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Title: A rapid test for the detection of influenza A virus including pandemic influenza A/H1N1 2009

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1 **A rapid test for the detection of influenza A virus including pandemic influenza A**  
2 **/ H1N1 2009**

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8 Running title: Rapid test for influenza A

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1 **Abstract**

2 A new rapid diagnostic test for detection of influenza A virus was evaluated with  
3 four sets of experiments: first, a comparison with a commercial diagnostic kit against  
4 a panel of virus strains was conducted; second, the kit was tested against a collection  
5 of 40 strains of influenza A virus isolated from five different host species and 26  
6 strains of other respiratory viruses used as controls; third, the kit was tested against  
7 specimens collected in the field obtained from human and chicken; and fourth, the kit  
8 was tested against the novel pandemic influenza A / H1N1 2009 clinical specimens  
9 obtained from admitted to hospital patients. The test kit displayed a sensitivity of 88%  
10 for both human specimens and avian specimens. The corresponding specificity was  
11 99.3% for human specimens and 96.5% for avian specimens. This test kit may be  
12 useful for rapid diagnosis of influenza A virus.

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14 **Key words:** influenza A; nucleoprotein; rapid test; Dot-ELISA

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1           Rapid tests for influenza are useful for the assessment of influenza-like illness in  
2 the clinic. Additionally, such tests can serve as a tool for disease surveillance  
3 (Pachucki, 2005). Indeed, the current epidemic of swine-origin influenza A virus  
4 H1N1 (S-OIV H1N1) (Dawood et al., 2009) now designated as pandemic influenza A  
5 / H1N1 2009 (WHO, 2009) has highlighted the need of rapid influenza diagnostic  
6 tests to facilitate early treatment of infected individuals.

7           Current rapid tests for influenza A virus are usually produced with the  
8 monoclonal antibody against nucleoprotein (NP) of 498 amino acids length encoded  
9 by influenza A virus RNA segment 5 (Yang et al., 2008). The antigenic specificity of  
10 NP is related to host species and divided into at least five distinct host-specific groups,  
11 namely, human, avian, swine, equine, and gull (Gorman et al., 1990). However,  
12 influenza A / H1N1 2009 is different from the common human influenza A virus  
13 because it contains genes from avian, human, and swine influenza viruses (Dawood et  
14 al., 2009). Hence recent studies evaluating commercial rapid antigen tests with the  
15 pandemic H1N1 viral antigen in clinical specimens showed that the sensitivity ranged  
16 from 10% to 69%, and declined substantially with lower viral titers (CDC., 2009;  
17 Chan et al., 2009; Faix et al., 2009; Ginocchio et al., 2009). Thus some specimens  
18 with low viral titers will probably be undetected by using the rapid antigen test.

19           To overcome this sensitivity issue, a new rapid influenza A test, Flu A  
20 Dot-ELISA was developed (Wantai Biological Pharmacy Enterprise company,  
21 Beijing, China). This is a flow-through immunoassay that uses two influenza A virus  
22 NP specific monoclonal antibodies (mAb). One of the antibodies, immobilized on a

1 nitrocellulose membrane, serves as the capture antibody, and the other labeled with  
2 horseradish peroxidase is used as the detecting antibody. The procedure involves  
3 mixing 200µl of specimen with 400µl of lysis buffer. This mixture was filtered in a  
4 microtiter-like well that had a permeable membrane located at the bottom of the well  
5 with the antigen captured during the filtration process. The entire test procedure can  
6 be completed in 30 minutes and the results can be read visually (Chen et al., 2008).

7 Four sets of experiments were conducted to determine the properties of this  
8 assay. The first set of experiments was performed to compare the results of this kit  
9 with another commercial kit, Quick Vue Flu A test (Quidel Corp., San Diego, CA)  
10 against 10 strains of H5N1 influenza A viruses (Table 1). The detection limits of Flu A  
11 Dot-ELISA for the different influenza A strains ranged  $\log_{10}$  2.4 to 4.8 TCID<sub>50</sub> (or  
12 0.002 to 0.025 HA), which was 80 times lower than the detection limits of Quick Vue  
13 Flu A test. This sensitivity range is superior to many of the current rapid influenza  
14 tests (CDC., 2009; Chan et al., 2009; Hurt et al., 2007).

15 The second set of experiments used 40 strains of influenza A virus isolated from  
16 human, swine, avian, equine, and gull species and for controls, 26 strains of other  
17 respiratory viruses including 18 strains of influenza B virus, two strains of human  
18 parainfluenza viruses (HPIV), two strains of respiratory syncytial virus (RSV), two  
19 strains of adenovirus, and two strains of Newcastle disease virus. The detection limits  
20 for these influenza A strains ranged 0.01 to 0.001 HA units/0.2ml of sample, and there  
21 was no difference for the limit of detection for the current influenza A H1N1 virus.  
22 Another notable feature was the broad range of influenza A viruses detected from

1 different host species. None of the 26 strains of non-influenza A respiratory virus  
2 described above (data not shown) reacted positively to the test.

3 The third set of experiments evaluated the performance of Flu A Dot-ELISA  
4 using nasal and throat swabs collected from patients in Southern China, and the  
5 tracheal and cloacal swabs collected from apparently healthy chickens in live poultry  
6 markets also in Southern China. Samples were tested in parallel by Flu A Dot-ELISA  
7 and by virus culture in 10-day-old embryonated SPF chicken eggs or MDCK cells  
8 (Chen et al., 2009). The sensitivity and specificity of Flu A Dot-ELISA for the human  
9 samples were 88.5% (69/78) and 99.3% (146/147), respectively; and the positive  
10 predictive and negative predictive values were 98.6% (69/70) and 94.2% (146/155),  
11 respectively. For avian specimens, the sensitivity and specificity of Flu A Dot-ELISA  
12 for the chicken samples were 88.1% (74/84) and 96.5% (83/86), respectively; and the  
13 positive predictive and negative predictive values were 96.1% (74/77) and 89.2%  
14 (83/93), respectively (Table 2).

15 The final set of experiments was an evaluation conducted in a cohort of real-time  
16 RT-PCR confirmed novel influenza A / H1N1 pharyngeal specimens obtained from  
17 hospitalized patients. The sensitivity was 94.5 (69/73, 95% CI: 85.8% to 98.2%).

18 These results show that the Flu A Dot-ELISA is highly sensitive and sufficiently  
19 specific against all the variants of influenza A virus examined. As the test can be  
20 accomplished rapidly and does not require extensive laboratory facilities, this may  
21 play a role for rapid diagnosis of influenza A virus.

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1 Table 1

2 Detection of influenza A H5N1 virus by the Quidel Quick Vue Flu A+B and the Flu A

3 Dot-ELISA

Influenza A H5N1 Virus	Limit of Detection			
	Log <sub>10</sub> TCID <sub>50</sub> <sup>a</sup>		HA titer <sup>b</sup>	
	Quidel	Dot-ELISA	Quidel	Dot-ELISA
Chicken/Hong Kong/YU22/02	5.9	3.9	0.256	0.002
Duck/Shantou/4231/03	6.4	4.4	2.56	0.025
Chicken/Malang/BBVET 4/04	4.9	2.9	1.024	0.010
Chicken/Guangxi/2439/04	4.4	2.4	2.048	0.020
Duck/Vietnam/283/05	6.1	4.1	0.128	0.001
Indonesia/5/05	4.1	3.1	0.204	0.020
Bar-headed Goose/Qinghai/15C/05	5.2	3.2	0.256	0.002
Little Egret/Hong Kong/718/06	5.1	3.1	0.256	0.002
Japanese White Eye/Hong Kong/1038/06	4.4	2.4	0.256	0.002
Goose/Guiyang/337/06	6.8	4.8	0.256	0.002
Mean	5.3	3.4	0.724	0.009
Relative sensitivity <sup>c</sup>		~80		~80

4 <sup>a</sup> TCID<sub>50</sub>, 50% tissue culture infective dose;

5 <sup>b</sup> HA titer = the reciprocal of the highest dilution of virus with complete  
6 hemagglutination;

7 <sup>c</sup> Relative sensitivity = detection limit of Quidel Quick Vue A+B/detection limit of Flu

8 A Dot-ELISA.

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1 Table 2

2 Detection of influenza A virus in field samples by the Flu A Dot-ELISA

Virus culture	Flu A Dot-ELISA					
	Human samples <sup>a</sup>			Chicken samples <sup>b</sup>		
	No.	Positive	Negative	No.	Positive	Negative
Influenza A positive	78	69	9	84	74	10
Influenza A negative	147	1	146	86	3	83
Total	225	70	155	170	77	93
Sensitivity	88.5% (69/78)			88.1% (74/84)		
Specificity	99.3% (146/147)			96.5% (83/86)		
Positive predictive value	98.6% (69/70)			96.1% (74/77)		
Negative predictive value	94.2% (146/155)			89.2% (83/93)		

3 <sup>a</sup> Human samples are nasal and throat swabs collected from patients in hospital and  
 4 were tested by virus culture in MDCK cells and by the Flu A Dot-ELISA.

5 <sup>b</sup> Chicken sample are tracheal and cloacal swabs collected from healthy and diseased  
 6 chickens in markets and poultry farms were tested by virus culture in SPF eggs and by  
 7 the Flu A Dot-ELISA.

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