

## Comparison of Four Rapid Diagnostic Kits of Immunochromatography for Detection of Influenza A and Influenza B Viruses

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### Research Article

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#### ABSTRACT

**Objective:** The objective was to compare the performance of four commercial rapid influenza virus antigen detection test kits, namely QuickNavi-Flu (Denka Seiken, Tokyo, Japan), Alere BinaxNOW Influenza A&B assay (Alere Scarborough, Scarborough Inc., Scarborough, ME, USA), KaiBiLi Genesis Influenza A&B Antigen Test (Genesis, Hangzhou, China) and Actim Influenza A&B (Medix Biochemica, Kauniainen, Finland).

**Methods:** CAP proficiency testing specimens and ultrasonic lysates of normal human lung fibroblast cell (MRC-5 at  $2.9 \times 10^6$ /mL) were taken as specimens. The supernatants of influenza A and influenza B were used as stock solutions, and, using saline, were diluted to 1:10 and then serially diluted to 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200. The dilutions were tested respectively to evaluate the sensitivity of these kits. Respiratory syncytial virus, adenovirus, Para influenza virus type-2 and MRC-5 cell were taken as samples to evaluate the specificity of these kits. Influenza A (H1N1) at a dilution of 1:200, influenza A (H3N2) at a dilution of 1:100 and influenza B at a dilution at 1:100 were used to detect the reproducibility. Each sample was tested nine times in total.

**Results:** In this experiment, the four commercial kits showed the same detection limit for influenza A (H3N2) where all the kits could not give positive result for dilutions lower than 1:100. On the contrary, different detection limits were obtained for influenza A (H1N1) and influenza B. For influenza A (H1N1), positive results could be obtained using a dilution at 1:800 by Quick Navi Flu yet positive results could only be obtained at the dilution of 1:200 using the other three kits. All the results showed that timely detection gave better sensitivity than delayed detection except detection for Influenza A (H1N1) by the Alere BinaxNOW kit where dried specimens gave slightly higher sensitivity. The Actim kit showed positive result for Influenza B when detecting the ultrasonic lysates of MRC-5 cell, illustrating that false positive result may be obtained when detecting specimens which contain components related to MRC-5 cell using Actim kit. There was no non-specific cross-reactivity for the other three kits. In general, all these kits provided good reproducibility except that results of of Influenza A (H1N1) detection using the Alere BinaxNOW kit were inconsistent.

**Conclusions:** Various performances were found for the detection of influenza A and B viruses using four rapid immunochromatography diagnostic kits most commonly used in China.

## INTRODUCTION

### Background

Influenza virus is an enveloped RNA virus and a member of the family Orthomyxoviridae. It can infect humans and many kinds of animals. Influenza virus infections are responsible for significant morbidity and mortality in both pediatric and adult populations worldwide, especially influenza viruses A and B [1-3]. Rapid-diagnostic kits able to detect influenza A and B virus by immunochromatography developed by different manufacturers, while useful in early diagnosis, may vary widely in sensitivities and specificities [2,4]. In this study, we compared the performance of four rapid influenza diagnostic kits currently used in China in order to give insights and recommendations on the choice of flu screening kits in hospital laboratories.

### Objectives

The objective was to compare the performance of four commercial rapid influenza virus antigen detection test kits, namely QuickNavi-Flu (Denka Seiken, Tokyo, Japan), Alere BinaxNOW Influenza A&B assay (Alere Scarborough, Scarborough Inc., Scarborough, ME, USA), KaiBiLi Genesis Influenza A&B Antigen Test (Genesis, Hangzhou, China) and Actim Influenza A&B (Medix Biochemica, Kauniainen, Finland). The study was performed in a clinical laboratory of a tertiary and teaching hospital in Beijing, China.

## STUDY DESIGN

### Specimens

CAP proficiency testing specimens (reconstituted with 2 mL sterile distilled water and stored at -70°C freezer) including Influenza A, New Caledonia/20/99 (Group H1N1, VR1-15, 2012), Influenza A, Wisconsin/67/05 (Group H3N2, VR1-08, 2015), Influenza B, Florida/04/06 (VR1-04, 2015), Respiratory syncytial virus (VR1-18, 2015), Adenovirus (VR1-17, 2015), and Parainfluenza Virus Type-2 (VR1-14, 2015) were tested in this study. Ultrasonic lysates of normal human lung fibroblast cell (MRC-5, or ATCC CCL-171, American Type Culture Collection, Manassas, VA, USA) at a concentration of  $2.9 \times 10^6$ /mL were also tested as control.

## METHODS

### Detection limit and stability assessment for dried specimens

Specimens of influenza A and influenza B were retrieved from -70°C freezer and then centrifuged at 2000 rpm for 5 min after they were thawed and mixed completely. The supernatants of influenza A and influenza B were used as stock solutions, and, using saline, were diluted to 1:10 and then serially diluted to 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200. The stock and diluted solutions were taken as samples and detected by the four test kits in parallel. Manipulations of all samples were performed inside a biosafety cabinet (Steril GARD III, Baker Company, USA). 50 ul of each sample were added to the test swab carefully and the swabs were allowed to absorb the fluid thoroughly. Two swabs were prepared for each test at the same time. One of the swab was tested immediately after sample addition and the other swab was tested 30 min later after air drying in the biosafety cabinet. The assays were performed according to each manufacturer's instructions.

### Specificity assessment

Specimens of respiratory syncytial virus, adenovirus, parainfluenza virus type-2 and ultrasonic lysates of normal human lung fibroblast (MRC-5) cell were retrieved from -70°C freezer and then vortexed after they were thawed completely. These specimens were taken as samples and tested without further dilution. 50 ul of each sample were added to the test swab carefully and the swabs were allowed to absorb the fluid thoroughly. Then the swab was tested immediately after sample addition. All the sample manipulations had been done inside a biosafety cabinet and the assays were performed according to each manufacturer's instructions.

### Reproducibility assessment

Influenza A (Group H1N1) at a dilution of 1:200, influenza A (Group H3N2) at a dilution of 1:100, influenza B at a dilution of 1:100 were taken as samples to detect the reproducibility of these four test kits. 50 ul of each sample were added to the test swab carefully and the swabs were allowed to absorb the fluid thoroughly. Three swabs were prepared and tested immediately for each test at the same time. Another three swabs were prepared and tested 30 min later and three further swabs were prepared and tested in 60 min later. Each sample was tested nine times in total.

## RESULTS

### Detection limit and stability assessment for dried specimen results

In this experiment, the four commercial kits showed the same detection limit for influenza A (H3N2) where all the kits could not give positive result for dilutions lower than 1:100. On the contrary, different detection limits were obtained for influenza A (H1N1) and influenza B. For influenza A (H1N1), positive results could be obtained using a dilution at 1:800 by Quick Navi Flu yet positive results could only be obtained at the dilution of 1:200 using the other three kits (**Table 1**).

**Table 1.** Results of the limit of detection and stability assessment for dried specimens, Note: w+, weak positive; vw+, very weak positive.

	QuickNavi-Flu		Alere BinaxNOW		KaiBiLi Genesis		Actim	
	0 min	30 min	0 min	30 min	0 min	30 min	0 min	30 min
Influenza A (H1N1) stock solution	+	+	+	+	+	+	+	+
1:10	+	+	+	+	+	+	+	+
1:100	+	+	w+	w+	w+	w+	w+	w+
1:200	+	w+	-	w+	w+	vw+	w+	vw+
1:400	w+	w+	-	-	-	-	-	-
1:800	vw+	-	-	-	-	-	-	-
Influenza A (H3N2)								
stock solution	+	+	+	+	+	+	+	+
1:10	+	+	+	+	+	+	+	+
1:100	w+	w+	w+	vw+	w+	vw+	vw+	vw+
1:200	-	-	-	-	-	-	-	-
1:400	-	-	-	-	-	-	-	-
1:800	-	-	-	-	-	-	-	-
Influenza B								
stock solution	+	+	+	+	+	+	+	+
1:10	+	+	+	+	+	+	+	+
1:100	+	+	+	+	+	+	v	w+
1:200	w+	w+	w+	w+	w+	w+	vw+	vw+
1:400	vw+	-	-	-	vw+	-	-	-
1:800	-	-	-	-	-	-	-	-

For stability assessment for dried specimens, all the results showed that timely detection gave better sensitivity than delayed detection except detection for Influenza A (H1N1) by the Alere BinaxNOW kit where dried specimens gave slightly higher sensitivity.

### Specificity assessment results

In this experiment, the Actim kit showed positive result for Influenza B when detecting the ultrasonic lysates of MRC-5 cell, illustrating that false positive result may be obtained when detecting specimens which contain components related to MRC-5 cell using Actim kit. As shown in **Table 2**, there was no non-specific cross-reactivity for the other three kits in this experiment.

### Reproducibility assessment results

In general, all these kits provided good reproducibility except that results of Influenza A (H1N1) detection using the Alere BinaxNOW kit were inconsistent as **Tables 2 and 3** shown.

**Table 2.** Results of specificity assessment, Note: w+, weak positive.

	QuickNavi-Flu	Alere BinaxNOW	KaiBiLi Genesis	Actim
CAP EQA specimens				
RSV	-	-	-	-
Adenovirus	-	-	-	-
Parainfluenza Virus Type-2	-	-	-	-
Ultrasonic lysates of MRC-5 cell	-	-	-	influenza B w+

**Table 3.** Results of reproducibility assessment; Note: w+, weak positive; vw+, very weak positive.

	QuickNavi-Flu			Alere BinaxNOW			KaiBiLi Genesis			Actim		
	1	2	3	1	2	3	1	2	3	1	2	3
Influenza A (H1N1) 1:200												
0 min	+	+	+	w+	+	-	vw+	vw+	vw+	w+	w+	w+
30 min	w+	w+	w+	+	w+	-	vw+	vw+	vw+	vw+	vw+	vw+
60 min	w+	w+	w+	vw+	-	-	vw+	vw+	vw+	vw+	vw+	vw+
Influenza A (H3N2) 1:100												
0 min	w+	w+	w+	+	+	w+	vw+	vw+	vw+	vw+	vw+	vw+
30 min	vw+	vw+	vw+	-	-	-	vw+	vw+	vw+	vw+	vw+	vw+
60 min	vw+	vw+	vw+	-	-	-	vw+	vw+	vw+	vw+	vw+	vw+
Influenza B 1:100												
0 min	+	vw+	vw+	vw+	vw+	vw+	w+	w+	w+	vw+	vw+	vw+
30 min	w+	w+	w+	-	-	-	w+	w+	w+	vw+	vw+	vw+
60 min	w+	w+	w+	-	-	-	w+	w+	w+	vw+	vw+	vw+

## DISCUSSION

The new H1N1 pandemic virus emerged in Mexico in April 2009 and was spread to China in May 2009 [5]. The virus had caused 793 deaths by February 28, 2010 (China CDC official data). Since that time, screening for this virus in patients with suspected influenza symptoms were widely conducted in the emergency laboratory or emergency department of many hospitals in China, which aimed for neuraminidase inhibitors treatment and nosocomial infection control such as the determination of whether pregnant women with suspected influenza infection could be hospitalized in the same ward with other pregnant women. Rapid influenza diagnostic tests (RIDTs) are easy to use and have rapid turnaround time. However, RIDTs have been shown to have suboptimal sensitivity (40–70%) compared to RT-PCR or viral culture [6-10]. Although RIDT have demonstrated variable sensitivities, they still remain the test of choice in most clinical virology laboratories around the world due to the speed in obtaining results and simplicity in assay procedure [11]. In a survey including 240 US hospital laboratories, RIDT was the only diagnostic test available for influenza in 67% of the laboratories [12].

In China, there are currently more than 30 kinds of domestic influenza rapid test kits and more than 10 kinds of imported influenza rapid test kits which have been approved by CFDA. However, the qualities of these kits are uneven. POCT influenza detection kits itself has the problem of low sensitivity, so the selection of kits that are of good quality (sensitivity/specificity) and reasonable price is very important. Most of the laboratories engaged in flu screening are not professional influenza laboratories and the method which they could use to select the kit is limited, sometimes the selection is blindly or randomly. The methods used in this study were simple but quite effective, and could be recommended for laboratories in general hospitals. If VR1 CAP EQA specimens are not available, laboratories could also use the PCR-confirmed influenza positive or negative specimens. In this study, we did not use the recent virus isolates for testing for two reasons. The first reason was that we did not have recent isolates of the above viruses. The second reason was that the results of VR1 PT samples were reliable because these results were the results from more than 200 virology laboratories that participating the CAP proficiency testing worldwide. In this study, we also want to determine if there is any possibility that the rapid immunochromatography diagnostic kits for influenza viruses could have cross-reaction with normal cells. We have normal cells of BGMK, Vero, HEp-2, McCoy, Hela229 and MRC-5 in our laboratory. MRC-5 cell line is human fetal lung fibroblast cell and may be more close to human respiratory tract epithelial tissue because respiratory tract samples are usually collected for influenza virus tests.

For evaluation of different kinds of kits, to choose the kit with the highest sensitivity is very important because there are large number of patients in fever clinic who need flu screening. The positive ones need further confirmation by PCR, treatment with drugs such as neuraminidase inhibitors, and appropriate isolation control measures 13, 14. On the other hand, selection of the most cost-effective one is also very important and the administrative procedures need so.

The testing steps of the four kits in this study are quite similar and easy to operate, but the time spent to operate and wait for result reading is varied. For QuickNavi TMFlu, it requires less than 1 min to preprocess the samples and another 8 min to read the result after sample addition into the strips. For Alere BinaxNOW and KaiBiLi Genesis, they also require less than 1 min to preprocess the sample but additional 15 min are required to read the result after sample addition into the strips. For Actim TM, it requires 5 min to preprocess the samples and another 10 min to read the result after sample addition into the strips. In our opinion QuickNavi TMFlu is the most convenient one in the four kits tested.

As many respiratory specimens are taken by physicians and the dried swabs are transferred to laboratories for testing some time later in our hospital, we intended to compare testing sensitivities between fresh swabs and dried swab samples. It was shown that the sensitivities were slightly decreased in three kits and slight increase in one kit in our study.

As a limitation of the present study, only preserved CAP PT specimens were included in the comparison tests while clinical specimens were not included. Also, these specimens may not represent all the virus types which could be contained in clinical samples in different seasons. In this study, we added uniform amount of simulation samples to the swabs and used uniform processing method and but in real clinical situation sample collection may not be so easy and may vary largely. Our results also implied that different kits may have various performance of detection. Using a second method to detect respiratory viruses such as molecular assays for critical patients who are suspected with respiratory viruses infection is essential and may improve patient care.

In conclusion, our survey suggests that the most sensitive kit for detecting influenza A (H1N1) among the four kits tested is Quick Navi Flu; whereas Alere BinaxNOW kit may be not very stable when detecting Influenza A (H1N1). The Actim kits may give false positive results when detecting the specimens which contain components related to MRC-5 cell. Our results implied that different kits may have varying performance of testing. It may be necessary to use molecular assay to detect or confirm respiratory viruses for critical patients who are suspected to be infected with influenza virus <sup>[12-14]</sup>.

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## FOOTNOTES

Author Contributions Y. Z. and A. N. were responsible for the conception and design of the study; Y. Z., Y. W. and A. N. performed laboratory work. Y. Z., Y. W., M. Z., Y. X. and A. N. analysed the results. Y. Z. and A. N. drafted the paper. All authors reviewed the manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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