

# M19 - THE RELATIVE PERFORMANCE OF 4 INFLUENZA ANTIGEN ASSAYS

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## ABSTRACT

A number of rapid immunoassays for influenza are now available to both clinicians and clinical microbiologists. However, it is difficult to derive a clear understanding of the comparative performance of these devices from published evaluations using clinical specimens because the relative amount of antigen is undefined in these specimens. The purpose of this study was to compare the performance of 4 Influenza A+B assays under controlled conditions. The following devices were used: Quidel QuickVue® Influenza A+B, Binax Now® Influenza A&B, Remel Xpct™ Flu A&B, and BD Directigen™ Flu A&B.

Five different Influenza A (2 H<sub>1</sub>N<sub>1</sub> and 3 H<sub>3</sub>N<sub>2</sub>) and five Influenza B virus serotypes were cultivated in primary rhesus monkey kidney (MK) cells. Lysates of the infected cell cultures were then prepared in PBS and titrated in MK cells. Subsequently, the lysates were also titrated in duplicate using each of the rapid test devices according to manufacturer's instructions for nasal wash specimens. Results are expressed as the reciprocal of the highest dilution that produced a positive result. Operator comments were also recorded for each kit. Virus lysate titers ranged from 32,768 to 131,072 TCID<sub>50</sub> for the Influenza A strains and 44,728 to 98,304 TCID<sub>50</sub> for the Influenza B strains. The analytical sensitivity of one assay, Quidel QuickVue® Influenza A+B, was greater than or equal to the sensitivity of other tests for both Influenza A and B. The Quidel QuickVue® Influenza A+B assay was analytically more sensitive in 38/40 (95%) instances and equivalent in 2/40 (5%; both Influenza B). Improved sensitivity with the Quidel QuickVue® Influenza A+B assay ranged from two to eight fold for all viruses tested. Remel Xpct™ Flu A&B was subject to occasional false positive results (2/120); no other kits had specificity problems. Overall, the Quidel QuickVue® Influenza A+B assay was analytically the most sensitive test and has added benefits in terms of ease of use and rapid time to positive result.

REVISED



- 1 min extraction step
- 10 min incubation
- generally easy to read
- CLIA waived

Fig. A: Quidel QuickVue® Influenza A+B



- no extraction step for nasal wash
- 15 min incubation
- generally easy to read
- CLIA waived

Fig. B: Binax NOW® Influenza A&B



- no extraction step for nasal wash
- incubation 15 to 30 min
- generally easy to read
- non-waived

Fig. C: Remel Xpct™ Flu A&B



- nasal wash is diluted
- multiple reagents
- multiple incubations
- can be difficult to read
- non-waived

Fig. D: BD Directigen™ Flu A&B

## CONCLUSIONS

Our studies described here show the analytical sensitivity of the Quidel QuickVue® Influenza A+B is greater than or equal to the sensitivity of the other tests for multiple Influenza A and B strains. The Quidel QuickVue® Influenza A+B and Binax NOW® Influenza A&B both required little to no specimen preparation, were rapid (10-15 minute incubation) and were generally easy to interpret. The Remel Xpct™ Flu A&B performed better in terms of sensitivity when the incubation period was extended to 30 minutes for 6 of the 10 isolates tested. However, the improved sensitivity at 30 minutes was marred by the development of 2 false positive lines that developed at this later time point. The BD Directigen™ Flu A&B test was the most difficult to interpret and was also the most cumbersome to perform. However, the BD Directigen™ Flu A&B tied with Quidel QuickVue® Influenza A+B for sensitivity of 2 of 10 viruses and seemed to perform better for Influenza B isolates than with Influenza A isolates.

## DISCUSSION

There have been no published studies comparing the performance characteristics of these 4 rapid influenza tests. Additionally, the literature lacks references utilizing Quidel QuickVue® Influenza A+B. We found significant differences in the analytical sensitivity of the 4 tests studied here. Other performance differences, including number of steps, incubation time and ease of interpretation were also found to vary among the tests used in this study. Challenges in interpretation of the BD Directigen™ Flu A&B test have been recently noted (1). Although we might predict a more analytically sensitive test to be more sensitive with patient specimens, these studies need to be done separately. Therefore, performance characteristics of these four rapid tests using clinical specimens are currently being evaluated by our laboratory.

Virus	QuickVue	Binax NOW	Xpct at 15 min	Xpct at 30min	Directigen
A/New Caledonia/20/99 (H1N1)	512	256	64/128	256	128/256
A/Korea/770/2002 (H3N2)	256	64	<64	64	64
A/Panama/2007/99 (H3N2)	512	256	64	128	64/128
A/H1N1/Taiwan/87 (H1N1)	256	128	64	128	64
A/H3N2/Philippines/86 (H3N2)	512	128	64	128	128
B/Shizuoka/15/2001	1024	256	128	256	512
B/Sichuan/379/99	512	256	128	128	256
B/HongKong/330/2001	256	128	64	64	256
B/Shanghai/361/2002	256	128	64	64	64
B/USSR/86	512	128	128	128	512

**Table 1. Titer Results for Each Kit Tested.** Results are expressed as the reciprocal of the highest dilution that produced a positive result. Yellow boxes are used to shade the test or tests giving the highest analytical sensitivity for each influenza strain tested. All tests were performed using the manufacturer's instructions for nasal wash specimens.

## MATERIALS AND METHODS

Five different Influenza A (2 H<sub>1</sub>N<sub>1</sub> and 3 H<sub>3</sub>N<sub>2</sub>) and five different Influenza B virus serotypes were cultivated in primary rhesus monkey kidney (MK) cells. Lysates of the infected cell cultures were then prepared in PBS and titrated in MK cells. TCID<sub>50</sub> values were calculated using the Reed-Muench method. Virus lysate titers ranged from 32,768 to 131,072 TCID<sub>50</sub> for the Influenza A strains and 44,728 to 98,304 TCID<sub>50</sub> for the Influenza B strains. Lysates were serially diluted in saline. These dilutions were tested in duplicate using each of the rapid test devices according to manufacturer's instructions for nasal wash specimens. The following devices were used: Quidel QuickVue® Influenza A+B, Binax NOW® Influenza A&B, Remel Xpct™ Flu A&B, and BD Directigen™ Flu A&B. Results are expressed as the reciprocal of the highest dilution that produced a positive result. Operator comments were also recorded for each kit. The Wilcoxon Signed Rank Test, a non-parametric test, was utilized to compare dilution thresholds for detecting 10 virus types for QuickVue® versus Binax NOW®, Remel Xpct™ at 15 minutes, and BD Directigen™.

## RESULTS

The Quidel QuickVue® Influenza A+B assay was analytically more sensitive than the other 3 kits for 8 of the 10 viruses tested and equivalent in 2/10 (both Influenza B; see Table 1). Improved sensitivity with the Quidel QuickVue® Influenza A+B assay ranged from two to eight fold for all viruses tested. Quidel QuickVue® was superior to Binax NOW® (Signed Rank Statistic = 27.5, p = .002), Remel Xpct™ at 15 minutes (Signed Rank Statistic = 27.5, p = .002), and BD Directigen™ (Signed Rank Statistic = 14, p = .008). Compared with results obtained at 15 minute incubation, the analytical sensitivity for the Remel Xpct™ test improved for 6 of 10 influenza isolates (including all 5 Influenza A isolates) when results were recorded at 30 minutes (Table 1). Remel Xpct™ Flu A&B was subject to occasional false positive results characterized by development of an incorrect type-specific line following 30 minute incubation (2/120); no other kits demonstrated specificity problems. Overall, the Quidel QuickVue® Influenza A+B assay was the most sensitive test and has added benefits in terms of ease of use and rapid time to positive result (see figures A-D).

## INTRODUCTION

Ideally, a rapid test for influenza would have all of the following characteristics: simple to set up and interpret, short incubation, and be both sensitive and specific. A number of rapid immunoassays for influenza are now available to both clinicians and clinical microbiologists. However, it is difficult to derive a clear understanding of the comparative performance of these devices from published evaluations using clinical specimens because the relative amount of antigen is undefined in these specimens. A variety of patient populations with different inclusion criteria, specimen types and rapid tests kits are used in these various studies, further complicating analysis of the literature. The purpose of this study was to compare the performance of 4 Influenza A+B assays under controlled conditions using known concentrations of cultured virus.

## REFERENCE

1. Weinberg, A. and M.L. Walker. Clin Diagn Lab Immunol. 2005. 12:367-370.

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