

Evaluation of Lateral Flow and Membrane Flow Immunoassays for the Rapid Detection of Influenza A and Influenza B Viruses

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ABSTRACT

OBJECTIVE: To compare the performance of three lateral flow chromatographic immunoassays (X-pect Flu A/B, Remel; Directigen EZ Flu A/B, BD Diagnostic Systems; Clearview Flu A/B, Wampole Laboratories) and one membrane flow immunoassay (Directigen Flu A/B, BD Diagnostic Systems) to R-Mix spin-amplified cell culture (Diagnostic Hybrids) for the detection of Influenza A and B viruses.

METHODS: One hundred (100) respiratory specimens submitted for Influenza A and B testing were evaluated by all four rapid immunoassay kits, following each specific package insert, and also by culture using R-Mix cells grown in 48-well tissue culture plates. Specimens were inoculated (0.2 mL) into duplicate R-Mix wells and the plates were centrifuged at 700 x g for 1 hour. Cultures were stained for Influenza A in one of the duplicate wells and Influenza B in the other duplicate well using monoclonal antibodies (DAKO, Inc.) after 36-48 hours incubation.

RESULTS: Of the 100 specimens, 56 were R-Mix culture positive for Influenza A or Influenza B. 53 of 56 were positive by X-pect; 54 of 56 were positive by Directigen EZ; 44 of 56 were positive by Clearview; 50 of 56 were positive by Directigen. 44 specimens were negative for Influenza A and B by all methods (Table 1). The decision to evaluate the Remel X-pect kit further was made based on this data. An additional 100 respiratory specimens were tested using the Remel X-pect kit versus R-Mix culture. 49 of 53 Influenza A positive R-Mix cultures were positive for Influenza A by X-pect, 23 of 23 Influenza B specimens were positive by both methods and 24 specimens were negative by both methods. The cumulative data for the Remel X-pect Flu A/B kit is found in Table 2.

CONCLUSION: The Remel X-pect Flu A/B and the BD Directigen EZ Flu A/B assays demonstrated a sensitivity of 94% and 96% respectively, with a specificity of 100% for both kits, compared to R-Mix culture, for the first 100 specimens evaluated. These are both rapid (15 minutes), easy to perform, sensitive and specific methods for the detection of Influenza A and B viruses. At the time of this evaluation, the BD Directigen EZ Flu A/B kit was not cleared by the FDA.

OBJECTIVE

Influenza A and B viruses are an annual cause of significant morbidity and mortality, with symptoms ranging from malaise, fever and sore throat to potentially fatal pneumonia. Because numerous other respiratory viruses can produce flu-like symptoms, rapid, differential diagnosis of influenza virus infection is important in early treatment and patient management. Anti-viral therapy can lessen the course of the disease as well as be used for chemoprophylaxis in outbreak situations. With the availability of immunochromatographic assays, our laboratory wanted to find an accurate, rapid diagnostic system that would allow for easier use, eliminating multi-step procedures.

Table 1. Comparison of Lateral Flow/Membrane Immunoassays to R-Mix culture for the detection of Influenza A&B Viruses

	Influenza A+/B-	Influenza A-/B+	Influenza A-/B-	Sensitivity to R-mix culture
R-Mix Culture	48	8	44	100% Flu A/100% Flu B
Remel X-pect	45	8	47	94% Flu A/100% Flu B
BD Directigen EZ*	46	8	46	96% Flu A/100% Flu B
Wampole Clearview	36	8	56	75% Flu A/100% Flu B
BD Directigen	42	8	50	88% Flu A/100% Flu B

n=100

* Not FDA cleared at time of evaluation

Specificity of all assays = 100%

Table 2. Remel X-pect Flu A/B Assay compared to R-Mix Culture

	Influenza A+/B-	Influenza A-/B+	Influenza A-/B-	Sensitivity to R-mix culture
R-Mix Culture	101	31	68	100% Flu A/100% Flu B
Remel X-pect	94	31	75	94% Flu A/100% Flu B

n=200

ACKNOWLEDGEMENTS

We gratefully acknowledge Fisher Scientific and Remel for providing test kits for this study.

NOTE: At the time of this evaluation, the Directigen™ EZ Flu A/B assay was not cleared by the FDA.

METHODS

One hundred (100) respiratory specimens submitted for Influenza A and B testing were evaluated by three lateral flow chromatographic immunoassays and one membrane flow immunoassay, and compared to R-Mix spin-amplified cell culture.

R-Mix cell culture

R-Mix cells (Diagnostic Hybrids), in 48-well plate format, were stored at room temperature and pre-incubated for at least 1 hour prior to inoculation. 0.8 mL of refeed media with trypsin (Diagnostic Hybrids) was used to replace shipping media. Two wells of R-Mix cells were inoculated with 0.2 mL of specimen per well. Plates were centrifuged at 700 x g for 60 minutes in a centrifuge equipped with biohazard containment plate carriers, and were then incubated at 35-37°C in 5% CO₂. Cultures were stained for Influenza A and B with monoclonal antibodies (Dako) after 36-48 hours incubation.

X-pect™ Flu A/B Test (Remel)

Specimens were vortexed and 0.2 mL of each specimen was added to 5 drops of Specimen Diluent. Specimens were mixed and, using the transfer pipette provided in the kit, 0.1 mL of specimen was added to the diluent. Using the same pipette, 0.2 mL of diluted specimen was added into the center of the sample well of the test device. Results were read and recorded after 15 minutes of room temperature incubation. Two black-colored bands, one in the test region and one in the control region, indicated a positive result; one band in the control region only indicated a negative result and proper test performance. (See Figures 1-3)

Figure 1.
Flu A Pos/Flu B Neg

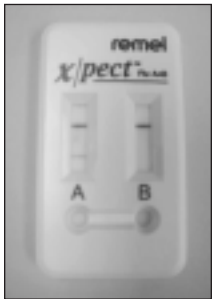


Figure 2.
Flu A Neg/Flu B Pos



Figure 3.
Flu A & B Neg



Directigen™ EZ Flu A&B Test (BD Diagnostic Systems)

Specimens were vortexed, 0.3 mL of each specimen was added to 4 drops of Extraction Reagent E and specimens were re-mixed. Dispenstube tips were inserted into each dilution tube; 3 drops of diluted specimen were dispensed into the center of both sample wells of the test device. Results were read and recorded after 15 minutes of room temperature incubation. Two dark-colored bands, one in the test region and one in the control region, indicated a positive result; one band in the control region only indicated a negative result and proper test performance.

Clearview Flu A/B Test (Wampole Laboratories)

Specimens were vortexed, 0.1 mL of each specimen was added to 3 drops of Specimen Diluent and specimens were re-mixed. 0.2 mL of diluted specimen was dispensed into the center of the sample well of the test device. Results were read and recorded after 15 minutes of room temperature incubation. Two black-colored bands, one in the test region and one in the control region, indicated a positive result; one band in the control region only indicated a negative result and proper test performance. (See Figures 4-6)

Figure 4.
Flu A Pos/Flu B Neg



Figure 5.
Flu A Neg/Flu B Pos



Figure 6.
Flu A & B Neg



Directigen™ Flu A/B Test (BD Diagnostic Systems)

Specimens were vortexed, 0.2 mL of each specimen was added to 8 drops of Extraction Reagent E and specimens were re-mixed. Dispenstube tips were inserted into each dilution tube. 4 drops of diluted specimen were dispensed into each A and B test wells. After absorption, the flow controller was removed. 2 drops of Wash Reagent 1 were added to both test wells. 2 drops of Detection Reagent 2 were added to the A well only; 2 drops of Detection Reagent 3 were added to the B well only. Test cartridge was incubated for 2 minutes at room temperature. 3 drops of Wash Reagent 4 were added to both wells followed by 3 drops of Wash Reagent 5. 3 drops of Substrate Reagent 6 were added to both wells and incubated for 5 minutes at room temperature. Test wells were read immediately. A purple triangle of any intensity indicated a positive reaction; negative wells exhibited a small purple control dot indicating proper test performance. (See Figures 7-9)

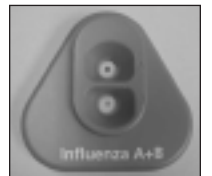
Figure 7.
Flu A Pos/Flu B Neg



Figure 8.
Flu A Neg/Flu B Pos



Figure 9.
Flu A & B Neg



RESULTS

R-Mix cell culture and staining with monoclonal antibodies directed against influenza A or B resulted in forty-eight (48) specimens positive for Influenza A, eight (8) specimens positive for Influenza B and forty-four (44) specimens negative for both Influenza A and B. X-pect™ Flu A/B detected 45/48 Influenza A positive specimens (FluA sensitivity=94%). Directigen™ EZ Flu A/B detected 46/48 Influenza A positive specimens (FluA sensitivity=96%), while the Directigen™ Flu A/B detected 42/48 Influenza A positive specimens (FluA sensitivity=88%). Clearview Flu A/B detected 36/48 Influenza A positive specimens (FluA sensitivity=75%). All test systems detected 8/8 Influenza B positive specimens for Flu B sensitivity of 100%. 44/44 specimens were negative for Influenza A and B by all test systems for an overall specificity of 100% (Table 1).

Based on this initial data, the decision was made to evaluate the X-pect™ Flu A/B kit further. An additional 100 respiratory specimens were evaluated with the X-pect™ Flu A/B test compared to R-Mix cell culture. 49/53 Influenza A positive R-Mix cell cultures were positive for Influenza A by X-pect™ Flu A/B, 23 specimens were positive for Influenza B by both methods and 24 specimens were negative for Influenza A and B by both methods (Table 2). The cumulative data of the entire 200 specimens tested by the X-pect™ Flu A/B test demonstrated sensitivities of 94% for Influenza A and 100% for Influenza B, with an overall specificity of 100%.

Table 1. Comparison of Lateral Flow/Membrane Immunoassays to R-Mix culture for the detection of Influenza A and B Viruses.

	Influenza A+/B-	Influenza A-/B+	Influenza A-/B-
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BD Directigen	42	8	50

n=100

* Not FDA cleared as of 11/5/04

Table 2. Cumulative data comparison of Remel X-pect™ Flu A/B Assay to R-Mix culture for the detection of Influenza A and B viruses.

	Influenza A+/B-	Influenza A-/B+	Influenza A-/B-
R-Mix Culture	101	31	68
Remel X-pect™	94	31	75

n=200

CONCLUSION

- Lateral flow chromatographic immunoassays for Influenza A and B viruses have eliminated the tedious, multi-step procedures associated with membrane flow immunoassays, which are time-consuming especially when dealing with large test volumes. The X-pect™ Flu A/B and the Directigen™ EZ Flu A/B assays demonstrated sensitivities of 94% and 96% respectively, with a sensitivity of 100% for both assays, compared to R-Mix cell culture. Both assays are rapid, easy to perform, sensitive and specific methods for the direct detection of Influenza A and B viruses.

