of molecular point-of-care testing for respiratory viruses in hospitalised adults with acute respiratory illness that involves a ‘test and treat’ strategy for influenza.

8.3 Viral load

High respiratory viral loads on nasopharyngeal swabs at the point of hospitalisation are strongly associated with prolonged hospital length of stay in adults with viral acute respiratory illness.[244] This supports existing evidence demonstrating that viral acute respiratory illness is often a viral

load driven process,[245, 246] and suggests that viral load could be used in clinical practice to predict prolonged hospitalisation and prioritise antivirals.

The ResPOC trial has both data on multiple clinically-relevant outcomes and nose and throat swabs in viral transport media from all patients in the POCT group. Quantitative PCR (qPCR, or real-time PCR) can be used to measure the viral load in the viral transport media from virus-positive patients and therefore we can explore the relationship between the magnitude of viral load at presentation in the nose and throat and subsequent clinical outcomes. This may lead to the potential to stratify the risk of adverse patient outcomes by viral load and therefore more appropriately direct treatment and resources and improve patient outcomes. Research ethics committee approval to test these samples by qPCR has already been granted.

Respiratory virus RNA has been found in blood in certain groups of patients. For example, influenza and RSV viraemia has been associated with adverse clinical outcomes in haematopoietic stem cell transplant recipients and rhinovirus can be found in young children with severe respiratory illness.[247-249] However, it is unknown whether adults presenting to hospital with respiratory virus infection are frequently viraemic, and how the presence and load of virus in blood may relate to clinical outcomes. The ResPOC trial has blood samples, and similar to the qPCR testing proposal for viral transport medium samples, ethical approval for investigation has already been granted.

Cepheid's GeneXpert platform, which has some molecular point-of-care potential albeit restricted by the very limited range of viruses currently detected, may have some benefit in future studies as it does produce a cycle-threshold value which is a reasonable surrogate measure of viral load.[112,113] This potentially negates the need for further laboratory RT-PCR if the cycle-threshold value was to be used as a measure of viral load, although qPCR provides a more accurate and reliable viral quantification.

8.4 Health economic analysis

Healthcare systems are subject to financial constraints. In order to change patient pathways, particularly by influencing national guidelines set by the National Institute for Health and Care Excellence (NICE) which include cost-effectiveness in their recommendations, a health economic analysis is mandatory.

ResPOC trial patient data collected includes duration of time in hospital, antibiotic and antiviral dose data, laboratory test (e.g. blood tests) type and frequency, radiology test information and critical care admission data. All these factors have a cost. Most of these costs are nationally

available information, for example, the British National Formulary contains standardised antibiotic and neuraminidase inhibitor costs. Therefore, it is feasible to calculate the mean cost per patient in the point-of-care test group and the routine clinical care group and compare these to see if routine syndromic molecular point-of-care testing is cost effective. It is hypothesised that as there was a shorter duration of hospital stay (a major determinant of healthcare costs) in the POCT group, then this cost-consequent analysis will show that a routine POCT strategy will be cost effective, despite the cost of consumables and operator time for the molecular POCT.

8.5 Patient-participant feedback and involvement

Patient and Public Involvement (PPI) has become integral to research study design and is expected for successful grant applications by many funding bodies. The ethos of PPI involves research being done 'with' or 'by' patients and the public rather than 'to' or 'for' them.[250] Future studies could both have a mechanism of obtaining feedback from study participants to improve the design of studies and their relevance to patients but ideally should include PPI involvement from the study conception stage. Patients, from groups such as airways disease charities or support groups, could offer insights and improvements into study design that make a genuine difference to patients, and they can improve the relevance of information sheets and assist in dissemination of research findings. Feedback forms can be given to study participants to both assist in future study design and gather patient reported outcome measures data.

8.6 Potential related trials

There are other molecular point-of-care tests for infectious diseases that warrant high-quality trials as there is the potential to improve clinical care, patient flow through the healthcare setting and be a cost saving to the health service.

Patients frequently present to hospital with loose stool, as their presenting complaint, as one symptom within their illness, or as a consequence of treatment. There is also a significant burden on hospital wards of norovirus and to an extent, *Clostridioides difficile* (formerly *Clostridium difficile*). As such, large numbers of patients in UK hospitals are routinely put into isolation rooms because of their loose stools, without a specific diagnosis, until laboratory stool results confirm or deny a gastrointestinal infectious pathogen. A rapid multiplex molecular point-of-care test for gastrointestinal pathogens may improve isolation facility use if infectious causes of diarrhoeal illness in hospitalised patients can be rapidly excluded, rather than waiting for the long turnaround time of laboratory methods. In addition, pathogen-directed antimicrobial therapy, including cessation of antibiotics, is possible. The FilmArray Gastrointestinal Panel offers such a

system that is deployable as a rapid, multiplex, molecular point-of-care test.[218] The GastroPOC trial (ISRCTN88918395) is a randomised controlled trial of molecular POCT, compared with standard care, in adults hospitalised with diarrhoea using the FilmArray GI Panel as a molecular POCT; this trial is ongoing in Southampton.

The FilmArray Meningitis/Encephalitis Panel has 14 different targets including *Neisseria meningitidis, Streptococcus pneumoniae, Cryptococcus neoformans* and Herpes simplex viruses. It operates similarly to the Respiratory Panel in that there is minimal hands-on time and it generates a result in about an hour.[219] Key potential outcome measures in a trial examining the benefits of such a system in an AMU or ED setting would include antibiotic use, antiviral use, antifungal use, isolation facility use and length of stay. However, the relatively low numbers of such presentations to even a large hospital would mean that a multi-centre trial would be necessary to achieve meaningful numbers of recruited patients. Strict infection control policies must also be considered when handling cerebrospinal fluid specimens to prevent potential infection of the operator and specimen contamination.

Molecular point-of-care testing for infectious diseases in other clinical settings apart from acute medical admissions, particularly primary care and critical care settings, currently have a very limited evidence base. The differences in these patient populations compared to the adults presenting to secondary care would affect the outcome measures chosen but antibiotics and antiviral medication outcome measures would still remain very important in these settings. With unrecognised recent influenza infection a comorbidity in around 10% of patients hospitalised with an acute myocardial infarction, there is even the potential to improve clinical outcomes in patients in coronary care units by molecular point-of-care testing and subsequent pathogen-directed neuraminidase inhibitor treatment.[29] Similarly, as ischaemic stroke admission in older adults is associated with respiratory virus infection, molecular point-of-care testing may improve outcomes in this patient group.[19,26] Therefore a wide range of clinical settings may benefit from future high-quality trials of molecular point-of-care testing for respiratory viruses and other infectious diseases.

8.7 Conclusion

The pioneering nature of the ResPOC trial has generated a collection of ongoing and potential future projects. This includes using the existing ResPOC data, and new high-quality trials, all of which have the potential to improve patient care.

Appendix A Additional tables and figures

Table 24: List of final clinical diagnoses in "Other" category

	POCT (n=47)	Control (n=47)
Congestive cardiac failure	10	9
Exacerbation of other lung diseases*	9	12
Tonsillitis/pharyngitis	6	1
Pulmonary embolism	3	6
Pleural effusion	3	0
Urinary sepsis	3	2
Gastroenteritis	3	0
Musculoskeletal chest pain	2	1
Empyema	1	1
Anaemia	1	0
Infected spinal metal work	1	0
Infectious mononucleosis	1	1
Lymphoma	1	0
Pericarditis	1	0
Atrial fibrillation	1	0
Pyogenic liver abscess	1	0
Sepsis/bacteraemia of unknown source	0	4
Viral meningitis	0	2
Sinusitis	0	1
Tuberculosis	0	1
Acute cholecystitis	0	1
Haemarthrosis	0	1
Inflammatory bowel disease	0	1
Lobar collapse of unclear aetiology	0	1
Transverse myelitis	0	1
Diabetic ketoacidosis	0	1

POCT=point-of-care test group.

*Includes bronchiectasis and interstitial lung diseases.

Table 25: Multivariable analysis for the primary outcome, receipt of any antibiotics

Variable	Odds ratio (95% CI)	p value
Point-of-care test	0.99 (0.57 to 1.70)	0.957
Age, years	1.03 (1.01 to 1.05)	0.001
Duration of symptoms prior to admission, days	0.96 (0.83 to 1.11)	0.579
Temperature, °C	1.34 (0.97 to 1.84)	0.073
Sex: male	0.71 (0.40 to 1.25)	0.235
Antibiotics <14 days prior*	1.54 (0.79 to 3.01)	0.204
Cardiovascular disease*	0.58 (0.27 to 1.23)	0.158
Respiratory disease*	2.25 (0.97 to 5.20)	0.059
Other comorbidity*	2.70 (1.17 to 6.25)	0.059
Current smoker*	1.11 (0.57 to 2.19)	0.755
Influenza vaccine*†	0.79 (0.42 to 1.48)	0.468
CRP, mg/L	1.02 (1.01 to 1.02)	<0.001
Diagnosis**		
Asthma	0.15 (0.04 to 0.54)	0.004
IECOPD	0.31 (0.08 to 1.25)	0.101
ILI/NPLRTI	0.16 (0.05 to 0.49)	0.001
Other	0.20 (0.06 to 0.70)	0.012

All continuous variables are mean-centred.

95% CI=95% confidence interval. CRP=C-reactive protein.

IECOPD=infective exacerbation of COPD. ILI=influenza-like illness.

NPLRTI=non-pneumonic lower respiratory tract infection

*Reference category: no.

**Reference category: pneumonia.

†For the current influenza season.

Table 26: Multivariable analysis for the duration of antibiotics

Variable	Incidence rate ratio (95% CI)	p value
Point-of-care test	0.92 (0.83 to 1.02)	0.119
Age, years	1.00 (1.00 to 1.00)	0.979
Duration of symptoms prior to admission, days	1.00 (0.97 to 1.030)	0.980
Temperature, °C	0.99 (0.94 to 1.04)	0.683
Sex: male	1.02 (0.92 to 1.13)	0.737
Antibiotics <14 days prior*	1.05 (0.93 to 1.19)	0.413
Cardiovascular disease*	0.97 (0.85 to 1.10)	0.614
Respiratory disease*	1.04 (0.92 to 1.19)	0.497
Other comorbidity*	1.03 (0.91 to 1.16)	0.674
Current smoker*	0.96 (0.85 to 1.09)	0.523
Influenza vaccine*†	1.00 (0.89 to 1.13)	0.952
CPR, mg/L	1.00 (1.00 to 1.00)	<0.001
Diagnosis**		
Asthma	0.52 (0.42 to 0.64)	<0.001
IECOPD	0.83 (0.71 to 0.98)	0.025
ILI/NPLRTI	0.69 (0.59 to 0.81)	<0.001
Other	0.99 (0.84 to 1.16)	0.873

All continuous variables are mean-centred.

95%CI=95% confidence interval. CRP=C-reactive protein.

IECOPD=infective exacerbation of COPD. ILI=influenza-like illness.

NPLRTI=non-pneumonic lower respiratory tract infection

*Reference category: no.

**Reference category: pneumonia.

†For the current influenza season.

Table 27: *Post hoc* analysis showing baseline characteristics in patients not prescribed antibiotics prior to randomisation and POCT result availability

	POCT (n=120)	Control (n=167)	p value
Age, years	54 (33-71)	57 (40-70)	0.479
Female sex	68 (56.7%)	82 (49.1%)	0.232
Co-morbidity			
CVD	36 (30.0%)	52 (31.1%)	0.897
Respiratory disease	72 (60.0%)	93 (55.7%)	0.471
Renal disease	3 (2.5%)	6 (3.6%)	0.739
Liver disease	4 (3.3%)	2 (1.2%)	0.241
Diabetes	17 (14.2%)	20 (12.0%)	0.596
Cancer	8 (6.7%)	9 (5.4%)	0.801
Observations			
Temperature, °C	36.8 (36.2-37.3)	36.6 (36.3-37.3)	0.649
CRP, mg/L	23 (4-76)	28 (8-86)	0.382
Final diagnosis			
Asthma	31 (25.8%)	32 (19.2%)	0.195
IECOPD	22 (18.3%)	40 (24.0%)	0.309
Pneumonia	20 (16.7%)	31 (18.6%)	0.755
ILI/NPLRTI	31 (25.8%)	37 (22.2%)	0.485
Other	16 (13.3%)	27 (16.2%)	0.616

All data are given as number (%), or median (IQR).

POCT=Point-of-care test group. CVD=cardiovascular disease.

CRP=C-reactive protein. IECOPD=infective exacerbation of COPD.

ILI, influenza-like illness. NPLRTI, non-pneumonic lower respiratory tract infection.

Table 28: *Post hoc* analysis showing antibiotic use in patients not prescribed antibiotics prior to randomisation and POCT result availability

	POCT (n=120)	Control (n=167)	Difference (95% CI)	Odds ratio (95% CI)	p value
Any antibiotic given	61/120 (50.8%)	107/167 (64.5%)	-13.7% (-25.2 to -2.2)	0.57 (0.35 to 0.91)	0.022
Single dose only	2/61 (3.3)	0/107 (0)	3.3% (-1.8 to 12.4)	9.0 (0.4 to 191.4)	0.135
<48 hours in total	9/61 (14.8)	3/107 (2.8)	12.0% (3.5 to 23.0)	6.0 (1.6 to 23.1)	0.009
Duration, days					
mean (SD)	8.0 (6.0)	8.7 (5.1)	-0.7 (-2.5 to 1.2)	-	0.477
median (IQR)	6.5 (5.5-9.8)	7.1 (5.9 -9.8)	-0.5 (-1.5 to 0.4)	-	0.279

All data are given as number (%), n/n (%), mean (SD), or median (IQR). POCT=Point-of-care test group.

95% CI=95% confidence interval. NNT=number needed to test.

Table 29: Antibiotic duration and length of hospital stay by POCT result

	POCT positive (n=158)	POCT negative (n=202)	Difference (95%CI)	Odds ratio (95%CI)	p value
Antibiotic use					
Any antibiotic given	129 (79.7%)	172 (85.1%)	-5.4% (-13.3 to 2.5)	0.73 (0.41 to 1.27)	0.206
Duration, days					
mean (SD)	6.2 (4.8)	8.0 (5.3)	-1.7 (-2.9 to -0.6)	-	0.003
median (IQR)	6.5 (2.1.8.2)	7.0 (5.7-9.2)	-0.5 (-1.8 to -0.2)	-	0.011
Length of stay					
Hospitalised	142 (89.9%)	190 (94.1%)	-4.2 (-9.7 to 1.3)	0.56 (0.26 to 1.22)	0.167
Length of stay, days§					
mean (SD)	4.7 (4.6)	6.5 (7.2)	-1.7 (-3.0 to- 0.4)	-	0.009
median (IQR)	3.1 (1.3-6.7)	4.0 (1.9-7.1)	-0.9 (-1.3 to 0.1)	-	0.059

Data are given as number (%), mean (SD) and median (IQR). †POCT negative compared with control group.

§Adjusted for in hospital mortality. POCT=point-of-care test.

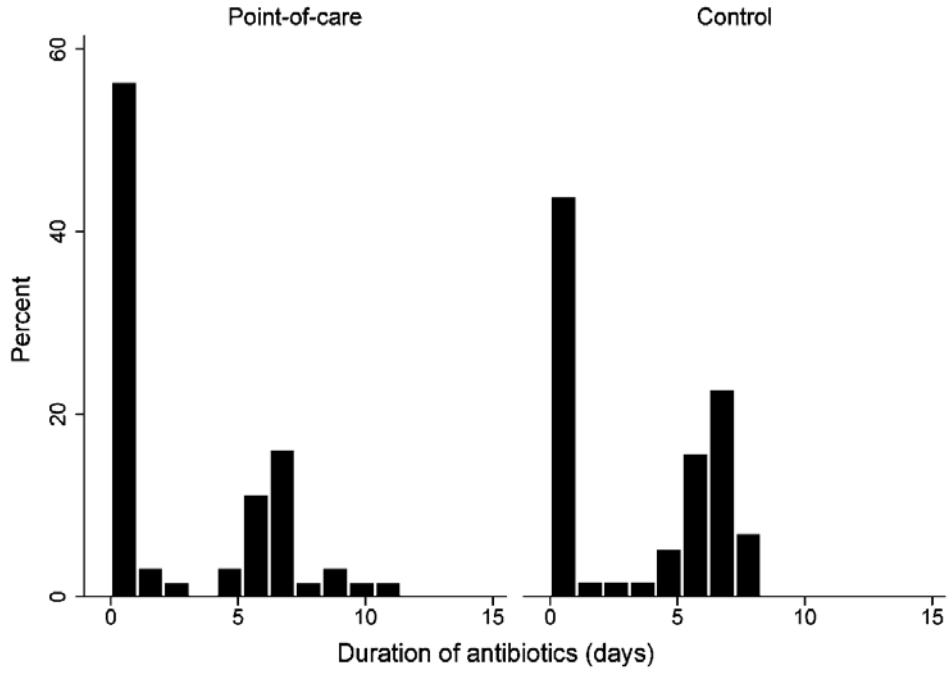


Figure 14: Distribution of antibiotic duration in patients with asthma

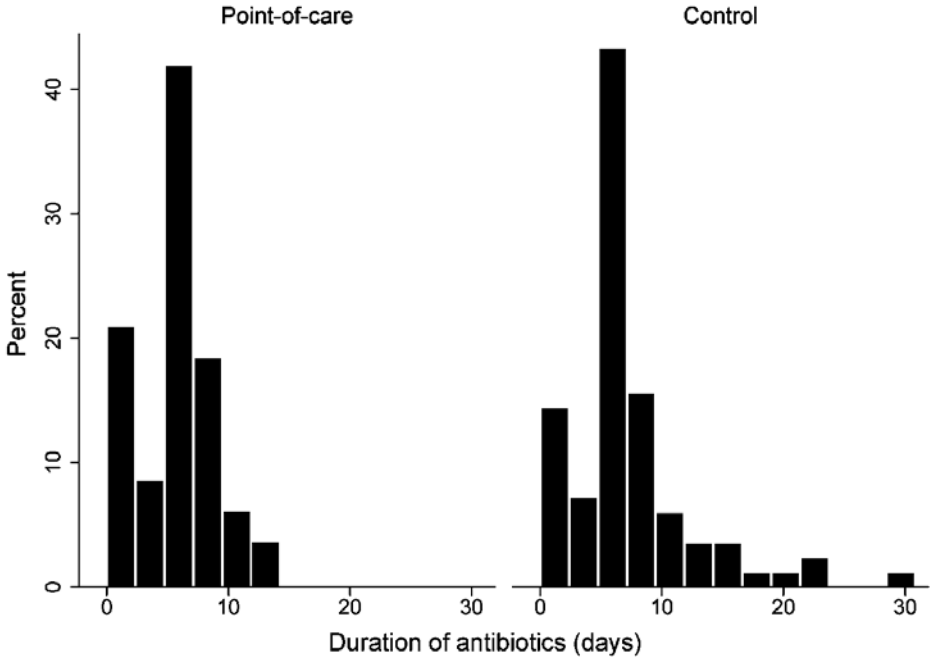


Figure 15: Distribution of antibiotic duration in patients with COPD

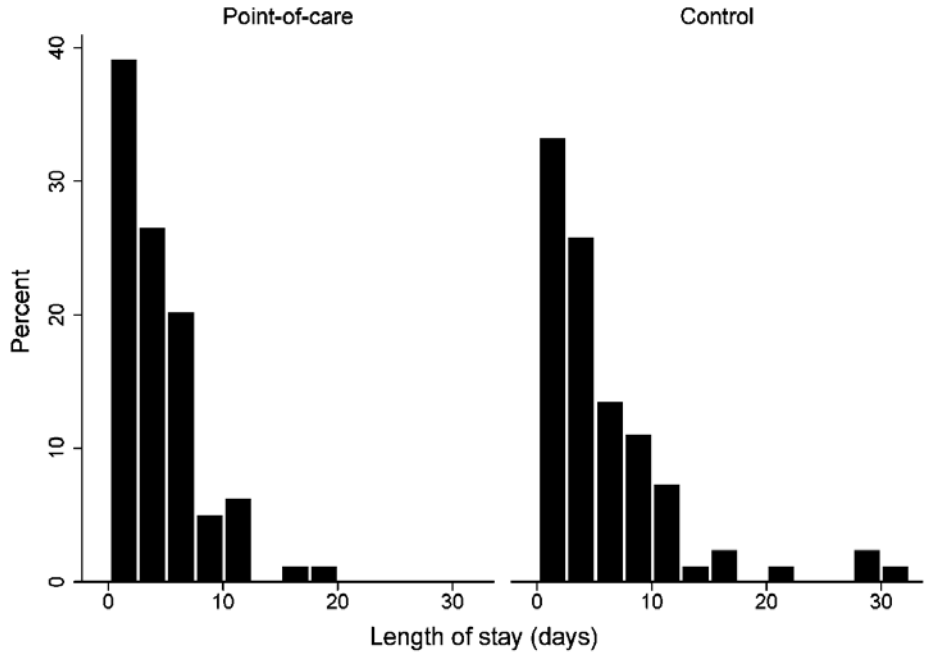


Figure 16: Distribution of length of stay in patients with COPD

Appendix B Participant information sheet and consent form

University Hospital Southampton NHS
NHS Foundation Trust

6th July 2015, Version 3.0

Participant Information Sheet

Point-of-care testing for respiratory viruses. Randomised controlled trial comparing POCT with standard clinical care, in adults presenting to secondary care with acute respiratory illness: a pilot study (ResPOC Trial).

Chief Investigator: Dr Tristan Clark

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?

Infection by respiratory viruses, including influenza ('flu'), account for a significant number of visits to emergency departments and admissions to hospitals in the UK. Current methods of detection of these viruses can take several days for the results to become available to the doctors looking after patients. If these tests are positive for respiratory viruses it may alter which treatments are offered (such as antibiotics or antiviral medication), or the place in which that the patient is cared for (such as a side room).

Rapid 'point-of-care' tests for respiratory viruses have been developed and can provide accurate results in 1 hour rather than several days. We wish to explore if this test improves patient care.

Why have I been asked?

The symptoms and clinical signs that you display are consistent with an 'acute respiratory illness' or influenza and so we would like to potentially test you for a wide variety of respiratory viruses which might have caused your illness. We would then collect data from your hospital case notes to see if testing you with this method – a rapid point-of-care test, made any difference to your care.

We are therefore asking for volunteers over the age of 18 who present to hospital with an acute respiratory illness and/or a fever.

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. 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. A computer program will assign you randomly to group one or group two. Group one will have a swab taken from the nose and throat, group two may or not have this procedure, alongside usual medical care.

If you are selected to have a swab taken then a swab (which is like a 'cotton bud') is briefly put up your nose and in the back of your throat. This is then taken to our analyser and analysed for many different viruses. You will be informed of the results, as will the doctors and nurses looking after you. You still have the right to discuss the results with the research team and the clinical team looking after you.

Once you have been discharged, whether you had a swab taken or not, a researcher will look at your hospital case notes to determine if having the test performed, or not has affected your management during your hospital stay. This includes how quickly you were discharged, whether you were nursed in a side room and decisions relating to antibiotic or antiviral medication.

If you have a swab that reveals a particular result, we may approach you again to ask if you would give us permission to take a blood sample and further nasal swabs for additional ethically approved research. You have the right to decline this, should you wish.

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.

Your GP would be informed that you are participating in this study.

The doctors and nurses treating you are told the results of the point-of-care test looking for respiratory viruses.

University Hospital Southampton NHS
NHS Foundation Trust

What are the risks?

The risks of having a simple swab taken from your nose and throat in this study are minimal. Having the swab taken could be mildly uncomfortable for some people, but it is over very quickly.

What happens when the research study stops?

Initially we are carrying out an initial 'pilot study' (this study). This will end when we have tested 200 people like you for respiratory viruses. By this time we hope to have collected all the information we need to decide if we can go on to test a lot more people.

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 is available on the internet. All results are 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 makers of the machines used to test for respiratory viruses are not sponsoring this study. The researchers are not funded or rewarded by the manufacturers.

Who has approved this study?

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
Tel: 02381218410. T.W.Clark@soton.ac.uk

Dr Nathan Brendish, Co-investigator
Tel: 02381204989. Nathan.Brendish@uhs.nhs.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

Patient trial ID:

Participant Consent Form

Point-of-care testing for respiratory viruses. Randomised controlled trial comparing POCT with standard clinical care, in adults presenting to secondary care with acute respiratory illness: a pilot study (ResPOC Trial). 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 agree that my GP will be informed about my participation in the study. ☐
4. I agree to have a nose and throat swab taken to be tested for respiratory viruses and certain bacteria to be analysed by a 'point-of-care' rapid diagnostic test. ☐
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 approached later depending on the results of my swab, for the purposes of taking further swabs and/or a blood test (I have the right to opt out of this, should it be asked). ☐
9. I understand that any samples collected from me may be kept for the purpose of further 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

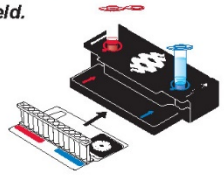
When completed 1 for volunteer, 1 for research file (both original signatures)
REC number 14/NW/1467 Study number RHM MED 1217 Version 2.0

Appendix C Standard Operating Procedures for the molecular POCT and swab acquisition

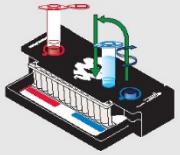
Instructions for FilmArray platform use as provided by the manufacturer.

FilmArray® Respiratory Panel Quick Guide
(Injection Vials)

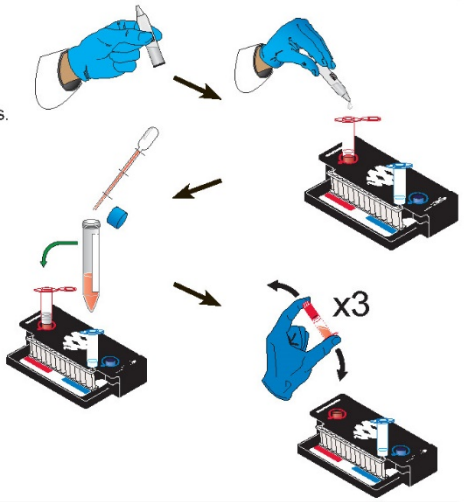
⚠ ***To avoid contamination always wear gloves and work behind a protective shield.***



Step 1: Prepare Pouch
- ❑ Insert pouch into Pouch Loading Station.
- ❑ Place Sample Injection Vial into red well.
- ❑ Place Hydration Injection Vial into blue well.

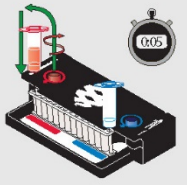


Step 2: Hydrate Pouch
- ❑ Unscrew Hydration Injection Vial, leaving cap in Pouch Loading Station, and insert into pouch hydration port.
- ❑ Forcefully push down to puncture seal.
- ❑ Wait as Hydration Solution is drawn into pouch.



Step 3: Prepare Sample Mix
- ❑ Add Sample Buffer to Sample Injection Vial.
  - Invert Sample Buffer Ampoule so that tip is facing up.
  - ***Note:*** *Do not touch the tip of the ampoule.*
  - Firmly pinch textured plastic tab on side of ampoule until seal snaps.
  - With the tip facing down, dispense Sample Buffer into Sample Injection Vial using a slow, forceful squeeze, followed by a second squeeze. Avoid generating excessive bubbles.
- ❑ Using Transfer Pipette, draw up specimen to third line.
- ❑ Add sample to Sample Injection Vial.
- ❑ Tightly close lid of Sample Injection Vial.
- ❑ Mix sample by gently inverting Sample Injection Vial **3** times.
- ❑ Return Sample Injection Vial to red well of Pouch Loading Station.

⚠ ***Warning: The Sample Buffer is harmful if swallowed and can cause serious eye damage and/or skin irritation.***



Step 4: Load Sample Mix
- ❑ Unscrew Sample Injection Vial from cap.
- ❑ Pause for 3-5 seconds, then remove Sample Injection Vial, leaving cap in Pouch Loading Station.
- ❑ Insert Sample Injection Vial into pouch sample port.
- ❑ Forcefully push down to puncture seal.
- ❑ Wait as Sample Mix is drawn into pouch.

Step 5: Run Pouch
- ❑ Follow instructions on computer for initiating a test.

BIOFIRE®
A BIOMÉRIEUX COMPANY

BioFire Diagnostics, LLC | 390 Wakara Way, Salt Lake City, Utah 84108, USA | +1-801-736-6354

FilmArray® Respiratory Panel Quick Guide
(Injection Vials)

FilmArray™ Respiratory Panel — BIOFIRE — www.BioFireDx.com

*The **Run Summary Section** displays information about the sample and a summary of the control and test results.*

1. Detected:
   - Names of any detected pathogens
   - If 'None', no pathogens were detected
   - If '⚠Invalid', RETEST SAMPLE
2. Equivocal (↔):
   - If 'None', no pathogens were equivocal
   - If a pathogen is listed, RETEST SAMPLE
   - If '⚠Invalid', RETEST SAMPLE
3. Controls:
   - If 'Passed', results are valid
   - If '⚠Failed', RETEST SAMPLE
   - If '⚠Invalid', RETEST SAMPLE

Run Summary			
Sample ID:	1-214	Run Date:	23 Jan 2013 11:30 AM
1. Detected:	Human Rhinovirus/Enterovirus	3. Controls:	Passed
2. Equivocal:	↔ Influenza A H1-2009		

*The **Results Summary Section** lists the test results for each pathogen targeted by the Respiratory Panel.*

4. If 'Not Detected', pathogen was not detected
5. If '✓Detected', pathogen was detected
6. If '↔ Equivocal', result could not be determined, RETEST SAMPLE

If '⚠Invalid', RETEST SAMPLE

***Note**: If repeated 'Invalid' results are obtained, contact BioFire Diagnostics, the local bioMérieux sales representative, or an authorized distributor.*

Result Summary		
4.	Not Detected	Adenovirus
	Not Detected	Coronavirus 229E
	Not Detected	Coronavirus HKU1
	Not Detected	Coronavirus NL63
	Not Detected	Coronavirus OC43
	Not Detected	Human Metapneumovirus
5.	✓ Detected	Human Rhinovirus /Enterovirus
6.	↔ Equivocal	Influenza A H1 2009
	Not Detected	Influenza B
	Not Detected	Parainfluenza Virus 1
	Not Detected	Parainfluenza Virus 2
	Not Detected	Parainfluenza Virus 3
	Not Detected	Parainfluenza Virus 4
	Not Detected	Respiratory Syncytial Virus
	Not Detected	*Bordetella pertussis*
	Not Detected	*Chlamydophila pneumoniae*
	Not Detected	*Mycoplasma pneumoniae*

*The **Run Details Section** displays information about the pouch, instrument, run status, and operator.*

7. Run Status:
   - If 'Completed', run is complete
   - If 'Incomplete', 'Aborted', or any other error message, RETEST SAMPLE

***Note**: If repeated 'Error' messages are obtained, contact BioFire Diagnostics, the local bioMérieux sales representative, or an authorized distributor.*

Run Details			
Pouch	Respiratory Panel	Protocol:	NPS v.2.0
7. Run Status:	Completed	Operator:	JDoe
Serial No.:	00026577	Instrument:	ITI FA "FA1115"
Lot No.:	100302A		

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RFIT-PRT-0436-02

Nose and throat swab acquisition standard operating procedure

WTCRF-BRU Southampton University Hospitals NHS Trust

Study Specific SOP to collect throat and nasal Swabs for ResPOC (RHM Med 1217)

SOP Number:	*WTCRF-BRU/GEN*	Effective Date: 30 Jan 2015
Version Number & Date:	*V1, 30 Jan 2015*	Review Date: 30 Jan 2017
Superseded Version Number & Date:	*V1*	

Author: *Sandy Pink* ***Designation:*** *Senior Research Nurse; Respiratory BRU*	***Signature***	***Date***
Authorised By: ***Designation:***		
Expert Authorisation: ***Designation:*** ***Contact Details:***		

Revision Chronology				
Version Number	Date	Changes	Author, Designation	Authorisation, Designation
V1	30 Jan 2015		Sandy Pink Senior Research Sister. Respiratory BRU	

PURPOSE

This standard operating procedure (SOP) describes to staff the process of how to obtain a nose and throat swabs in a safe and consistent manner. Please note that this procedure differs from obtaining a nasopharyngeal sample.

SCOPE

This procedure can only be conducted by doctors and nurses who are trained in this procedure and assessed as competent to do so.

RESPONSIBILITIES

It is the responsibility of staff to read and use this SOP prior to carrying out a nasal swab for study RHM MED 1217.

PROCEDURE

Equipment

- 1 x Viral culture swab with medium such as Σ-Virocult® (M40 compliant)
- Tongue depressor
- Gloves (latex free)
- Apron
- Surgical mask and eye protectors or a splash resistant mask if it covers the entire face
- Tissues
- Specimen bag
- WTCRF lab Specimen record form
- Container suitable in transporting specimens

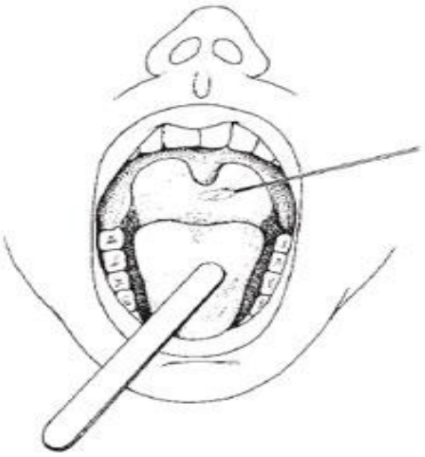
Throat swab and nasal swab

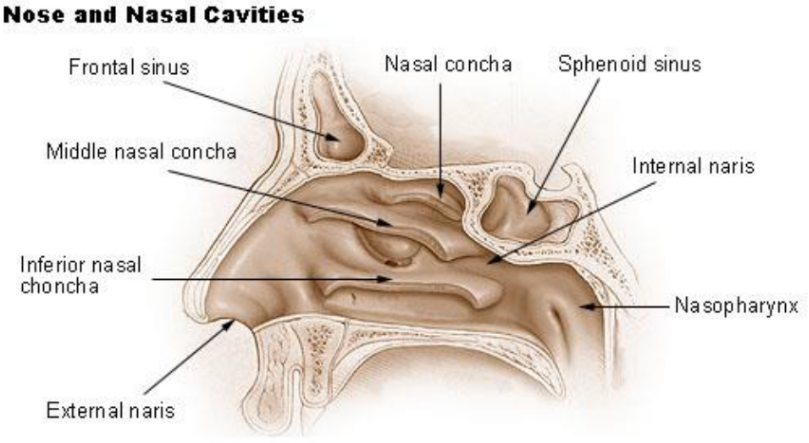
- Ensure the participant is fit for the procedure
- Obtain informed consent from the participant
- Wash hands according to UHS policy and put on gloves, apron, surgical mask and eye protection (or splash resistant visor if it covers the entire face).
- Unwrap packaging of the swab.
- Ask patient to tilt their head back and open their mouth wide. Use tongue depressor if required.
- With the swab rub the soft pallet at the back of the patient's mouth on each side rotating the swab as you do it.

The same swab can be used for the nasal swab or alternatively an additional swab can be used according to the patient's preference. If two swabs are used, they must both be placed in the same tube containing VTM

- Have the participant sit in a chair or on a bed with their head supported by a 2nd person or pillow if needed to ensure the head remains still
- Ask the participant to put his/her hands below the leg to prevent hands interfering with the procedure
- Tilt the participant's head backwards. Holding the shaft of the swab between the thumb and index finger carefully insert the tip of the swab into the nostril and push backwards (rather than upwards) until resistance is felt (inferior nasal conchae) - this is usually no more than 3-5 cm inside to the nasal cavity. Rotate the swab, and then remove.

Throat swab





- Place the swab into the collecting medium and snap off the shaft so that the lid can be replace. Ensure that the lid is securely fastened.
- Fill in the participant's details appropriately on the container.
- Ensure the participant is comfortable post procedure, offering them a tissue as it can make their eyes water a little.
- Enter procedure into the participant's medical notes.
- Take samples to the laboratory for processing.

RELATED DOCUMENTS

UHS Standard infection control precautions policy 22 March 2013
UHS Hand Hygiene Policy April 2010

Appendix D Example result from FilmArray Respiratory Panel

FilmArray®
Respiratory Panel

BIOFIRE
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Run Summary			
Sample ID:	ID redacted	**Run Date:**	13 Feb 2015 10:43 AM
Detected:	Influenza A H3	**Controls:**	Passed
Equivocal:	None		

Result Summary	
Not Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
✓ Detected	Influenza A H3
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus
Not Detected	*Bordetella pertussis*
Not Detected	*Chlamydophila pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

Run Details			
Pouch:	Respiratory Panel v1.7	**Protocol:**	NPS v2.0
Run Status:	Completed	**Operator:**	Redacted
Serial No.:	02029393	**Instrument:**	ITI FA "FA3405"
Lot No.:	182914		

Appendix E Neuraminidase inhibitor prescribing questionnaire

Southampton | University Hospital Southampton NHS

Neuraminidase inhibitor prescribing by physicians in the UK: a questionnaire-based survey of practice (expanded)

Introduction

Dear Doctor

Thank you for participating in this ethically approved questionnaire-based survey examining neuraminidase inhibitor prescribing by hospital-based adult physicians in the UK. In light of the recent controversy regarding the efficacy of the neuraminidase inhibitors (Oseltamivir and Zanamivir) for the treatment of influenza, we are seeking to explore current prescribing patterns for adults presenting to secondary care. Defining the current use of neuraminidase inhibitors is important for several reasons including planning of future studies of novel anti-influenza therapies.

As the clinicians who frequently assess, give advice on, or care for patients with suspected influenza we are asking specialists (consultant or registrar level) in infectious diseases, microbiology, acute medicine, respiratory medicine, emergency medicine and geriatric medicine to complete this short questionnaire, which should take no more than 5 minutes.

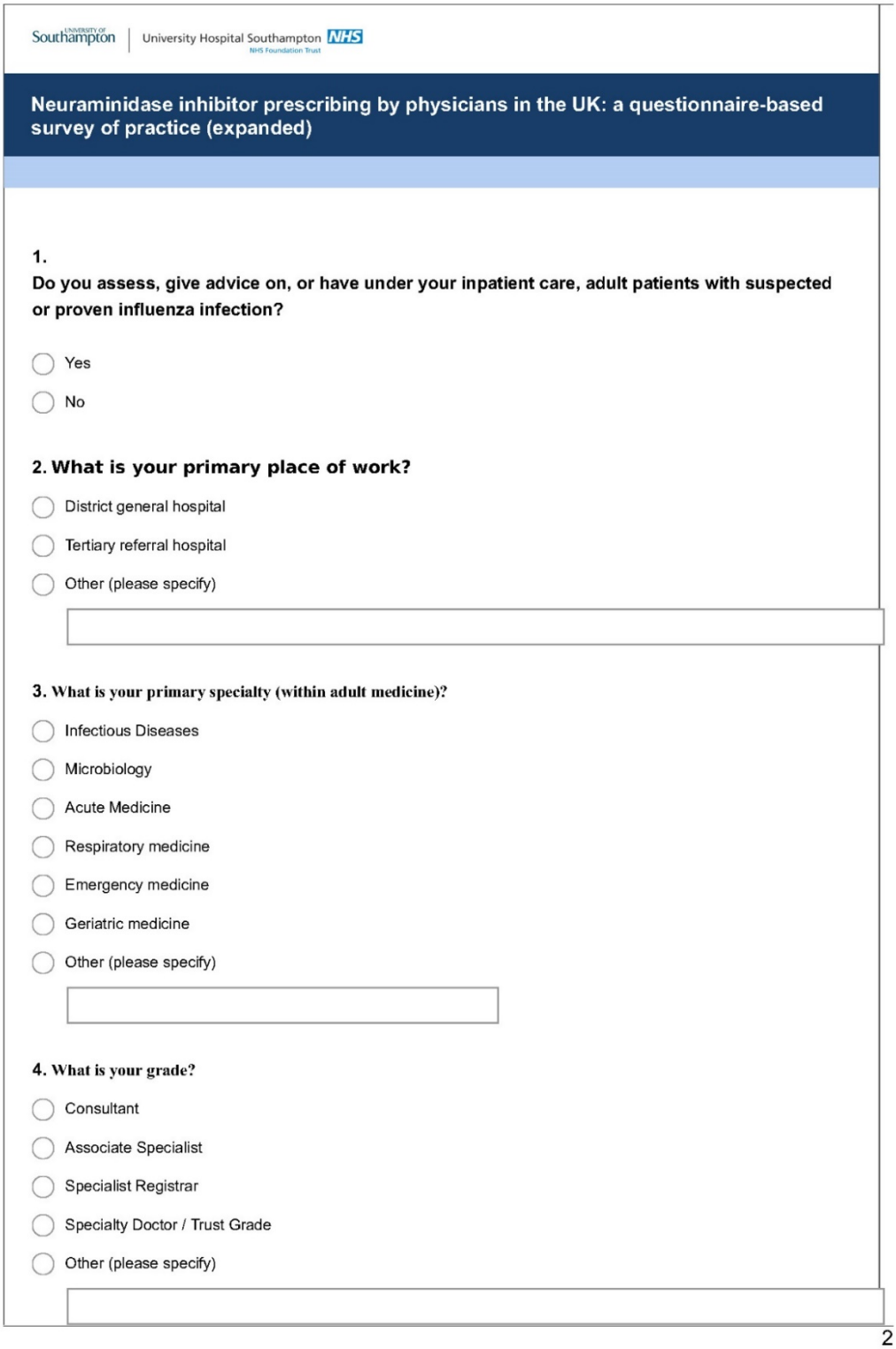
We would discourage looking up any reference material or guidelines prior to completing the questionnaire as we are trying to ascertain current knowledge and prescribing practice. We ask you to please answer the questions honestly as related to your current personal practice. All answers are fully anonymised.

As an incentive to complete this questionnaire, all participants will be offered entry into a prize draw. The first prize is an iPAD Air2 (worth £399) with 4 runner-up prizes of amazon vouchers worth £50 each. To inform you of the results of this study we will contact the professional society that sent you this email with an anonymised summary of the results in due course.

Yours sincerely

Dr Tristan Clark
Associate Professor and Honorary Consultant in Infectious diseases
University of Southampton

1



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Neuraminidase inhibitor prescribing by physicians in the UK: a questionnaire-based survey of practice (expanded)

1.
Do you assess, give advice on, or have under your inpatient care, adult patients with suspected or proven influenza infection?

- ◯ Yes
- ◯ No

2. What is your primary place of work?

- ◯ District general hospital
- ◯ Tertiary referral hospital
- ◯ Other (please specify)

3. What is your primary specialty (within adult medicine)?

- ◯ Infectious Diseases
- ◯ Microbiology
- ◯ Acute Medicine
- ◯ Respiratory medicine
- ◯ Emergency medicine
- ◯ Geriatric medicine
- ◯ Other (please specify)

4. What is your grade?

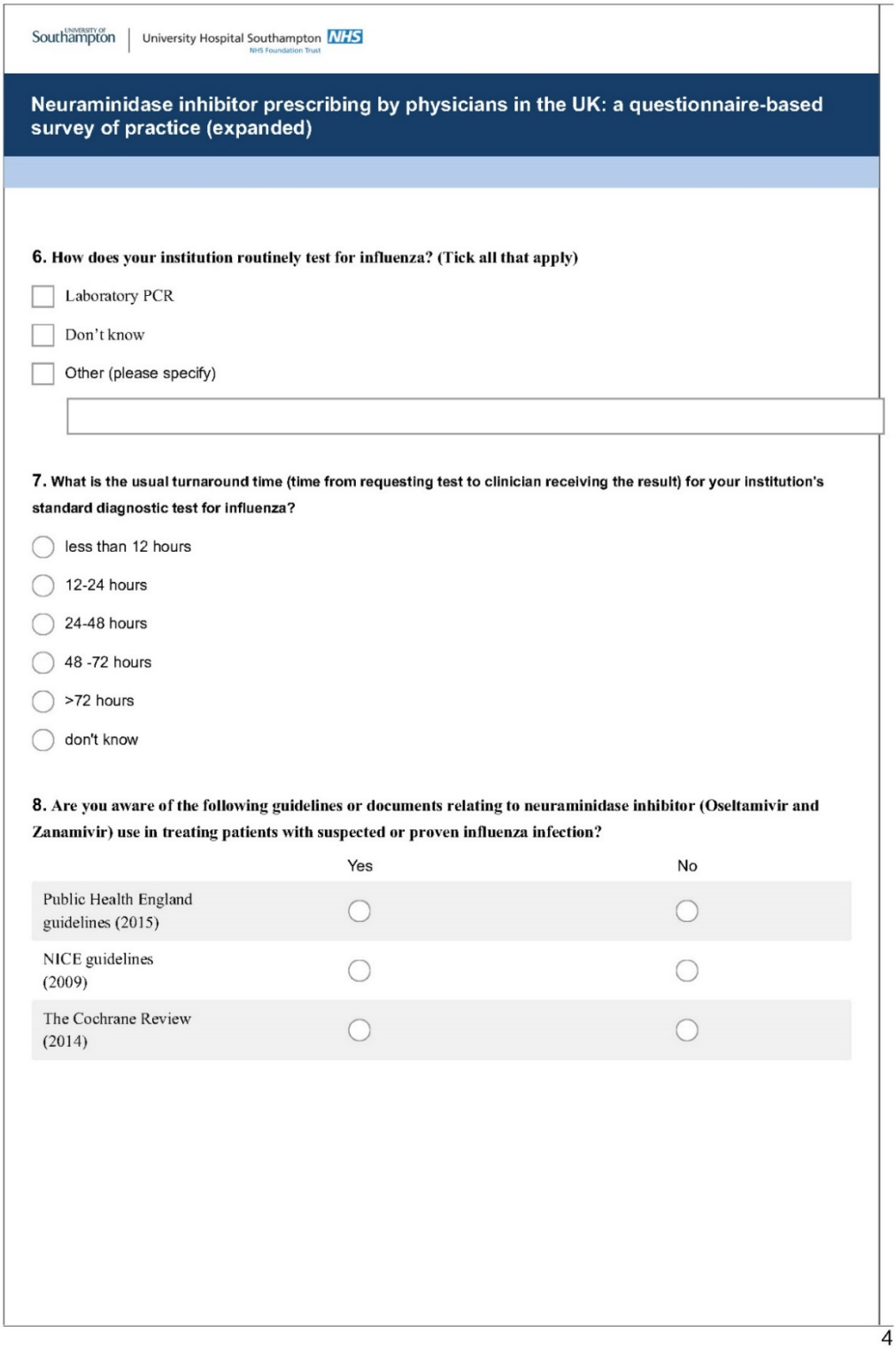
- ◯ Consultant
- ◯ Associate Specialist
- ◯ Specialist Registrar
- ◯ Specialty Doctor / Trust Grade
- ◯ Other (please specify)

2

5. How long have you been qualified as a doctor for?

- ◯ ≤10 years
- ◯ 11 to 20 years
- ◯ 21 to 30 years
- ◯ 31 to 40 years
- ◯ >40 years

3



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Neuraminidase inhibitor prescribing by physicians in the UK: a questionnaire-based survey of practice (expanded)

6. How does your institution routinely test for influenza? (Tick all that apply)

- ☐ Laboratory PCR
- ☐ Don't know
- ☐ Other (please specify)

7. What is the usual turnaround time (time from requesting test to clinician receiving the result) for your institution's standard diagnostic test for influenza?

- ○ less than 12 hours
- ○ 12-24 hours
- ○ 24-48 hours
- ○ 48 -72 hours
- ○ >72 hours
- ○ don't know

8. Are you aware of the following guidelines or documents relating to neuraminidase inhibitor (Oseltamivir and Zanamivir) use in treating patients with suspected or proven influenza infection?

	Yes	No
Public Health England guidelines (2015)	○	○
NICE guidelines (2009)	○	○
The Cochrane Review (2014)	○	○

4

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Neuraminidase inhibitor prescribing by physicians in the UK: a questionnaire-based survey of practice (expanded)

The following clinical scenarios all takes place during the winter months during periods of peak influenza circulation, according to national surveillance schemes.

9.

Scenario 1

A fit and healthy 24 year old man presents to the emergency department with a 36 hour history of fever, chills, myalgia, sore throat, dry cough and malaise. He is febrile but haemodynamically stable with normal oxygen saturations and a normal chest radiograph.

Regarding testing for influenza and treatment with neuraminidase inhibitors, which of the following best describes your current practice?

- ◯ Treat empirically with oseltamivir and do not send a test for influenza
- ◯ Send a test for influenza and treat empirically with oseltamivir whilst awaiting the result
- ◯ Send a test for influenza and withhold oseltamivir treatment whilst awaiting the result
- ◯ Do not treat with oseltamivir at any stage but send a test for influenza for other reasons (eg infection control)
- ◯ Do not treat with oseltamivir and do not send a test for influenza
- ◯ Other practice (please specify)

10. Comments and justification for scenario 1 answer (optional)

5

11. Scenario 2

A 48 year old woman with type 2 diabetes and chronic kidney disease presents to the emergency department with a 36 hour history of fever, chills, myalgia, sore throat, dry cough and malaise. She is febrile but is haemodynamically stable with normal oxygen saturations and a normal chest X-ray.

Regarding testing for influenza and treatment with neuraminidase inhibitors, which of the following best describes your current practice?

- ◯ Treat empirically with oseltamivir and do not sent a test for influenza
- ◯ Send a test for influenza and treat empirically with oseltamivir whist awaiting the result
- ◯ Send a test for influenza and withhold oseltamivir treatment whilst awaiting the result
- ◯ Do not treat with oseltamivir at any stage but send a test for influenza for other reasons (eg infection control)
- ◯ Do not treat with oseltamivir and do not send a test for influenza
- ◯ Other (please specify)

12. Comments and justification for scenario 2 answer (optional)

13. Scenario 3

A 38 year old woman with asthma is hospitalised with a severe exacerbation. Her symptoms started 36 hours ago with fever, chills, myalgia, sore throat, dry cough and malaise followed by wheeze and dyspnea. She is treated with nebulised bronchodilators and oral glucocorticoids.

Regarding testing for influenza and treatment with neuraminidase inhibitors, which of the following best describes your current practice?

- ◯ Treat empirically with oseltamivir and do not sent a test for influenza
- ◯ Send a test for influenza and treat empirically with oseltamivir whist awaiting the result
- ◯ Send a test for influenza and withhold oseltamivir treatment whilst awaiting the result
- ◯ Do not treat with oseltamivir at any stage but send a test for influenza for other reasons (eg infection control)
- ◯ Do not treat with oseltamivir and do not send a test for influenza
- ◯ Other (please specify)

6

14. Comments and justification for scenario 3 answer (optional)

15. Scenario 4

A previously healthy 62 year old man is hospitalised with community acquired pneumonia. His symptoms started 72 hours ago with fever, chills, myalgia, sore throat, dry cough and malaise followed by shortness of breath and productive cough. His chest X-ray shows bilateral infiltrates. He is treated with supplementary oxygen and antibiotics and referred to the intensive care unit.

Regarding testing for influenza and treatment with neuraminidase inhibitors, which of the following best describes your current practice?

- ◯ Treat empirically with oseltamivir and do not sent a test for influenza
- ◯ Send a test for influenza and treat empirically with oseltamivir whist awaiting the result
- ◯ Send a test for influenza and withhold oseltamivir treatment whilst awaiting the result
- ◯ Do not treat with oseltamivir at any stage but send a test for influenza for other reasons (eg infection control)
- ◯ Do not treat with oseltamivir and do not send a test for influenza
- ◯ Other (please specify)

16. Comments and justification for scenario 4 answer (optional)

7

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Neuraminidase inhibitor prescribing by physicians in the UK: a questionnaire-based survey of practice (expanded)

17. In addition to the occurrence during time periods of known influenza circulation, what other factors influence your decisions to prescribe neuraminidase inhibitors to patient with suspected or confirmed influenza?

- [ ] Duration of illness
- [ ] Presence of risk factors for complicated disease (age >65, chronic cardiovascular, respiratory, renal, liver or neurological diseases, diabetes mellitus or immune suppression)
- [ ] Presence of already complicated or severe diseases (e.g. pneumonia or exacerbation of airways disease)
- [ ] Concerns over the efficacy of oseltamivir
- [ ] Concerns over the side effects or oseltamivir
- [ ] Concerns over resistance to oseltamivir
- [ ] A history of influenza vaccination for the current season
- [ ] Other (please give details)

18. Comments and justification for Question 17 (optional)

19. If you wish to state any additional comments relating to your current practice of prescribing or advising on the prescribing of neuraminidase inhibitors, please state them here:

20.

Thank you for completing this questionnaire. If you wish to be in the draw for the prizes, please enter your email address here:

8

21. Please state which institution you are from:

Researcher contact details: Principal Investigator Dr T W Clark, Associate Professor & Honorary Consultant in Infectious Diseases. t.w.clark@soton.ac.uk;

Research Sponsor: University Hospital Southampton NHS Foundation Trust

9

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