A flu optical immunoassay (ThermoBioStar's FLU OIA): a diagnostic tool for improved influenza management

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ThermoBioStar's and Biota's flu optical immunoassay (FLU OIA) is a rapid test designed to diagnose influenza A and B infection using a variety of specimen types. The assay uses highly sensitive thin-film detection methods, coupled with specific monoclonal antibodies to the nucleoprotein. The test is simple to perform, requires no instrumentation and is intended to provide a result within 15 min of test initiation in the 'point-of-care' environment. In initial clinical studies, the assay was demonstrated to be equivalent to culture in identifying infected individuals. Subsequent independent studies using a variety of sample types have demonstrated sensitivity ranging from 48 to 100% and specificities ranging from 93 to 97%. In addition to detecting human strains, this assay has been demonstrated to be capable of detecting a variety of avian and non-human mammalian influenza viruses. The FLU OIA test has been used in large-scale surveillance schemes intended to provide rapid epidemiological data during normal influenza seasons and has demonstrated the potential for fulfilling a similar role for multispecies surveillance in, for example, conditions that offer challenges for conventional virus isolation methods. Conceivably, such use should facilitate the timely recognition of influenza outbreaks and prioritization of positive specimens for more conventional, laboratory characterization, leading to improved interpandemic surveillance and rapid reaction in the face of the next pandemic.

Keywords: influenza; diagnostic; FLU OIA; rapid; optical; immunoassay

1. INTRODUCTION

Influenza diagnosis in the primary-care setting plays a key role in disease management at several levels. In the first instance, accurate diagnosis facilitates appropriate measures to be taken in treatment for the infected patient and limitation of spread of the disease within the community. Second, recognition of influenza in the clinic precedes specimen collection, laboratory isolation and subtyping of the virus in support of national or international surveillance efforts. The former process obviously has immediate benefit for disease alleviation and containment whereas the latter is crucial for the recognition of emerging subtypes for determination of annual vaccine composition and timely identification of viruses with pandemic potential.

Influenza symptoms are similar to those caused by other respiratory pathogens, which can be prevalent during the influenza season (Drinka *et al.* 1999). Differentiation of true influenza from diseases caused by other respiratory pathogens therefore presents a challenge for both patient and health-care practitioner. Although the clinical diagnosis of influenza is reported to be correct in 65–85% of cases in the controlled setting of clinical trials, when rigorous case definitions are applied and when influenza has been identified in the community (reviewed

by Cram *et al.* 1999; Newton *et al.* 2000), the accuracy of influenza diagnosis based on symptoms alone may be lower in the more general community. Indeed, several studies have highlighted the difficulties encountered distinguishing influenza symptoms from those caused by other respiratory pathogens in health practices contributing to influenza surveillance networks, let alone routine health care. For example, recent Australian surveillance data reveal that the clinical suspicion of influenza in general practice is confirmed by laboratory isolation of influenza in only 49–54% of cases (Kelly *et al.* 2000). Similar studies in England and Wales, France, and Scotland have reported confirmation of clinical diagnosis of influenza ranging from 6 to 67% of notified cases (Joseph 1995; Carrat *et al.* 1997).

Laboratory methods used to confirm influenza are varied but generally involve virus isolation using one of a number of susceptible cell lines. Although of benefit for disease-surveillance purposes, methods such as cell culture, that require days to weeks to resolve, can provide only retrospective diagnosis in most cases and have no immediate benefit for the patient from whom the sample originated. In order to benefit the patient and/or health-care provider, a diagnostic result must be available quickly enough and provide sufficient information to lead to some action during or immediately after the patient consultation that offers an improvement over the outcome of clinical diagnosis alone. The turn-round time required

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Figure 1. FLU OIA. Two developed tests are shown. (a) A negative test (no influenza antigen detected), with the central positive control dot indicating that the correct procedure was followed. (b) A positive test (influenza antigen detected) with the large circle corresponding to captured influenza A or B antigen.

for the diagnostic result to be useful is in part a function of the environment under which the consultation occurs. For example, for viral respiratory tract infections in the hospital environment semi-rapid (1 day or less) laboratory confirmation of clinical diagnoses has been reported to lead to both clinical and financial benefits for patients and health-care providers (Woo *et al.* 1997; Barenfanger *et al.* 2000). Benefits include reductions in mortality, length of stay and hospital costs, and the more appropriate use of antibiotics. Although it is conceivable that such a semi-rapid test may have benefit in general practice, the realities of patient numbers, short consultation time and difficulties involved in ensuring patient recall for treatment suggest that a much more rapid, 'point-of-care' (POC) test is required.

POC diagnostic tests have been defined as rapid (less than 30 min), easy to use assays that can be performed near the patient without the aid of additional equipment and provide immediate results to the health-care professional (reviewed by Hesterberg *et al.* 1995; Hesterberg & Crosby 1996). Typical POC environments include general practitioner and paediatric offices, labour and delivery suites, emergency rooms, walk-in clinics and student health clinics (Hesterberg & Crosby 1996). In hospital and managed-care environments POC assays have a role wherever rapid confirmatory diagnosis is considered beneficial and are often referred to as 'bedside tests'. In the case of influenza, the probable benefits of rapid tests in such environments are considered to include early

institutional outbreak recognition, improved focused diagnosis (particularly in patients with complex medical history), earlier discharge and reduced prescription of inappropriate antibiotics (Newton *et al.* 2000).

2. REVIEW OF THE FLU OPTICAL IMMUNOASSAY

The flu optical immunoassay (FLU OIA) (Thermo-BioStar, Inc., Boulder, CO, USA) (figure 1) is an optical immunoassay POC test designed to detect both influenza A and B from a variety of sample types. The test requires no instrumentation, is performed at room temperature and provides a result within 15 min that is distinguishable by the human eye. The test is based on antibody-mediated detection of influenza nucleoprotein via a silicon chip-based thin-film biosensor (reviewed by Ostroff *et al.* 1999). Tests are self-contained, single units with internal performance controls. Although each is designed for single use the result is stable and can be stored for retrospective analysis if desired.

The FLU OIA test has been approved by the US Food and Drug Administration and is available in most countries worldwide. Users of the FLU OIA test perform a limited number of steps according to the procedure outlined in figure 2. A positive result yields a purple coloured disc covering a significant portion of the biosensor chip whereas a negative, properly conducted test yields a small purple spot in the centre of the chip (figure 1). The following sections will review the

Figure 2. FLU OIA procedure card. (a) From left to right the panels depict the steps involved in testing a specimen for the presence of influenza A or B using the FLU OIA test. A more detailed description of the procedure is included in the test kit along with all reagents and consumables required. (b) The range of results that may be obtained, from negative (bottom), weak positive (middle) through to strong positive (top).

performance of the FLU OIA test in the laboratory and clinic with reference to peer-reviewed research where available.

(a) Analytical sensitivity

Analytic sensitivity was determined using a variety of influenza A and B strains grown in either embryonated chicken eggs or MDCK cells. Graded concentrations of virus samples prepared in extraction buffer were applied to a series of FLU OIA tests, which were developed according to the manufacturer's instructions. The lowest concentration of virus that yielded a result detectable by the operator's eye was considered to be the limit of sensitivity. Sensitivity ranged from 5×10^2 to 1.2×10^4 50% tissue culture infective dose (TCID₅₀) units for influenza A and 9.4×10^2 to 1.4×10^4 TCID₅₀ units for influenza B (mean 4.4×10^3 and 7.4×10^3 , for A and B, respectively) (table 1). The test detected influenza A H1N1, H2N2, H3N2, H5N1 and influenza B viruses with comparable sensitivities. Reactivity tests against a wider selection of influenza viruses have revealed that the FLU OIA test is capable of detecting multiple influenza A subtypes of human, swine, equine and avian origin (table 2). Watanabe *et al.* (1999) have reported the sensitivity for the FLU OIA test to be in the range of $3\text{--}6.5 \times 10^4$ plaque-forming units (PFU) for a variety of human A and B, swine and avian influenza viruses.

(b) Cross-reactivity and interference

The FLU OIA test is intended for use with a variety of patient specimens isolated from sites within the respiratory tract. Since specimens may be contaminated with a variety of organisms and substances present at this site, it

Table 1. FLU OIA analytical sensitivity.

(Duplicate dilutions of each virus were prepared in phosphate-buffered saline and tested according to the FLU OIA test procedure. The detection limit is expressed as TCID₅₀ units per test.)

virus	detection limit (TCID ₅₀)
A/Texas/36/91 (H1N1)	7.1×10^3
A/Bayern/7/95 (H1N1)	2.3×10^3
A/Singapore/1/57 (H2N2)	5.0×10^2
A/Hong Kong/68 (H3N2)	2.6×10^3
A/Shangdong/9/93 (H3N2)	1.2×10^4
A/Wuhan/359/95 (H3N2)	1.3×10^3
A/Hong Kong/156/97 (H5N1)	5.3×10^3
B/Victoria/2/87	1.2×10^3
B/Panama/45/90	1.3×10^4
B/Beijing/184/93	9.4×10^2
B/Guangdong/5/94	6.0×10^3

was important to establish that the FLU OIA test does not react (i.e. yield a false-positive signal) with probable contaminants or that its ability to detect influenza is not inhibited by these factors. Reassuringly, no cross-reactivity or interference was observed in experiments involving a comprehensive panel of potential contaminants (table 3). Others have confirmed the excellent cross-reactivity profile of the FLU OIA test using specimens from the respiratory tract of patients infected with pathogens other than influenza A or B (Greene & Morris 1999; Turnbow *et al.* 1999; Hindiyeh *et al.* 2000).

Table 2. FLU OIA virus reactivity and cross-reactivity.

(Viruses were grown in culture or embryonated hen's eggs according to published methods to titres of between 1×10^6 and 1×10^{10} TCID₅₀ ml⁻¹ (or haemagglutination titre of 1:128 for some influenza viruses). All of the viruses on the reactivity panel provided a positive result in the FLU OIA test, whereas all of the viruses in the cross-reactivity panel gave a negative result.)

reactivity panel	cross-reactivity panel
A/NWS/33 (H1N1)	adenovirus 4
A/New Jersey/8/76 (HswN1)	adenovirus 5
A/Japan/170/62 (H2N2)	adenovirus 7A
A/Puerto Rico/8/34 (H1N1)	coronavirus 229E
A/Taiwan/1/64 (H2N2)	coxsackievirus A9
A/Port Chalmers/1/73 (H3N2)	coxsackievirus A21
A/Victoria/3/75 (H3N2)	coxsackievirus B5
A/Sydney/97 (H3N2)	cytomegalovirus AD-169
A/swine/1976/31 (H1N1)	echovirus 11
A/equine/Prague/1/56 (H7N7)	herpes simplex virus 1
A/duck/Ukraine/1/63 (H3N8)	herpes simplex virus 2
A/duck/Czechoslovakia/56 (H4N6)	influenza C
A/duck/England/56 (H11N6)	measles virus
A/duck/Alberta/60/76 (H12N6)	parainfluenza virus 1
A/duck/Pennsylvania/10218/82 (H5N2)	parainfluenza virus 2
A/chick/Germany/N/48 (H10N7)	parainfluenza virus 3
A/chicken/Hong Kong/1203/97 (H5N1)	rhinovirus 2
A/turkey/Ontario/6118/68 (H8N4)	rhinovirus 16
A/turkey/Oregon/1/74 (H7N3)	respiratory syncytial virus
A/shearwater/Australia/1/72 (H6N5)	varicella zoster virus
A/gull/Maryland/704/77 (H13N6)	—

Table 3. FLU OIA bacterial cross-reactivity.

(Organisms were grown in culture according to published methods and tested at a concentration of at least 1×10^7 organisms per test. All organisms gave a negative result in the FLU OIA test.)

<i>Acinetobacter calcoaceticus</i>	<i>Lactobacillus fermentum</i>	<i>Serratia marcescens</i>
<i>Bacteroides fragilis</i>	<i>Lactobacillus plantarum</i>	<i>Staphylococcus aureus</i>
<i>Bordetella pertussis</i>	<i>Legionella pneumophila</i>	<i>Staphylococcus aureus</i> (Cowen I)
<i>Candida albicans</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus epidermidis</i>
<i>Chlamydia trachomatis</i> LGV-2	<i>Moraxella catarrhalis</i>	<i>Stomatococcus mucinolagens</i>
<i>Corynebacterium diphtheriae</i>	<i>Mycobacterium avium</i>	<i>Streptococcus</i> group A
<i>Enterococcus faecalis</i>	<i>Mycobacterium tuberculosis</i>	<i>Streptococcus</i> group B
<i>Enterococcus gallinarum</i>	<i>Neisseria gonorrhoeae</i>	<i>Streptococcus</i> group C
<i>Escherichia coli</i>	<i>Neisseria meningitidis</i>	<i>Streptococcus</i> group D
<i>Gardnerella vaginalis</i>	<i>Neisseria mucosa</i>	<i>Streptococcus</i> group F
<i>Haemophilus aphrophilus</i>	<i>Neisseria sicca</i>	<i>Streptococcus</i> group G
<i>Haemophilus influenzae</i>	<i>Neisseria subflava</i>	<i>Streptococcus viridans</i>
<i>Haemophilus parainfluenzae</i>	<i>Proteus mirabilis</i>	<i>Streptococcus mutans</i>
<i>Haemophilus paraphrophilus</i>	<i>Proteus vulgaris</i>	<i>Streptococcus pneumoniae</i>
<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus sanguis</i>
<i>Lactobacillus casei</i>	<i>Salmonella choleraesuis</i> sub minnesota	<i>Torulopsis glabrata</i>

Similarly, substances that could potentially contaminate specimens from patients with influenza-like illness (table 4) did not adversely affect the performance of the FLU OIA test.

(c) Clinical sensitivity

The FLU OIA test is approved for use with a number of different sample types, including throat swabs, nasal aspirates, nasopharyngeal swabs and sputa. Investigators have also shown that the FLU OIA test is capable of

detecting virus antigen in other sample types including frozen specimens stored in viral transport media (VTM), nasal swabs, nasal washes, tracheal aspirates, bronchial washes and bronchial alveolar lavages (Greene & Morris 1999; Turnbow *et al.* 1999; Casciato *et al.* 2000; Hindiyeh *et al.* 2000; Lynch 2000). The health-care worker may therefore use a sample type that is considered most appropriate and acceptable for the patient in a given consultation. Although this provides a useful element of flexibility it should be emphasized that not all sample

Table 4. FLU OIA interference by potential contaminants.

(Various widely available (in USA) over-the-counter products were tested for interference with the ability of the FLU OIA test to detect influenza virus antigen. No interference was observed at the concentrations tested. Interference was also not observed for organisms tested from the bacterial cross-reactivity panel or viral cross-reactivity panel.)

potential contaminant	number of types tested	concentration tested (%)
whole blood	1	2
mouthwash	4	25
throat spray	3	25
cough lozenges	3	25
nasal spray	3	25
cough and cold elixir	3	25

types yield equivalent performance. For example, throat swabs are considered to be a convenient sample type for many patients but are not necessarily the best source for the isolation of influenza virus (see below (§ 2c(i)) and Covalciuc *et al.* (1998, 1999) and Kaiser *et al.* (1999)).

(i) Clinical trial results

Clinical trials of the FLU OIA test were designed to include a variety of sample types and to compare the performance of the FLU OIA test with that of the gold standard: 14-day tissue culture. A three-site study was conducted during the 1998 season. One hundred and eighty-three patients were recruited across three geographically distinct sites after presentation with influenza-like illness at various health-care providers including physicians' offices, emergency rooms and health clinics. Patients were asked to provide any combination of dual nasopharyngeal swabs, dual throat swabs, nasal aspirates and sputum specimens. A total of 404 specimens were collected. The results of this trial have been published previously (Covalciuc *et al.* 1999) and will be summarized below.

Trial results with the FLU OIA test indicated that nasal aspirates and nasopharyngeal swabs were the most sensitive source for antigen detection whereas throat swabs and sputa yielded the poorest sensitivity when compared directly with influenza isolation in tissue culture (table 5 and Covalciuc *et al.* (1999)). Some differences were apparent between the FLU OIA data generated solely at the POC site compared with the FLU OIA results obtained at both the POC and in clinical laboratories (table 5). Similar observations have been made by others (Lynch 2000) and it seems probable that these differences can be attributed to variables in survival of influenza virus and antigen during transport to the laboratory.

The trial design permitted the comparison of culture isolation and FLU OIA results from individual samples to be compared with an expanded gold standard. Because multiple specimen types were obtained from each patient an expanded gold standard was established wherein the ability of tissue culture or the FLU OIA test to accurately predict the influenza status of patients known to be

influenza positive was quantified. Using this approach Covalciuc *et al.* (1998, 1999) reported that throat swabs are the worst sample type, of those examined, for isolation of culturable influenza virus or reactivity with the FLU OIA test (51.8% in both cases). The best sample type for virus isolation was sputum (90.2%) and the nasal aspirates were the most frequently positive specimens by the FLU OIA test (79.6%).

Interestingly, the FLU OIA test and 14-day culture were found to yield statistically indistinguishable results for all sample types when compared with the expanded gold standard (figure 3 and Covalciuc *et al.* (1999)). Notably, there were differences in the results obtained from direct comparison of the FLU OIA test and tissue culture for each specimen (table 5) and the ability of each to accurately predict the influenza status of known influenza-positive patients (figure 3). It seems probable that these discrepancies are caused by different requirements for isolation of culturable virus and preservation of reactive antigens in a given specimen type. Indeed, the retrospective detection of influenza in several culture-negative and FLU OIA test-positive specimens using other approaches (table 5) supports this hypothesis. Nonetheless, both analyses agree that the choice of specimen type has a substantial impact on the ability of the diagnostic test to establish the influenza status of a patient with influenza-like illness.

(ii) Performance of the FLU OIA assay in clinical practice

FLU OIA tests are used in a wide range of clinical settings worldwide and there is an increasing body of literature available on the performance of the test in these real-world environments. A summary of published work on the FLU OIA test is provided in table 6.

It is interesting to note the wide range of specimen types listed in table 6. It is unclear whether this wide range is a consequence of different physician or clinical laboratory preferences, institutional practices, or variation in specimen practicality and acceptability between patient groups (see, for example, Covalciuc *et al.* 1999). However, it highlights the desirability for a POC diagnostic to accommodate multiple specimen types. Overall it appears that the specimen types yielding the highest FLU OIA sensitivity in clinical practice are aspirates (nasal or tracheal), sputa and nasopharyngeal swabs. Although comparative data were not available in all cases, throat swabs were reported to be the least sensitive sample by several investigators. These observations are in agreement with the results obtained during clinical trial of the FLU OIA test (see § 2a(i)).

A number of published studies have incorporated specimens transported or stored in VTM. Although the use of VTM is expected to dilute the sample and is not recommended by the manufacturer, the published data indicate that VTM does not significantly interfere with detection of influenza antigens by the FLU OIA test. As noted by Hindeyeh *et al.* (2000), this offers the potential for collection of a single specimen from a patient for testing by both the FLU OIA test at the POC and transport to the laboratory for confirmatory culture or subtyping should this be required.

The proportion of influenza A and B virus specimens reported in most studies have been significantly skewed in

Table 5. FLU OIA performance characteristics in the 1998 clinical trial.

(Four hundred and four specimens were collected from 183 patients recruited at health-care providers in the Rocky Mountain, Midwest and southwestern regions of the USA. FLU OIA tests were performed at the POC by registered nurses or nursing students. Clinical laboratory FLU OIA results were obtained from samples transported in sterile containers and tests were performed by clinical laboratory staff. Performance of the FLU OIA test was assessed by reference to 14-day tissue culture performed according to published methods.)

specimen type	combined clinical laboratory and POC		POC only	
	sensitivity (%) ^a	specificity (%) ^a	sensitivity (%)	specificity (%)
nasal aspirate	88.4 (89.4)	69.4 (78.1)	90.0	63.6
nasopharyngeal swab	83.3 (86.2)	76.2 (83.7)	95.5	64.1
sputum	81.1 (84.1)	51.5 (65.4)	73.7	40.0
throat swab	62.1 (66.7)	79.5 (83.5)	80.0	70.5

^aWhere sufficient sample was available, FLU OIA test-positive, culture-negative specimens were tested by DFA and reverse transcriptase-PCR. Numbers in parentheses include these data (FLU OIA test versus combined culture, direct fluorescence assay (DFA) and reverse transcriptase-PCR).

Figure 3. Percentage of specimens influenza-positive by FLU OIA test or 14-day tissue culture from patients confirmed as influenza-positive by culture from any specimen type. Influenza virus was cultured from specimens provided by 92 out of the 183 patients (50%) recruited with influenza-like illness. Specimens collected from the 92 confirmed positive patients included 54 nasal aspirates (NA), 65 nasopharyngeal swabs (NPS), 56 throat swabs (TS) and 41 sputum (SP) samples. These specimens were tested by the FLU OIA test and 14-day tissue culture. The percentage of each specimen and test accurately predicting influenza infection in this known influenza-positive population was calculated from these data. FLU OIA data, filled bars; 14-day culture data, open bars. Asterisk, range bars indicate 95% confidence intervals.

favour of one or the other, making comparison of A and B results in a single study problematical. This presumably reflects the low level of influenza A and B co-circulation during the seasons and locales in which specimens were isolated. The majority of studies appear to have involved specimens principally from influenza A-infected patients. However, two reports have documented FLU OIA performance characteristics using specimens that were predominately influenza B positive (Mitamura *et al.* 1999; Yamazaki *et al.* 1999). In both cases the sensitivity and specificity of the FLU OIA test were consistent with the results for influenza A-positive specimens reported by others (table 6). Data obtained using cultured influenza A and B viruses also indicate that the FLU OIA test is not biased towards either A or B (table 1 and Watanabe *et al.* (1999)). In addition, on the basis of the limited co-circulation data available, it appears that the FLU OIA test may have a clinical detection limit for influenza B that is similar to that of influenza A virus (Herrmann

et al. 2001). Taken together, these data indicate that the FLU OIA test has equivalent performance characteristics for influenza A or B virus.

The clinical manifestations of influenza and viral shedding patterns are known to differ between adults and children (reviewed by Hayden & Palese 1997). Although the data available to date are limited and further study is warranted, it appears that the FLU OIA test may be *ca.* 10% more sensitive for paediatric specimens (Mulford *et al.* 1999; Lynch 2000).

3. DISCUSSION

POC testing has had a major impact on human health care and has helped revise management practices for a variety of human diseases (reviewed by Hesterberg *et al.* 1995; Hesterberg & Crosby 1996). In the case of influenza virus, treatment options have been limited until recently, which has probably moderated the level of interest in

rapid tests to confirm a clinical suspicion of influenza. Confirmatory laboratory diagnoses have been predominantly limited to surveillance networks and associated with subtyping in support of annual vaccine reformulation efforts. This work is an essential component of a comprehensive influenza management system, but it provides little immediate benefit for the infected patient or health-care provider because of the delays associated with confirmation of the clinical diagnosis. Nonetheless, a limited number of studies have shown that rapid laboratory-based confirmatory testing for influenza can confer substantial benefits for patients and the health-care system (Woo *et al.* 1997; Barenfanger *et al.* 2000).

With the advent of neuraminidase inhibitors, POC testing for influenza A and B virus is expected to play an increasingly important role in diagnostic and treatment decision-making (reviewed by Newton *et al.* 2000). A reliable diagnosis of influenza on clinical grounds alone is challenging under most circumstances, and particularly so during the early stages of an outbreak when intervention is likely to be most effective. Since laboratory tests are expensive, require expert staff and provide results that are too late to impact the doctor–patient interaction, there is a role for rapid, sensitive POC tests for influenza A and B to augment clinical impressions. Herein we have reviewed the performance characteristics of one such test, the FLU OIA test. The FLU OIA test is a unique POC test based on silicon chip biosensor-mediated detection of influenza A and B nucleoprotein. The test is broadly reactive with influenza A and B viruses and is used in a wide range of health-care organizations worldwide. It has been found to be compatible with a large number of specimen types, including mucoid specimens such as sputum, which can present problems for ‘dipstick’-type POC tests relying on chromatographic technology or flow-through membrane-based tests.

The data gathered to date indicate that the performance of the FLU OIA test, like any diagnostic test, is dependent on the quality of the specimen type. For example, throat swabs, which have been found to be a comparatively poor specimen for isolation of influenza virus by culture, are also poor performers in the FLU OIA. The best samples for the FLU OIA test have been found to be nasopharyngeal swabs, aspirates and sputa. Sensitivity of the test using the best specimens from studies which examined multiple specimen types is reported to range from 79 to 100% (mean 91%, table 6). However, it is recognized that flexibility in the choice of sample type is an important consideration for both health-care workers and patients. The overall high specificity of the FLU OIA test (84 and 94% for all sample types and sample types yielding highest specificity, respectively) confers confidence in a positive test result even in those circumstances where less than optimal specimens are available. This conclusion is reinforced by the observation that throat swabs are often listed as the specimen type yielding highest specificity (table 6).

Although the present review has focused on human health-care applications, the FLU OIA test also has relevance for the veterinary field. For example, H. G. G. Townsend, S. L. Moore (Department of Veterinary Medicine, University of Saskatchewan, SK, Canada S7N 5B4), J. R. Bogdan, D. M. Haines (Department of

Veterinary Microbiology, University of Saskatchewan), P. W. Dowling and J. S. Youngner (Department of Molecular Genetics and Biochemistry, School of Medicine, University of Pittsburgh, PA 15261, USA) (personal communication) have shown that the FLU OIA test is able to detect influenza in infected horses with high sensitivity (83%) and specificity (93%). The rapid detection of influenza in horses is important for effective outbreak control and the FLU OIA test is considered to be a cost-effective option for field-testing horses via their nasal secretions with significant benefits over slow, variable and costly laboratory diagnosis (Horse Health Lines 2000).

(a) POC tests and influenza management in the interpandemic and pandemic periods: a proposal

We propose that rapid influenza testing when combined with clinical judgement has an important role in human and veterinary medicine during the interpandemic period. Indeed, a recent report on the use of the FLU OIA test in clinical practice indicated that physicians found routine POC testing to be clinically useful in augmenting clinical impressions (Musgnug & Chapin 2000). Benefits described included improved patient satisfaction, informed discussion of intervention measures and increased incidence of appropriate therapy. From the institutional perspective, rapid diagnosis of influenza at the outset and during an outbreak provides a useful tool in the timely and informed application of appropriate disease-containment measures. A similar outcome is envisaged for veterinary applications of the FLU OIA test. All of these applications take advantage of the rapid feedback of a diagnostic result to the health-care worker afforded by a POC test.

POC testing when used as an adjunct to clinical judgement can lead to improved disease management; however, it should be emphasized that POC testing for influenza is not a replacement for routine laboratory culture for influenza surveillance and typing purposes. Although of little immediate benefit for the patient suffering from influenza, the individuals, institutions and government organizations performing this function play a vital role in influenza management on a community, national and international basis. Rather than supplanting this established network, we contend that the widespread use of POC tests provides an opportunity to extend awareness of influenza in the community and offers the potential for more diverse and cost-effective surveillance. For example, kits such as the FLU OIA test that are appropriate for ‘field use’ may be used to gather data on influenza incidence in remote locations or in communities that lack convenient access to clinical laboratories. In addition, the testing of samples by the FLU OIA test before shipment to the laboratory for tissue culture offers interesting options for increasing the frequency of influenza-positive specimens and reduced laboratory costs.

What might be the role of POC tests at the onset and during the next pandemic? In the event of an influenza pandemic it seems probable that demand for diagnostic confirmation of suspected cases will become widespread, and in those areas where laboratories are available they are likely to quickly become overloaded. POC tests are expected to prove useful in facilitating increased surveillance through patient screening prior to submission of

Table 6. Performance of FLU OIA in clinical practice.

(NPS, nasopharyngeal swab; TS, throat swab; DFA, direct fluorescence assay; NASP, nasal aspirate; TASP, tracheal aspirate; SPUT, sputum; IFA, indirect fluorescence assay; NWSH, nasal wash; BWSH, bronchial wash; BAL, bronchoalveolar lavage; MISC, miscellaneous; PPV, positive predictive value; NPV, negative predictive value.)

author	reference method	sample types	influenza type ^a	sensitivity (%)			specificity (%)			PPV (%)	NPV (%)	comments
				all samples	best sample	all samples	best sample	all samples	best sample			
Gander <i>et al.</i> (1998); University of Texas SW Medical Centre, Dallas, TX, USA	culture, DFA and RT-PCR	NPS, TS, NASP, SPUT	37/153 A; 23/153 B	76.7	95.5	92.5	97.6	92.5	97.6	86.8	86	sputum most sensitive (95.5%) and NPS most specific (97.6%); DFA sensitivity 41%
Green & Morris (1999); Hershey Medical Centre, Hershey, PA, USA	culture, and IFA	NPS, TS, NASP, TASP, SPUT, NWSH	— ^b	65.8	100	93.8	100	93.8	100	71.4	92.1	nasal and tracheal aspirates most sensitive (100%), TS least sensitive (0%) and NPS, TS and nasal aspirate most specific (100%); all specimens were frozen in VTM
Mulford <i>et al.</i> (1999); Washington University, St Louis, MO, USA	culture and IFA	paediatric NASP, NPS	—	81	100	89	93	89	93	—	—	NPS most sensitive (100%) and specific (93%) NPS FLU OIA performance equivalent to culture
		adult NPS, TS, SPUT	—	70	83	78	92	78	92	—	—	NPS and sputum most sensitive (83%), TS least sensitive (47%) and TS most specific (92%); adult NPS FLU OIA performance superior to culture; adult TS FLU OIA and paediatric NPS FLU OIA performance equivalent to culture
Longfellow <i>et al.</i> (1999); University of Colorado Health Science Centre, Boulder, CO, USA	culture, DFA and RT-PCR	MISC	—	86.9	—	69	—	69	—	74.6	83.3	FLU OIA cost considered roughly equivalent to DFA but DFA less sensitive (26.7%) and slower (1 h)
Turnbow <i>et al.</i> (1999); ARUP Laboratories and University of Utah Health Center, Salt Lake City, UT, USA	culture and DFA	TS, NWSH, SPUT, BWSH, MISC	—	56	—	95	—	95	—	—	—	
Yamazaki <i>et al.</i> (1999); Isehara Kyoda Hospital, Isehara, Japan	culture	NASP	all B	88.5	—	65.2	—	65.2	—	—	—	all specimens were influenza B
Mitamura <i>et al.</i> (1999); Nippon Kokan Hospital, Kawasaki, Japan	RT-PCR culture	NASP TS	5/75 A; 35/75 B	81.6 36.7	— 91.4	72.2 83.9	— 92.3	72.2 83.9	— 92.3	—	—	paediatric patients (largely influenza B)
		NASP TS (VTM)		80.0 50	—	68.8 91.7	—	68.8 91.7	—	—	—	paediatric patient samples frozen in VTM
		NASP (VTM)		91.4	—	92.3	—	92.3	—	—	—	

(Continued.)

Table 6. (Continued.)

author	reference method	sample types	influenza type ^a	sensitivity (%)		specificity (%)			PPV (%)	NPV (%)	comments
				all samples	best sample	all samples	best sample	best sample			
Watanabe <i>et al.</i> (1999); Kanagawa Prefectural Public Health Laboratory, Asahi, Japan	culture	NASP	—	89.7	—	76	—	—	—	—	detection limit for 25 human A, 12 human B, 2 swine and 3 avian strains were 3–6.5 × 10 ⁴ PFU per assay
Covalciu <i>et al.</i> (1999); ThermoBiosar, Inc., Boulder, CO, USA	culture	NPS, TS, NASP, SPUT	—	80.1	88.4	73.1	79.5	—	—	—	NASP most sensitive (88.4%), TS least sensitive (62.1%) and TS most specific (79.5%); FLU OIA equivalent to culture for all sample types examined
Lewis <i>et al.</i> (1999); Washington School of Medicine, St Louis, MO, USA	culture and IFA	Adult NSP, TS, SPUT, NASP	—	66	—	80	—	—	79	59	throat swabs and nasopharyngeal swabs were obtained from all subjects; sputum and nasopharyngeal aspirates were obtained where possible
Casciato <i>et al.</i> (2000); Providence Portland Medical Centre, Portland, OR, USA	culture and R-MIX shell vials	NPS, TS, NASP, SPUT, BAL	46/243 A no flu B reported	74.3	—	96.6	—	—	—	—	—
Lynch (2000); Fairview University Medical Centre, Minneapolis, MN, USA	culture and IFA	NPS, TS, NWSH, BAL	123/703 A; 1/703 B	77	—	84	—	—	—	—	87.5% sensitivity and 64% specificity when FLU OIA conducted at the principal surveillance site. FLU OIA exhibited 86% sensitivity and 89.5% specificity for paediatric samples
Musgnug & Chapin (2000); Lahey Clinic, Burlington, MA, USA	—	NPS	—	85	—	100	—	—	—	—	study conducted under POC conditions to measure outcome of test use on diagnosis and treatment
Hindeyeh <i>et al.</i> (2000); University of Utah Health Sciences Centre and ARUP Laboratories, Salt Lake City, UT, USA	culture and DFA	NPS, TS, NWSH, SPUT, BAL, MISC	47/145 A; 9/145 B	54	90	97	100	91	91	77	sputum most sensitive (90%) and specific (100%), TS least sensitive (25%); FLU OIA results not impaired by VTM
Herrmann <i>et al.</i> (2001); University Hospital, Uppsala, Sweden	culture, IFA and RT-PCR	NPS, NASP	79/184 A; 13/184 B	55.7	78.6	90.8	95.9	—	—	—	aspirates were collected in saline and concentrated by centrifugation (free virus may have been lost); sensitivity and specificity for NPS alone were 78.6 and 84.2%, respectively
Boivin <i>et al.</i> (2001); Centre Hospitalier University Quebec, Canada	culture and RT-PCR	TS	80/143 A; 0/143 B	54	—	74.1	—	—	—	—	—
		mean:		72	91	84	94				

^a Number of influenza A or B diagnoses confirmed by reference methods per specimen number.^b —, no data available (not provided in primary publication).

specimens for culture, reverse transcription polymerase chain reactions (RT-PCR), etc. (see, for example, A Framework for an Australian Influenza Pandemic Plan 1999). In communities that lack access to laboratories, POC testing may be the only option available. Because the simplicity of POC tests such as the FLU OIA test and their self-contained design is consistent with use by non-medical staff, surveillance activities could be extended to include non-traditional sampling sites and personnel. Indeed, it is conceivable that rapid POC tests could become a front-line tool with broad applications for disease management and containment not only in the health-care setting, but also in places such as farms, quarantine centres, military barracks, airports and other locations wherever an on-the-spot diagnosis is required.

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