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POINT-OF-CARE TESTS FOR THE RAPID DIAGNOSIS OF RESPIRATORY VIRAL INFECTIONS.

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INTRODUCTION

Although the three different types of influenza viruses (A, B and C) were discovered between 1933 and 1949, it was not until *in vitro* cell culture techniques were applied to clinical virology during the 1960s that the full extent of respiratory viral disease became apparent [1]. Members of the adenovirus group were isolated from tonsil and adenoids by Rowe and his colleagues in 1953. This was followed by the discovery of the parainfluenza viruses by Chanock and his associates in the USA in 1958 and by BEALE in Canada in the same year [2]. Perhaps the most significant finding was the association of respiratory syncytial virus (RSV) with acute infection in childhood also by CHANOCK *et al.* in 1961 [3]. MORRIS *et al.* [4] had previously identified a similar virus in 1956 as one that had caused a coryzal illness in a colony of laboratory chimpanzees (chimpanzee coryza agent).

Hospital-based studies have been useful in defining the different clinical categories of diseases

caused by these viruses. In the UK, such studies were carried out at four centres during the 1960s: Newcastle (GARDNER *et al.* 1960 [5]), Manchester (HOLZEL *et al.* 1963 [6]), Bristol (CLARKE *et al.* [7]) and Glasgow (STOTT *et al.* 1967 [8]). In a report to the Medical Research Council Subcommittee on RSV vaccines (1978), RSV was identified as the infection most often responsible for bringing children in the UK into hospital and is one of the main causes of acute respiratory failure in infancy.

Forty years on, there continues to be no effective means to control these infections. PROBER [9] estimated that approximately two-thirds of all infants are infected with RSV during the first year of life, and almost all have been infected by the time they are 2 yrs old. In a separate study, estimates of direct medical expenditure (US\$) were collected from a prospective cohort study of children admitted to hospital with proven RSV infection and from national and provincial databases of children aged <2 yrs. This Canadian-based investigation estimated that the

annual cost of RSV-associated illness was \$18 million (1997) [10]. The largest component of direct expenditure (62%) was for inpatient care, for the estimated 0.7% of all children in the study who were ill enough to require hospital admission. Similar studies in the USA suggest that an estimated 100,000 children were admitted for inpatient care with RSV infection at a cost of \$300 million [11]. RSV is now also recognised as an important cause of adult respiratory tract infection where signs and symptoms are less distinctive than in children and the diagnosis is often missed [12].

A point-of-care test (POCT) is defined as any analytical test performed for a patient by a healthcare professional outside of the conventional laboratory setting. The current tests available for respiratory viral infections are only limited to disposable non-instrumental systems, which test for RSV, influenza virus and adenovirus.

An example of how a POCT might be applied is given with RSV ►

infections. RSV causes annual winter epidemics, which result in hundreds of infants with bronchiolitis and pneumonia presenting to hospital over a relatively short period of time. There is currently no widely used vaccine or antiviral agent to control these infections. Routine POCTs screening for RSV have helped in the process of segregating infected patients and facilitating policies that minimise healthcare associated infections [13, 14]. We have utilised Binax NOW® RSV Test (Binax Inc, Maine, USA), an immunochromatographic assay used for the qualitative detection of the RSV fusion protein in nasal wash specimens from symptomatic patients. The principle of this assay utilises anti-RSV antibody, which is adsorbed onto a nitrocellulose membrane forming the “sample line”. On a separate part of the membrane, anti-RSV antibody is conjugated to visualising particles that are dried onto an inert fibrous “conjugate pad”. The sample to be tested is added to the top of the test strip and flows through the porous matrix. RSV antigen present in the clinical sample binds to the conjugated antibody, which is then captured and immobilised on the ‘sample line’. Test results are interpreted by the presence or absence of visually detectable coloured lines. The procedure for carrying out the test requires only one step and results are read after 15 min of incubation at room temperature. These features, in particular, have significant time saving advantages for staff working in a busy accident and emergency unit. The principle of this assay is similar to other assays that are commercially available.

It is essential for potential users to establish whether there is a well-identified need before implementing such a POCT (table 1).

The implementation of a POCT system must be part of a clinical total quality assurance plan. Normally, this would be led by a

Table 1. Identifying a need for point-of-care testing (POCT)

Questions to consider [15, 16, 17]
<ul style="list-style-type: none"> • Which group of patients need testing and what tests need to be performed? • How is the current service currently provided and does it meet the clinical need? • If clinical need has not been met, what has been done to try to rectify the problem? • Is access to laboratory service difficult for patients for patients requiring frequent monitoring? Has this been discussed with the laboratory? • Will POCT enable more rapid/effective diagnosis or treatment? • Can you provide evidence that POCT will provide a measurable clinical and economic benefit? • Will POCT provide a cost-effective alternative to laboratory testing?

senior grade healthcare scientist in collaboration with clinicians, nurses and hospital administrators. A number of useful guidelines have been established and provide a framework to introduce such assays [15–17]. Once a need has been established, the next step is to validate a suitable device by comparing its laboratory performance characteristics with the current local laboratory based tests. In other words, the accuracy limits and imprecision of POCT results should be established. Generally, laboratory based testing will have better performance characteristics compared with POCT.

CURRENT LABORATORY TESTING FOR RESPIRATORY VIRUS INFECTIONS

The past 5–10 years have witnessed a transformation in the laboratory diagnosis of respiratory virus infections in the UK and other industrialised countries. Novel test systems, based mainly on molecular diagnostic techniques (real-time PCR) have greatly enhanced the sensitivity and positive predictive value of test results compared with conventional procedures, such as cell culture and direct immunofluorescence. Also, the

range of viruses and atypical organisms that can be routinely screened has been expanded to include: influenza A/B, RSV, parainfluenza viruses types 1–4, metapneumovirus, adenoviruses, rhinoviruses, coronaviruses, bocavirus; the atypical bacteria such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*. These assays have been multiplexed and very small volumes of reagents and templates (clinical sample) are used. By using real-time PCR it has been estimated that the ratio of the number of positive by real-time PCR to the number of specimens positive by conventional cell culture techniques is 2.6:1 for RSV ($p < 0.0001$) and 1.6:1 for influenza A/B B ($p < 0.01$) [18].

Direct and indirect immunofluorescence tests (IF) on respiratory secretions have been the clinically most useful laboratory tests prior to the introduction of real-time PCR. Currently, the limitation in the performance of IF compared with molecular assays has been illuminated in recent comparative studies. For example, KUYPERS *et al.* [19] used an indirect immunofluorescence technique to test for six viruses in a variety of respiratory samples. The results of the individual PCR assays showed

differences among the six viruses in the direct IF panel in the proportion that was positive. While 90% of the RSV and influenza A positive by PCR was also positive by IF, a much smaller proportion of the specimens was positive for parainfluenza virus, metapneumovirus and adenovirus. They also demonstrated, by both PCR and IF, that the mean number of virus copies per mL in specimens was significantly higher, at 6.7×10^7 , than that in specimens positive only by PCR at 4.1×10^4 ($p < 0.001$). The PCR assay also identified specimens with multiple viral infections with a 10-fold greater frequency than by IF. The viral load detectable by the current range of POCTs for RSV and influenza is likely to be even higher than that observed for DIF, *i.e.* less sensitive than IF [19].

However, there are disadvantages to current real-time PCR format. In a recent study, the limitations of these novel assays were highlighted. The cost for real-time PCR, including labour costs, cost of reagents, depreciation of equipment and overhead costs (20%) was estimated at €330.00 per sample. In a randomised controlled trial, nasopharyngeal and oropharyngeal swab specimens from patients admitted for antibiotic treatment of lower respiratory tract infection (LRTI) were evaluated by means of real-time PCR for respiratory viruses and atypical pathogens, as well as conventional diagnostic procedures. Real-time PCR results for patients in the intervention group were reported to the treating physician; results for patients in the control group were not made available [20].

Real-time PCR increased the diagnostic yield significantly. In the intervention group, results for real-time PCR were reported as a mean duration (\pm SD) of 30 ± 13 h after sampling and 25% patients had positive results. Implementation of real-time PCR in the diagnostic work-up of

Table 2. Performance of Binax NOW RSV compared with direct immunofluorescence [21]

NOW RSV (point-of-care test)	Direct immunofluorescence		Total
	Positive	Negative	
Positive	99	12	111
Negative	15	180	195
Total	114	192	306

Sensitivity = $99/114 = 87\%$, point-of-care test compared with direct immunofluorescence; specificity = $180/192 = 94\%$; positive predictive value = $99/111 = 89\%$; negative predictive value = $180/195 = 92\%$; and prevalence = $114/306 = 37\%$.

patients in hospital with community-acquired LRTI increased the aetiological diagnosis from 21 to 43%. However, clinical treatment of patients hardly changed. In fact, test results hardly influenced clinical management of patients and did not reduce additional diagnostic procedures, antibiotic use, antibiotic costs or duration of hospital stay [20].

SENSITIVITY AND SPECIFICITY

The usual way to evaluate the effectiveness of a POCT, giving a positive or negative result (categorical data), is to compare it with a well-established assay, such as IF, RT-PCR or viral culture (gold standard or criterion standard).

Sensitivity = Number positive by POCT and the gold standard / Number positive by gold standard

Specificity = Number negative by POCT and the gold standard / Number negative by gold standard

In other words, the sensitivity is a proportion (%) of gold standard positives that are also POCT positive, and specificity is the proportion of gold standard negatives that are POCT negative.

We can also estimate the positive predictive value (PPV), the probability that a POCT positive

result will be a true positive, and the negative predictive value (NPV), the probability that a negative POCT will be a true negative. These depend on the prevalence of infection in the group being examined, as well as the sensitivity and the specificity.

The most convenient way to analyse data like these, is to include them in a 2x2 table and compare the POCT with the gold standard (table 2).

POINT-OF-CARE TESTS FOR RESPIRATORY VIRUSES

The list of US Food and Drug Administration-cleared and Clinical Laboratory Improvement Amendments-waived point-of-care tests for RSV and influenza is expanding (table 3)

These assays are primarily validated for use on nasopharyngeal samples in neonatal and paediatric groups. There may be significantly lower performance in adults and local validation should be undertaken prior to testing this patient group. Adults tend to have lower levels of virus in respiratory secretions compared with young children.

We carried out a prospective study to compare the performance characteristics of one of these assays, Binax NOW RSV, with laboratory-based direct ►

Table 3. List of point-of-care tests for respiratory syncytial virus (RSV)

Test	Principal of assay	Time for result min
QuickVue RSV	Dipstick immunoassay	15
ClearView RSV	Immunochromatographic	15
SAS RSV Alert	Immunochromatographic	15
Binax NOW RSV	Immunochromatographic	15
Directigen EZ RSV	Immunochromatographic	15–60
QuickVue Influenza A/B	Dipstick immunoassay	15
Binax NOW Influenza	Immunochromatographic	15
OSOM Influenza A & B	Immunochromatographic	15
Directigen A+B	Immunochromatographic	15

immunofluorescence [21] (table 4). The POCT was performed by registered nurses, assistant nurses and auxiliary nurses in a side-room, adjacent to a paediatric accident and emergency department. The main advantage of this type of immunochromatographic assay was its technical simplicity. However, a potential disadvantage of such an apparently simple test is that less emphasis was placed upon training and consequently there was less restriction in the number of healthcare workers allowed to carry out tests. There were 27 out of 306 discordant results, of which 16 were reversed when the POCT was repeated by laboratory trained staff. When these corrected results were incorporated into 2 × 2 tables to calculate performance characteristics, there was an improvement in sensitivity and PPV.

An important consideration in the decision to use a POCT is the prevalence of infection likely to be encountered. In this study the prevalence of RSV infection among infants attending an accident and emergency unit was nearly 40%. The performance of a POCT will be reduced substantially if the prevalence fell to <10%. GRIJALVA *et al.* [18] examined the accuracy and interpretation of rapid influenza tests in children aged <5 yrs who

were admitted to hospital over two winters. The prevalence of influenza varied markedly between these two periods and this was reflected in the performance of the rapid tests that were utilised at different sites (Directigen A+B, QuickVue A/B and NOW Flu A/B). They concluded that, before ordering an influenza rapid test, it

is important to have an estimate of influenza prevalence in the community. When the prevalence of infection is low, *i.e.* <10%, positive test results are more likely to be false-positive. They estimated that, when influenza prevalence is ~5%, a positive rapid-test will be correct 50% of the time. By contrast, when influenza prevalence is >10%, a positive rapid-test result will predict influenza infection correctly >70% of the time [18].

Few studies have determined whether POCTs for respiratory viruses actually have a beneficial impact on patient care. This could be measured objectively in terms of the number of additional investigations requested and treatment for acute respiratory illness. One recent study randomised children with acute respiratory illness to receive a POCT or not to have such a test performed. Subsequent diagnostic test ordering and antibiotic prescribing were compared for the

Table 4. Laboratory characteristics of Binax NOW® RSV compared with laboratory-based direct immunofluorescence

Characteristic	Binax NOW® RSV performed at the point of care	Binax NOW® RSV performed at the on-site laboratory [#]
Sensitivity	87 (81-93)	92
Specificity	94 (91-97)	94
PPV	89 (83-94)	90
NPV	92 (88-96)	95
False-positive rate	11	10
False-negative rate	8	5
Binax NOW® RSV positive	114	120
Binax NOW® RSV negative	192	186
Prevalence	37.2	39.2
Total n	306	306

Data are presented as % (95% CI), unless otherwise stated. #: corrected discordant results, *i.e.* where Binax NOW® RSV was repeated in the laboratory and there was concordance with the direct immunofluorescence result.

Table 5. Performance of point-of-care tests for respiratory syncytial virus (RSV), influenza and adenovirus

Kit	Compared with	Sensitivity %	Specificity %	PPV %	NPV %	Prevalence %
Clearview RSV [24] N = 120	IF	93	95	97	90	62
Directigen RSV [24] N = 119	IF	81	100	100	77	61
QuickVue Influenza [25] N=102	RT-PCR	85	97	98	82	60
Rapid tests [18] N=270	Culture & RT-PCR	63	97	81	94	15
QuickVue Influenza [22] N=205	Culture & RT-PCR	82	99	98	94	25
SAS Adeno Test [23] N=138	Culture	95				88
SAS Adeno Test [23] N=138	PCR	91				93

PPV: positive predictive value; NPV: negative predictive value; IF: immunofluorescence.

groups. The prevalence of influenza was 19% among 468 enrolled to the study. Fewer children in the POCT group had additional diagnostic tests ordered than in the group where no POCT was performed (39 *versus* 51%; $p=0.33$). There was no difference in antibiotic prescribing. Other benefits in receiving a rapid POCT result included reducing parental anxiety and public health decisions, such as closing schools during outbreaks, but the study was not designed to measure their impact [26].

FUJIMOTO *et al.* [23] showed good laboratory performance characteristics of the SAS Adeno Test when compared with conventional cell culture and PCR techniques. However, in that study, the prevalence of infection was exceptionally high (88 and 93%, respectively), only 16 culture-negative samples were evaluated and the specificity of the assay was

not provided. They found that when SYBR green real-time PCR was used, the detection limit of the SAS Adeno Kit was $10^{4.6}$ to $10^{5.8}$ virus genome copies- mL^{-1} for adenovirus types 1, 2, 3, 5, 6 and 8. These viral loads were surprisingly low when compared with similar comparisons with more sensitive assays, such as IF [23].

Table 5 summarises the performance of a selected number of POCT for RSV, influenza and adenovirus. Most of the studies showed a very high prevalence of infection which will provide the optimum sensitivity and PPV results for the POCT being evaluated. Generally, the specificity of the POCTs is in the range of 95–100%, but the sensitivity is lower. Other studies, not included in this table, have reported a range in sensitivity from 44 to 95%.

CONCLUSION

POCT for RSV, influenza and adenovirus help to provide a rapid test result close to the patient. These types of assay work best during periods of high prevalence, for example during the peak of a winter RSV epidemic or during epidemic and pandemic influenza. Most of these assays have been validated in paediatric patients and care is required if adult screening is anticipated. It is prudent to estimate the accuracy of a newly introduced POCT compared with existing laboratory based systems.

The development of more advanced POCTs for respiratory viruses is important for the control of these infections in the community and in hospital. Most of the technology that exists in this area is based on lateral flow strip technology, with moderate levels of test performance. ►

Recently inexpensive electronic readers have been developed for interrogating lateral flow strip results, avoiding the variation and subjectivity of visual assessment;

however, these are currently not available for routine use. There are also interesting commercial moves to bring real-time PCR closer to the patient. The ultimate

aim is to allow testing for a wider range of pathogens with a high level of sensitivity, next to the patient, by using nucleic acid based assays. ■

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