



Short communication

Analytical sensitivity of rapid influenza antigen detection tests for swine-origin influenza virus (H1N1)

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ABSTRACT

Background: A novel swine origin influenza virus (S-OIV) (H1N1) is spreading worldwide and threatens to become pandemic.**Objectives:** Determine analytical sensitivity of selected commercially available rapid influenza antigen detection tests in detecting S-OIV H1N1.**Study design:** Serial dilutions of two S-OIV isolates, one seasonal influenza A (H1N1) isolate and a nasopharyngeal aspirate from a patient with S-OIV disease were tested in five commercially available influenza antigen detection tests and by virus isolation in cell culture. Viral M gene copy number was determined by quantitative PCR methods.**Results:** The analytical sensitivity of the five influenza antigen detection tests for S-OIV (tissue culture infectious dose 50 (TCID₅₀) log₁₀ 3.3–4.7) was comparable with that of seasonal influenza (TCID₅₀ log₁₀ 4.0–4.5).**Conclusion:** The analytical sensitivity of the selected influenza A antigen detection tests for detection of S-OIV was comparable with that of seasonal influenza H1N1.

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1. Background

A novel influenza A (H1N1) virus emerged in Mexico in early 2009 and has to date caused 9830 human infections with 79 deaths (<http://www.who.int/csr/don/2009.05.19/en/index.html>). The virus is transmitting from human-to-human at least as efficiently as seasonal influenza viruses.¹ While human disease outside Mexico appears to be relatively mild, there is still significant morbidity and hospitalization, especially in those with underlying diseases.²

RT-PCR remains the method of choice for clinical diagnosis of S-OIV H1N1 virus in respiratory specimens and for differentiating it from seasonal influenza viruses (http://www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf). However, such tests are of high-complexity and cannot be readily performed in primary health care settings. It is therefore necessary to establish whether currently commercially available rapid antigen detection tests for influenza A can detect S-OIV as efficiently as they detect seasonal

influenza viruses. These tests do not differentiate between S-OIV and seasonal influenza A or even between subtypes H1 and H3, but they provide a rapid diagnosis of influenza A or B infection to aid clinical management. Such tests have moderate clinical sensitivity in detection seasonal influenza infections.^{3,4} Many are membrane immunoassay tests that target the virus nucleoprotein. S-OIV is believed to have arisen through the reassortment of two of more swine influenza viruses related to the H1N1 or H1N2 triple reassortant viruses of swine previously reported in North America and viruses belonging to the Eurasian swine (H1N1) lineage⁵ and the nucleoprotein of S-OIV appears to be derived from classical swine H1N1 viruses that are found in North America and Asia.

2. Objectives

To compare the analytical sensitivity of selected commercially available rapid influenza antigen detection tests in detecting S-OIV H1N1 and seasonal influenza A H1N1.

3. Study design

3.1. Viruses

We used two isolates of S-OIV, A/California/4/09 (H1N1) isolated in the USA in April 2009 and A/HK/415742/09 (H1N1) isolated

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Table 1
Analytical sensitivity of influenza antigen detection tests on seasonal influenza A H1N1 and swine origin influenza (S-OIV) A (H1N1) virus cultures.

Influenza antigen detection test	Log ₁₀ detection limit								
	S-OIV A/HK/415742/09 (H1N1) ^a			S-OIV A/California/4/09 (H1N1) ^a			Seasonal influenza A/HK/403946/09 (H1N1) ^a		
	Dilution of virus culture	TCID ₅₀ /mL	M gene copies/mL	Dilution of virus culture	TCID ₅₀ /mL	M gene copies/mL	Dilution of virus culture	TCID ₅₀ /mL	M gene copies/mL
QuickVue A + B	3.0	3.5	6.3	3.0	4.5	7.1	3.0	4.5	7.1
Directigen EZ A + B	3.2	3.3	6.1	3.3	4.2	6.8	3.2	4.3	6.9
Binax NOW A + B	2.5	4.0	6.8	2.8	4.7	7.3	3.0	4.5	7.1
Espline	3.0	3.5	6.3	3.5	4.0	6.6	3.5	4.0	6.6
Wondfo	3.2	3.3	6.1	3.6	3.9	6.5	3.2	4.3	6.9

^a The undiluted virus culture aliquots had the following TCID₅₀ and M gene copy titres. A/HK/415742/09 (H1N1): TCID₅₀ log₁₀ 6.5/mL; M gene copies log₁₀ 9.3/mL. A/California/4/09 (H1N1): TCID₅₀ log₁₀ 7.5/mL; M gene copies log₁₀ 10.1/mL. A/HK/403946/09 (H1N1): TCID₅₀ log₁₀ 7.5/mL; M gene copies log₁₀ 10.1/mL.

in Hong Kong in May 2009. For comparison, we used a seasonal influenza A isolate A/HK/403946/09 (H1N1). The viruses were cultured in Mardin Darby Canine Kidney (MDCK) cells and aliquots were frozen at -80°C .

3.2. Test evaluation

The rapid antigen detection tests evaluated were QuickVue influenza A + B (Quidel Corporation, CA, USA), BinaxNow Influenza A + B (Emergo Europe, The Netherlands), Directigen EZ Flu A + B (Becton Dickinson), Espline influenza A & B, N (Fujirebio Inc, Tokyo) and Wondfo (Wondfo Biotech Co., Ltd, Guangzhou, PR China). All these tests were carried out according to the manufacturer's instructions. An aliquot of each virus was thawed and serial 10-fold dilutions of the virus stock were tested. Further twofold dilutions were done in duplicate from the end point above, to determine the limit of detection of each rapid antigen test more accurately. The same virus dilutions were also inoculated onto MDCK cells to determine TCID₅₀. The viral M gene genome copy number of each virus preparation was determined using quantitative RT-PCR methods.⁶

3.2.1. Clinical specimens

Serial dilutions of a nasopharyngeal aspirate (NPA) specimen of one an S-OIV patient collected on the third day of illness was also tested in these rapid diagnostic assays. The M gene viral load of the original specimen was quantitated for comparison.

4. Results and discussion

The limit of detection of each rapid antigen test for the two S-OIV and one seasonal influenza A virus is shown in Table 1 expressed as lowest TCID₅₀ virus dose required for detection. It is recognized that the antigen targeted by these assays is the virus nucleoprotein. However, since the M gene copy number is commonly used for detection and quantification of influenza A virus in RT-PCR tests, it provides a clinically relevant reference-point for the relative analytical sensitivity of different tests. We therefore quantified the M-gene copy number in each virus preparation and calculated the expected M gene copies in the highest virus dilution that remained detectable in the rapid antigen test. The limit of detection of the different antigen detection tests for seasonal influenza A/HK/403946/09 (H1N1) virus ranged from log₁₀ TCID₅₀ 4.0 to 4.5 and M gene copy numbers of log₁₀ 6.5–7.1. The limit of detection of the swine-origin influenza H1N1 isolate A/California/4/09 ranged from log₁₀ 3.9–4.7 TCID₅₀ and that of A/HK/415742/09 (H1N1) ranged from log₁₀ 3.3 to 4.0. The comparable limits of detection in relation to M gene copies for the two viruses was log₁₀ 6.5–7.3 and log₁₀ 6.1–6.8, respectively.

While there may be marginal differences in the sensitivity of different test kits evaluated, we have not done sufficient replicates to

ascertain whether these inter-test differences are statistically significant. We and others have previously evaluated the analytical sensitivity of QuickVue influenza A + B (Quidel Corporation, CA, USA), BinaxNow Influenza A + B (Emergo Europe, The Netherlands), Directigen EZ Flu A + B (Becton Dickinson) for seasonal influenza viruses H1N1 and H3N2 and for avian influenza viruses H5N1. The TCID₅₀ detection limit of for H3N2 and H1N1 seasonal influenza viruses in our previous study⁷ are comparable with the results reported in the present study. Taken together, we can conclude that the rapid antigen tests we have evaluated in this study have comparable sensitivity for detection of S-OIV and seasonal influenza viruses.

Data on analytical sensitivity for detection of different viruses does not directly reflect clinical sensitivity on patient specimens. For example, while the analytical sensitivity some of these tests for the detection of avian influenza H5N1 was similar to that of seasonal influenza⁷ the performance of these tests on clinical specimens was poor.⁸ This may be related to differences in viral load in the upper respiratory tract of patients with avian influenza H5N1 infection compared to seasonal influenza⁹ and may in turn be related to differences in tropism of these viruses to the upper and lower respiratory tract.¹⁰ Our preliminary understanding of S-OIV disease suggests that it is primarily an upper (rather than lower) respiratory illness. Thus, given the comparable analytic sensitivity of rapid antigen tests for S-OIV and seasonal influenza, one may expect their sensitivity and specificity for this novel virus to be comparable to that seen with seasonal influenza. In hospitalized patients in California, 16 of 21 patients tested positive in rapid influenza antigen tests although the authors note that both false positives and negatives were found.²

Serial dilutions of a NPA specimen from a patient with S-OIV disease collected on day three of illness was tested in the panel of rapid antigen detection tests. The specimen (which had a M gene viral load of log₁₀ 8.5) remained detectable by these assays up to dilutions of log₁₀ 1.7–2.7 of the clinical specimen (i.e. M gene viral load log₁₀ 5.8–6.8) (Table 2). Thus detection limits of rapid anti-

Table 2
Limit of detection of influenza A antigen detection tests on a nasopharyngeal aspirate of a patient with swine origin influenza virus H1N1 disease.

Influenza antigen detection test	Log ₁₀ detection limit		
	Dilution of specimen ^a	TCID ₅₀ /mL	M gene copies/mL
QuickVue A + B	2.0	3.5	6.5
Directigen EZ A + B	2.4	3.1	6.1
Binax NOW A + B	1.7	3.8	6.8
Espline	2.7	2.8	5.8
Wondfo	2.7	2.8	5.8

^a The NPA specimen had a TCID₅₀ titre of log₁₀ 5.5 and M gene copy number of log₁₀ 8.5/mL.

gen tests on this clinical specimen was comparable to that seen in the cell cultured virus. The nasopharyngeal aspirate specimen from another S-OIV patient was only tested undiluted in the Directigen EZ A + B test kit and was negative. As this undiluted specimen had a M gene virus load of $\log_{10} 6$ which is at the lower range of the analytical sensitivity of these tests, the inability to detect this specimen by rapid antigen tests is not unexpected.

In summary, we find that the analytical sensitivity of the selected influenza A antigen detection tests for detection of S-IOV was comparable with that of seasonal influenza H1N1. The clinical sensitivity of these tests for S-OIV infected patients is likely to be comparable to that of seasonal influenza but more clinical studies are needed.

Conflicts of interest

None.

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