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Original Article

Performance of influenza rapid point-of-care tests in the detection of swine lineage A(H1N1) influenza viruses

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Background In April 2009, an A(H1N1) influenza virus of swine lineage was detected in humans in the USA, and in just over a month has infected over 10 000 people in more than 40 countries.

Objectives To determine the performance of the Binax Now, BD Directigen EZ, and the Quidel QuickVue influenza rapid point-of-care (POC) tests for the detection of the recently emerged swine lineage A(H1N1) virus.

Methods Swine lineage A(H1N1) and human seasonal influenza strains were cultured and then diluted to specific infectivity titres. Viral dilutions were assayed by the rapid POC tests and by real-time RT-PCR.

Results All three of the rapid POC tests successfully detected the swine lineage A(H1N1) viruses at levels between 10^3 and 10^5 $TCID_{50}$/ml (tissue culture infectious $dose_{50}$), with the BD Directigen test demonstrating marginally greater sensitivity than the other two tests. Viral infectivity and RNA load data for viruses at the detection limit of the rapid test kits, suggested that both the Quidel and the Binax tests were less sensitive for the detection of swine lineage A(H1N1) viruses than for human seasonal strains. In comparison the BD Directigen demonstrated similar sensitivity when detecting swine lineage A(H1N1) and human seasonal viruses.

Conclusions The three rapid POC tests all detected the emergent swine lineage A(H1N1) virus when it was present at high virus concentrations. Early diagnosis of infection can assist in the rapid treatment. However the tests are significantly less sensitive than PCR assays and as such, negative results should be verified by a laboratory test.

Keywords Immunoassays, point-of-care, rapid tests, swine origin influenza A(H1N1).

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Introduction

Although influenza virus was first isolated from pigs in 1930,[1] it was not until 1974 that swine influenza viruses were confirmed to infect humans.[2] Since that time there have been numerous reports of human infections with swine influenza viruses,[3,4] including triple-reassortant swine viruses which contain gene segments originally derived from human, swine and avian influenza viruses.[5] In April 2009, a novel A(H1N1) influenza virus caused outbreaks in humans both in Mexico and the USA.[6,7] Analysis of the viruses from these cases revealed that six gene segments were derived from the previously described North American triple-reassortant virus, while the remaining segments (neuraminidase and matrix) were most similar to Eurasian swine viruses. In just over a month from the first detected case in the USA (15th April 2009), the swine lineage A(H1N1) virus had spread to over 40 countries with more than 10 000 reported cases,[8] causing the WHO to raise the global pandemic alert to phase 5, only one phase short of declaring a pandemic. The mortality rate following infection with the virus, as of 21st May 2009, has been significantly higher in Mexico (2·0%; 3648 cases and 72 deaths), compared to the USA (0·1%; 5469 cases and six deaths).[8]

In an effort to slow the spread of the virus, countries have endeavoured to achieve early detection of infected patients and implement quarantine and contact tracing measures. Newly designed real-time PCR assays for the specific detection of swine lineage A(H1N1) influenza have been implemented by specialist influenza laboratories, and are currently considered the 'gold-standard' for confirming cases of swine influenza infection. However, such assays require a high level of expertise to perform, and often specimens can take many days to reach the laboratory for testing, particularly if they are being shipped from remote locations.

For cases where there is a high level of suspicion that a symptomatic patient is infected with swine influenza, either as a result of travel to an affected area or contact with a confirmed case, there are significant benefits of an early influenza diagnosis, both for the patient's wellbeing and for prevention of spread of the virus throughout the community. In addition, an early diagnosis enables the immediate administration of a neuraminidase inhibitor, such as oseltamivir, which is most effective if administered within 48 hours post-infection.[9]

Currently the fastest diagnostic tools for the detection of influenza viruses are rapid point-of-care (POC) tests which can generate a result in 15 minutes or less.[10] Most influenza rapid POC tests are immunoassays, where clinical specimens are applied to a lateral flow strip that contains monoclonal antibodies against influenza A or B viruses. The presence or absence of viral antigen is indicated by a colour change on the test strip, allowing a physician to test, diagnose and then offer the appropriate treatment before the patient leaves the surgery. The major drawback of the rapid POC tests is their relatively poor sensitivity, usually in the range of 60–70% for influenza A, compared to traditional laboratory-based assays such as cell culture or real-time PCR.[11] A further weakness of the rapid POC influenza tests is their inability to differentiate between influenza A subtypes.[10] Nevertheless, in combination with a subsequent confirmatory PCR assay, rapid POC tests have become a useful tool, particularly in closed communities such as nursing homes, to enable early antiviral drug treatment. We have therefore evaluated the performance of three widely available rapid POC tests in detecting the new swine lineage A(H1N1) virus.

Methods

Viruses

Viruses A/Auckland/1/2009 A(H1N1) swl (swine lineage) and A/Auckland/3/2009 A(H1N1) swl were isolated from clinical specimens submitted to the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, by Auckland Hospital, New Zealand. The viruses were confirmed by HA, NA and M sequence analysis (sequences available on GenBank) to be similar to the A(H1N1) reference strain A/California/4/2009 A(H1N1) swl. The reference strain A/California/4/2009 A(H1N1) swl was kindly provided by CDC, Atlanta, USA. Human seasonal strains A/Brisbane/59/2007 A(H1N1), A/Philippines/2676/2007 A(H1N1), and A/Perth/27/2007 A(H3N2) were submitted to the WHO Collaborating Centre as part of the WHO Global Influenza Surveillance Network (GISN).

Cell culture and viral infectivity

All viruses were isolated and passaged in Madin-Darby canine kidney (MDCK) cells (American Type Culture Collection (CCL-34)) maintained in maintenance medium (DMEM (Dulbecco's Modified Eagle's Medium) Coon's basal medium containing sodium bicarbonate (3%) with the addition of 2 mm glutamine, 1% non-essential amino acids, 0·05% $NaHCO_3$, 0·02 M HEPES, 4% penicillin and streptomycin, 2 μg/ml amphotericin B and 4 μg/ml trypsin (CSL Biotherapies)). To determine the viral infectivity titre for each of the strains MDCK cells were seeded into 96-well plates (Cellstar; Greiner Bio-one, Frichenhausen, Germany) ($1{\cdot}5 \times 10^4$ cells per well) and grown to near confluence overnight at 37°C, in a 5% CO_2 incubator. Monolayers were washed twice with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline before inoculation with six replicates of 10-fold dilutions of each virus. After incubation for 4 days at 35°C in 5% CO_2, each well was scored for virus growth by cytopathic effect and haemagglutination of turkey red blood cells and the dose required to infect 50% of wells ($TCID_{50}$, tissue culture infectious $dose_{50}$) determined by the Reed–Muench method.[12] Dilutions of the virus were prepared in maintenance medium, and were tested for the detection of influenza A virus by rapid POC test and real-time RT-PCR. All culture and handling of swine lineage A(H1N1) strains was completed at biosecurity level BSL2 with enhanced personal protective equipment.[13]

RNA extraction and real-time RT-PCR assay

Two hundred microlitre of each viral dilution was lysed with MagnaPure extraction buffer and RNA was extracted using the MagnaPure extraction kit and robot (Roche Indiana, USA) according to the manufacturer's protocols. Real-time RT-PCR detection was performed using SuperScriptTM III Platinum® One-Step qRT-PCR System (Invitrogen, California, USA), utilizing the influenza A matrix primers and probe as supplied by CDC, Atlanta, USA,[7] on an Applied Biosystems 7500 Real-Time PCR-System. The cycle threshold (Ct) value derived by a real-time PCR assay provides a relative quantitation of the amount of viral RNA present in each of the sample dilutions and is defined as the cycle number at which the fluorescence generated within a reaction crosses a set threshold. Therefore the Ct value assigned to a virus reflects the point during the reaction at which a sufficient number of amplicons have accumulated to be at a statistically significant point above the baseline. The Ct value is inversely proportional to the number of RNA copies in the sample.

Rapid POC tests

Three influenza rapid POC tests, Binax Now Influenza A&B (Inverness Medical, Waltham, MA, USA), BD Directigen EZ Flu A+B (BD EZ; Sparks, MD, USA) and Quidel QuickVue Influenza A+B Test (Quidel, San Diego, CA, USA), were purchased and evaluated for their ability to detect influenza A antigen by following each of the manu-

facturer's own protocols. The rapid POC tests were performed in singular on cell culture supernatant diluted in maintenance media to a log range of viral titre (10^6–10^1 $TCID_{50}$/ml). Based on the singular results following analysis of the log dilutions, half-log dilutions were prepared around the point of detection and viruses were retested by rapid POC test in duplicate. All tests were read by two operators.

Results

Analysis by the three rapid POC tests demonstrated that viral concentrations of 10^3–10^5 $TCID_{50}$/ml were necessary for the detection of the swine lineage A(H1N1) strains, whereas human seasonal influenza strains could be detected at viral titres between 10^2 and 10^3 $TCID_{50}$/ml (Table 1). Based on the limit of detection of the rapid POC tests in

Table 1. Relative performance of three influenza rapid POC tests in the detection of swine lineage A(H1N1) and human seasonal A(H3N2) and A(H1N1) influenza viruses

Influenza virus		Viral titre in sample tested ($TCID_{50}$/ml)	Rapid POC test			Real-time RT-PCR Ct value*
			Quidel QuickVue	BD Directigen EZ	Binax Now	
Swine lineage A(H1N1) viruses	A/Auckland/1/2009 A(H1N1) swl	10^6	++	++	++	NT
		10^5	++	++	++	22·5
		10^4	–	+	–	26·6
		10^3	–	–	–	30·2
		10^2	–	–	–	33·3
		10^1	–	–	–	36·4
	A/Auckland/3/2009 A(H1N1) swl	10^6	++	++	++	NT
		10^5	+	++	+	24·1
		10^4	–	–	–	28·3
		10^3	–	–	–	31·7
		10^2	–	–	–	34·2
		10^1	–	–	–	Negative
	A/California/4/2009 A(H1N1) swl	10^6	++	++	++	NT
		10^5	++	++	++	14·8
		10^4	++	++	++	20·4
		10^3	–	++	–	25·4
		10^2	–	–	–	29·5
		10^1	–	–	–	33·4
Human seasonal viruses	A/Brisbane/59/2007 A(H1N1)	10^6	++	++	++	NT
		10^5	++	++	++	15·5
		10^4	++	++	++	20·8
		10^3	+	+	+	25·4
		10^2	–	–	–	29·9
		10^1	–	–	–	33·5
	A/Philippines/2676/2007 A(H1N1)	10^6	NT	NT	NT	NT
		10^5	++	++	++	14·4
		10^4	++	++	++	18·7
		10^3	++	++	++	24·5
		10^2	+	+	–	28·8
		10^1	–	–	–	32·1
	A/Perth/27/2007 A(H3N2)	10^6	NT	NT	NT	NT
		10^5	++	++	++	14·2
		10^4	++	++	++	20·3
		10^3	+	++	+	25·1
		10^2	–	–	–	29·1
		10^1	–	–	–	31·9

*Mean Ct of duplicate real-time RT-PCR results.
++ indicates a strong positive result that was clearly visualized when held at a distance of 1 m from the eye.
+ indicates a weak positive that required close examination to determine the presence of a band.
NT, not tested; Ct, cycle threshold; POC, point-of-care.

Table 2. Limit of detection of three influenza rapid POC tests for the swine lineage A(H1N1) and human seasonal A(H1N1) influenza viruses

Influenza virus		Viral titre in sample tested ($TCID_{50}$/ml)	Rapid POC test					
			Quidel QuickVue		BD Directigen EZ		Binax Now	
			Rep1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Swine lineage A(H1N1) viruses	A/Auckland/1/2009 A(H1N1) swl	10^5	++	++	++	++	++	++
		$10^{4.5}$	+	+	++	++	+	–
		10^4	–	–	+	+	–	–
		$10^{3.5}$	–	–	–	–	–	–
		10^3	NT	NT	–	–	–	–
	A/California/4/2009 A(H1N1) swl	10^4	++	++	++	++	++	++
		$10^{3.5}$	+	+	++	++	+	–
		10^3	–	–	++	+	–	–
		$10^{2.5}$	–	–	–	–	–	–
		10^2	NT	NT	–	–	–	–
Human seasonal virus	A/Brisbane/59/2007 A(H1N1)	10^4	++	++	++	++	++	++
		$10^{3.5}$	+	+	++	++	+	+
		10^3	+	+	+	+	+	–
		$10^{2.5}$	–	–	–	–	–	–
		10^2	NT	NT	–	–	–	–

++ indicates a strong positive result that was clearly visualized when held at a distance of 1 m from the eye.
+ indicates a weak positive that required close examination to determine the presence of a band.
NT, not tested; Rep, replicate; POC, point-of-care.

these analyses (Table 1), half-log dilutions were prepared for two of the swine lineage A(H1N1) viruses and one of the human seasonal A(H1N1) strains and tested in duplicate by the three rapid POC tests. The A/Auckland/1/2009 swl strain was detected at a viral titre of 10^4 $TCID_{50}$/ml by the BD Directigen EZ test, although a slightly higher titre of $10^{4.5}$ $TCID_{50}$/ml was necessary for the Quidel QuickVue and Binax Now tests to achieve an influenza A positive result (Table 2). The BD Directigen EZ test detected the A/California/4/2009 swl strain at a viral titre one log lower than the A/Auckland/1/2009 swl strain, and again was approximately half a log more sensitive than the Quidel QuickVue and Binax Now tests. All of the rapid POC tests detected the human seasonal A/Brisbane/59/2007 A(H1N1) strain at a titre of 10^3 $TCID_{50}$/ml. No clear difference in sensitivity was seen between the rapid POC tests for the detection of either this strain (Table 2) or the other two human seasonal strains (Table 1).

Apart from the A/Auckland/3/2009 swl 10^1 $TCID_{50}$/ml sample, the real-time RT-PCR assay detected the full range (10^1–10^6 $TCID_{50}$/ml) of viral titres for all strains (Table 1). Because real-time RT-PCR can be used to quantitate viral RNA, the real-time RT-PCR Ct values were correlated with the ability of the rapid POC tests to detect the presence of influenza A antigen (Figure 1). Cycle threshold values for the swine lineage A(H1N1) virus dilutions that were at the limit of detection of the BD Directigen EZ test ranged from 24·1 to 26·6, indicating that any virus with a Ct value lower than this range (ie, with more virus present in the sample) would be expected to be detected by the rapid POC test, while a virus with a higher Ct value may not be detected. Comparison with the Ct values from the three human seasonal strains at the detection limits of the same POC test, indicates that the BD Directigen EZ kit had similar sensitivity for detecting swine lineage A(H1N1) viruses (mean Ct ± SD, 25·4 ± 1·3) compared with human seasonal A(H1N1) or (H3N2) viruses (mean Ct ± SD, 26·4 ± 2·1) (Figure 1). However both the Quidel QuickVue and the Binax Now kits could detect human seasonal strains at a higher mean Ct value (lower viral RNA load) than the swine lineage strains (Figure 1), supporting the viral infectivity data which found that the sensitivity of these kits was marginally higher for the human seasonal strains than for the swine lineage A(H1N1) viruses. Analysis of 10 clinical specimens from patients infected with swine lineage A(H1N1) virus gave Ct values ranging from 18–35·5 (Figure 1) using the same influenza A (matrix gene) real-time RT-PCR assay as used to assay the cell culture dilutions. Comparing these Ct values to the limit of detection for the most sensitive rapid POC test, the BD Directigen EZ (mean Ct of 25), it is likely only 3 of the 10 specimens would have been detected.

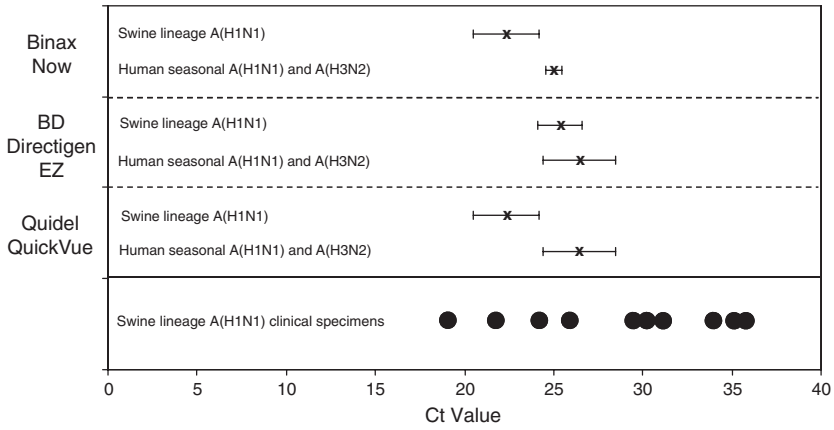

Figure 1. Comparison of the sensitivity of rapid point-of-care (POC) tests with viral RNA levels in swine lineage A(H1N1) virus-positive clinical specimens. Mean cycle threshold (Ct) values ± standard deviations were derived from the Ct values determined by real-time RT-PCR for either the three swine lineage viruses or the three human seasonal strains at the detection limit for the respective rapid POC test. Circles indicate the Ct values (derived from the same influenza A matrix real-time RT-PCR assay as used for the other experiment) for 10 different clinical specimens that were swine lineage A(H1N1) virus-positive.

Discussion

The majority of rapid POC influenza tests have been designed to detect seasonal influenza viruses (A(H1N1), A(H3N2) and influenza B) and only some have been tested against a wide range of influenza A subtypes (eg, Quidel QuickVue), while others have had limited testing (eg, Binax Now: A(H5), A(H7)) or very little testing (eg, BD Directigen EZ) (see package inserts). When influenza rapid POC tests (BD Directigen Flu A+B, x/pect Flu A+B) have been independently tested in the past against non-human seasonal influenza strains such as A(H5N1) (A/Vietnam/1203/2004), they have performed poorly compared to seasonal influenza A viruses, only detecting virus when it was present at high concentrations (10^6 $TCID_{50}$/ml).[14] This low sensitivity has also been reported in other studies using clinical samples containing A(H5N1) viruses.[15] The detection limit for a previous swine A(H1N1) influenza virus, A/New Jersey/8/76, reported within the package inserts from the three kits ranged from 10^2 to 10^3 pfu or $CEID_{50}$/ml, compared with the newly emergent swine lineage A(H1N1) strain which could only be detected at viral titres of 10^4–$10^{4.5}$ $TCID_{50}$/ml, although the assay methods used to quantify virus concentrations in these comparisons differed (pfu, $CEID_{50}$ and $TCID_{50}$).

Most of the rapid POC tests are based on antibodies to influenza nucleoprotein (NP) which is relatively conserved. However there is some genetic variability in the NP gene between strains, with a 7–8% amino acid difference between human seasonal A(H1N1) and A(H3N2) viruses, and approximately a 10% difference between the NP genes of swine lineage A(H1N1) and human seasonal A(H1N1) viruses. Because the antibodies used in the rapid POC tests have not been mapped it is not possible to predict how these individual sequence changes may impact on the antigen–antibody interaction and therefore the sensitivity of the various tests.

Many factors such as type and quality of the specimen, time of specimen collection post-infection and level of viral shedding can influence the performance of the rapid POC tests. Unlike the A(H5N1) human infections where throat or lower respiratory tract samples contained a higher viral load than nasal swabs,[15] preliminary data relating to human infections with the swine lineage A(H1N1) virus suggests that the preferred specimens for diagnostic testing (including rapid POC tests) are the traditional ones such as nasopharyngeal swabs and nasal swabs.[7] While the peak concentrations of virus in nasopharyngeal samples at 48 hours post-infection with human seasonal influenza has been found to be between 10^3 and 10^7 $TCID_{50}$/ml,[16] these levels have not been established for the emergent swine lineage A(H1N1) viruses, nor has the period of virus shedding been clearly determined.

A recent report suggests that children and young adults seem to be more commonly infected with the swine lineage A(H1N1) virus than the elderly[7] which, given the increased duration and high level of viral shedding associated with these age groups,[17,18] may improve the performance of the rapid POC tests.[11,19,20] Analysis of clinical specimens from patients infected with swine lineage A(H1N1) virus influenza in the current study revealed a wide range of viral loads in nasal swabs when measured by real-time RT-PCR. When compared to the detection limits of the rapid POC tests, it is probable that only 20–40% of these would have been detected. While this level of detection may improve as a result of increased awareness of clinical symptoms, and therefore earlier sampling when viral loads are likely to be higher, it highlights the need for more sensitive methods such as RT-PCR to be used for the unequivocal determination of infection with swine lineage A(H1N1) influenza.[21] Further evaluation of the rapid POC tests to determine their sensitivity and specificity with clinical samples of the swine lineage A(H1N1) virus in a clinical setting is essential.

It is hoped that the next generation rapid POC tests will address the sensitivity issues as well as allowing influenza A subtyping for existing and emerging influenza viruses that are infecting humans. Nevertheless rapid POC tests may still prove useful to investigate outbreaks of influenza-like illness in the community so that, if influenza A is detected, containment or treatment can be prescribed until it can determined whether the virus is an A(H1N1) swine lineage strain or a human seasonal A(H1N1) or A(H3N2) virus. Unfortunately if negative results are obtained with the rapid POC tests, influenza A (or influenza B) can not be confidently excluded and further testing would be recommended.

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References

1 Shope RE. Swine influenza: I. Experimental transmission and pathology. J Exp Med 1931; 54:349–359.
2 Smith TF, Burgert EO Jr, Dowdle WR *et al.* Isolation of swine influenza virus from autopsy lung tissue of man. N Engl J Med 1976; 294:708–710.
3 Komadina N, Roque V, Thawatsupha P *et al.* Genetic analysis of two influenza A (H1) swine viruses isolated from humans in Thailand and the Philippines. Virus Genes 2007; 35:161–165.
4 Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. Clin Infect Dis 2007; 44:1084–1088.
5 Shinde V, Bridges CB, Uyeki TM *et al.* Triple-reassortant swine influenza A (H1) in humans in the United States, 2005–2009. N Engl J Med 2009; Available at: http://www.ncbi.nlm.nih.gov/pubmed/19423871. (Accessed 21 May 2009).
6 Fraser C, Donnelly CA, Cauchemez S *et al.* Pandemic potential of a strain of influenza A (H1N1) : early findings. Science 2009; Available at: http://www.sciencemag.org/cgi/content/abstract/1176062. (Accessed 21 May 2009).
7 Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; Available at: http://www.ncbi.nlm.nih.gov/pubmed/19423869. (Accessed 21 May 2009).
8 WHO. Available at: http://www.who.int/csr/don/2009_05_17/en/index.html accessed on 21 May 2009. Website 2009.
9 Aoki FY, Macleod MD, Paggiaro P *et al.* Early administration of oral oseltamivir increases the benefits of influenza treatment. J Antimicrob Chemother 2003; 51:123–129.
10 Storch GA. Rapid diagnostic tests for influenza. Curr Opin Pediatr 2003; 15:77–84.
11 Hurt AC, Alexander R, Hibbert J *et al.* Performance of six influenza rapid tests in detecting human influenza in clinical specimens. J Clin Virol 2007; 39:132–135.
12 Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Hyg 1938; 27:493–497.
13 WHO. Available at: http://www.who.int/csr/resources/publications/swineflu/LaboratoryHumanspecimensinfluenza/en/index.html accessed 21 May 2009. Website 2009.
14 Fedorko DP, Nelson NA, McAuliffe JM *et al.* Performance of rapid tests for detection of avian influenza A virus types H5N1 and H9N2. J Clin Microbiol 2006; 44:1596–1597.
15 Abdel-Ghafar AN, Chotpitayasunondh T, Gao Z *et al.* Update on avian influenza A (H5N1) virus infection in humans. N Engl J Med 2008; 358:261–273.
16 Murphy BR, Baron S, Chalhub EG *et al.* Temperature-sensitive mutants of influenza virus. IV. Induction of interferon in the nasopharynx by wild-type and a temperature-sensitive recombinant virus. J Infect Dis 1973; 128:488–493.
17 Frank AL, Taber LH, Wells CR *et al.* Patterns of shedding of myxoviruses and paramyxoviruses in children. J Infect Dis 1981; 144:433–441.
18 Hall CB, Douglas RGJ, Geiman JM *et al.* Viral shedding patterns of children with influenza B infection. J Infect Dis 1979; 140:610–613.
19 Ruest A, Michaud S, Deslandes S *et al.* Comparison of the Directigen Flu A+B Test, the QuickVue influenza test, and clinical case definition to viral culture and reverse transcription-PCR for rapid diagnosis of influenza virus infection. J Clin Microbiol 2003; 41:3487–3493.
20 Cazacu AC, Chung SE, Greer J *et al.* Comparison of the directigen flu A+B membrane enzyme immunoassay with viral culture for rapid detection of influenza A and B viruses in respiratory specimens. J Clin Microbiol 2004; 42:3707–3710.
21 WHO. Available at: http://www.who.int/csr/resources/publications/swineflu/diagnostic_recommendations/en/index.html. Website 2009. (Accessed 21 May 2009).

