

Evaluation of the Quidel QuickVue® Influenza A+B Kit in Detecting Avian and Swine Influenza Virus

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Background: Pandemic influenza A viruses are often zoonotic. Rapid influenza virus diagnostics can play a role in reducing pandemic virus spread. However, most diagnostic tests have not been evaluated against animal influenza viruses. **Objective:** To evaluate a leading rapid diagnostic test against zoonotic influenza A virus strains. **Study Design:** We evaluated the performance of the QuickVue® Influenza A+B Kit in detecting 19 influenza A viruses of avian, human, and swine origin. Cell culture supernatant solutions were serially diluted (1:10-1:100,000), blanks were added, and the panel was arranged in a random order. A physician ran the 100 assays in a blinded fashion. **Results:** The QuickVue® Influenza A+B Kit detected every influenza A virus with no false positives. Virus detection limits varied from 2.2×10^0 TCID₅₀/ml (AvH6N2) to 1.0×10^5 TCID₅₀/ml (SwH1N2). The average TCID₅₀/ml's for avian, human and swine virus detections were 2.4×10^3 , 1.7×10^3 , and 3.5×10^4 , respectively. **Conclusion:** The QuickVue® Influenza A+B Kit readily detected all influenza A viruses but the kit required a higher viral titer in detecting swine H1N2 virus. When viral titers are relatively high, the QuickVue® Influenza A+B Kit may be useful in rapidly detecting the presence of avian and swine influenza A viruses.

Introduction

Influenza is a highly infectious respiratory virus that causes significant morbidity and mortality worldwide [1]. In developed countries, the disease has been estimated to explain 10–12% of all work absences. It likely contributes to the morbidity of a number of chronic diseases and directly and indirectly escalates medical costs and lost wages each influenza season [2]. In the US alone each influenza season an estimated 36,000 influenza-related deaths occur [3]. In recent years, commercially available, minimally complex, rapid influenza diagnostic tests have become available. While DFA, RT-PCR, and cell culture for influenza viruses have better diagnostic sensitivity, they require significant laboratory resources and significant turnaround time [4]. Hence, minimally complex, rapid influenza tests have frequently been used in outpatient clinical settings where prompt office-based testing may influence treatment decisions [5-7]. France, Switzerland, and certain US states have experimented with integrating such rapid tests into their influenza surveillance systems [2]. Rapid influenza tests' high specificity, simplicity, and increased reporting speed can provide early warning in some influenza

surveillance systems, especially those without advanced medical laboratory support [8]. A number of rapid influenza detection tests are now available that are minimally complex, quick (generate a result in 15 min), and can be performed without sophisticated laboratory support [6]. In controlled laboratory trials and in medical practices, rapid influenza tests have generally demonstrated good sensitivity and excellent specificity in detecting influenza virus [6, 9-11]. One of these tests, the QuickVue® rapid test, a lateral flow immunoassay that detects both influenza virus types A and B nucleoproteins, promises to be useful in clinical outpatient settings. [2, 6, 12-17]. The next pandemic influenza A virus is very likely to be zoonotic in origin. Rapid influenza A diagnostic tests may play roles in detection and the control of the spread of such novel influenza A viruses. However, generally rapid diagnostic tests have been developed to detect human influenza A viruses, and they have not been evaluated against zoonotic strains. In this study we sought to evaluate a leading rapid diagnostic test against a collection of zoonotic influenza A virus strains.

Methods

Nineteen prototypic influenza A viruses were tested with the Quidel QuickVue® Influenza A+B Kit per the manufacturer's instructions. The viruses (13 avian, 3 human, and 3 swine) were grown in MDCK cells or embryonated eggs. Reed-Muench TCID₅₀/ml infectious unit calculations for each virus were determined using trypsin in the neutralization assays. PBS blanks were added as negative controls. Samples were serially diluted from 1:10 to 1:100,000 with PBS. Five negative control specimens were added to the 95 specimens containing influenza A virus. All samples were then ordered by random-number and tested blindly. The 100 assays were run using 250 µl of sample in accordance with the Quidel QuickVue® Influenza A+B Kit package insert instructions.

Results

The Quidel QuickVue® Influenza A+B Kit detected all subtypes of influenza A virus (Table 1). There were no false positives. The detection limits varied from 2.2×10^0 TCID₅₀/ml (AvH2N2) to 1×10^5 TCID₅₀/ml (SwH1N2). The average TCID₅₀/ml's by virus type were 2.4×10^3 , 1.7×10^3 and 3.5×10^4 (avian, human and swine respectively, Table 2).

Table 1. Detection limits of avian, human, and swine influenza A viruses. 19 influenza A viruses tested with the Quidel QuickVue® Influenza A & B Kit 0=negative, +1=VERY light positive, +2=very light positive, +3=light positive, +4=positive.

sample/strain/ TCID50/ml	dilution	Result	sample/strain/ TCID50/ml	dilution	Result	sample/strain/ TCID50/ml	dilution	Result	sample/strain/ TCID50/ml	dilution	Result
AvH1N1 Duck/Alberta/25/76 1.0E+02	1:10	+4	AvH6N2 Turkey/MA/65 2.2E+00	1:10	+4	AvH11N9 Duck/Memphis/546/74 2.2E+03	1:10	+4	SwH1N1 Panama/2007/99 3.9E+03	1:10	+4
	1:100	+3		1:100	+4		1:100	+4			
	1:1000	+3		1:1000	+2		1:1000	+3			
	1:10000	0		1:10000	0		1:10000	0			
AvH2N2 Mallard/NY/6750/78 7.7E+00	1:100000	0	AvH7N2 Turkey/VA/4529/2002 1.6E+03	1:100000	0	AvH12N5 Duck/Alberta/60/76 1.8E+01	1:100000	0	SwH1N1 Sw/WI/238/97 5.6E+00	1:100000	0
	1:10	+4		1:10	+4		1:10	+4			
	1:100	+4		1:100	+4		1:100	+4			
	1:1000	+2		1:1000	+2		1:1000	+3			
AvH3N8 Duck/Ukraine/1/63 1.8E+04	1:10000	+2	AvH8N4 TY/Ontario/68 3.2E+02	1:10000	0	AvH13N2 Gull/MD/704/77 5.6E+01	1:10000	+2	SwH1N2 Sw/WI/R33F/01 1.0E+05	1:10000	0
	1:100000	0		1:100000	0		1:100000	0			
	1:10	+4		1:10	+4		1:10	+4			
	1:100	+4		1:100	+4		1:100	0			
AvH4N6 Duck/Cz/1/56 1.8E+02	1:1000	+2	AvH9N2 TY/MN/38391-6/95 1.5E+03	1:1000	+2	HuH1N1 NewCaldonia 1.7E+02	1:1000	+2	SwH1N2 Sw/MN/593/99 3.7E+03	1:1000	+2
	1:10000	0		1:10000	0		1:10000	0			
	1:100000	0		1:100000	0		1:100000	0			
	1:10	+4		1:10	+4		1:10	+4			
AvH5N2 Chucker/MN/14591-7/98 4.2E+03	1:100	+3	AvH10N7 Chicken/Germany/49 3.2E+03	1:100	+4	HuH3N2 Nanchang/933/95 1.2E+03	1:100	+4		1:100	+4
	1:1000	0		1:1000	+3		1:1000	+2			
	1:10000	0		1:10000	0		1:10000	0			
	1:100000	0		1:100000	0		1:100000	0			

19 influenza A viruses tested with the Quidel QuickVue® Influenza A & B Kit
 0=negative, +1=VERY light positive, +2=very light positive, +3=light positive, +4=positive

Options for the Control of Influenza VI

Table 2. Mean limit of detection by virus type.

Virus type	TCID ₅₀ /ml
Avian (n=13)	2.4x10 ³
Human (n=3)	1.7x10 ³
Swine (n=3)	3.5x10 ⁴ *

*SwH1N2 included.

Whe SwH1N1 excluded, swine value=1.9x10³

Discussion

The QuickVue® Influenza A & B Kit has been shown to be effective for detecting human influenza viruses with sensitivities ranging from 67% to 95% [6, 12, 14, 17-19]. None of these studies quantified the amount of virus in the clinical samples. The specificity of the test has often been found to be very close to 100% with one exception where a specificity of 76% was reported [7]. There has been only one report in the medical literature evaluating the test's performance against animal influenza viruses. Woolcock et.al examined 5 commercial immunoassay kits in detecting avian influenza virus (AIV) type A and uniformly concluded the kits were insensitive compared to the gold standard of virus isolation [20]. In Woolcock's evaluation, the QuickVue® Influenza A test did detect purified virus but failed to detect any clinical AIV specimens. However, Woolcock only looked at one avian virus. Following a controlled, blinded experimental designed, the QuickVue® Influenza A & B test detected each purified human, avian, and swine influenza virus we examined. There were no false positive assay results. We looked at the hemagglutinin type, neuraminidase type or species and there was no recognizable distribution pattern of detection limits (2.2x10⁰ to 1x10⁵ TCID₅₀/ml). Since Woolcock's clinical evaluation was limited to AvH6N2, additional testing of the QuickVue® Kit on clinical AIV samples of other subtypes is needed. This was the first evaluation of the QuickVue® assay against swine influenza A viruses. The Quidel QuickVue® Influenza A+B Kit has great potential for field use. Technicians in swine or poultry facilities might employ the QuickVue® Kit as a rapid indicator of influenza infection in accessing ill pigs or birds. If the test is positive, then immediate interventions might be taken to protect the rest of the herd or flock. However, because of imperfect sensitivity and non-validated test on clinical samples, a negative rapid influenza A test should be followed with either culture, DFA, or RT-PCR in further evaluating the ill pig or bird. In conclusion, the QuickVue® Influenza A+B Kit readily detected human, swine, and avian influenza A viruses. Its performance was comparable for human and avian influenza A subtypes. The kit required a higher viral titer for detecting swine H1N2 virus, but its performance against commonly circulating swine H1N1 and H3N2 virus, was similar to that for human and avian viruses. When swine and avian influenza A viral titers are relatively high, the QuickVue® Influenza A+B Kit may be useful in rapidly and accurately detecting influenza A viruses.

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