# EVALUATION OF THE DIRECTIGEN™ FLU A & B TEST USING CLINICAL SPECIMENS SUBMITTED IN VIRAL TRANSPORT MEDIA


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### Abstract

**Objective:** To compare the performance of Directigen™ Flu A & B (Becton Dickenson, Sparks, MD.) to tissue culture using a Mink Lung cell line in 48 well plate format (Diagnostic Hybrids, Inc., Athens, Ohio). This was a retrospective study for the detection and differentiation of Influenza A and B viruses in clinical specimens submitted in Starswab Multi Trans transport media (SMS) (Starplex Scientific, Etobicoke, Ontario, Canada).

**Methods:** Eighty five (85) respiratory specimens submitted in Starswab Multitrans transport media were cultured for Influenza A and Influenza B using Mink Lung cells which were grown in a 48 well microtiter tissue culture plate. Specimens were inoculated into duplicate wells of Mink Lung cells, (0.2mL per well). Refeed media with trypsin was used to replace the shipping media in plates; the plates were centrifuged at 700 x g for 60 minutes in a centrifuge equipped with biohazard containment plate carriers. Cultures were stained for Influenza A and B with monoclonal antibodies (Dako Inc., Carpinteria, CA) after 24 hours incubation. Specimens were frozen at –70ºC for storage. After quick-thawing, the specimens were tested for Influenza A and B by the Directigen™ Flu A & B rapid detection kit following package insert.

**Results:** From 85 specimens, 24 of 25 Influenza A Mnk Lung culture positive specimens were positive for Influenza A by Directigen™ Flu A & B; 10 specimens were positive for Influenza B by both methods; 50 specimens were negative for Influenza A and B by both methods (Table 1).

Table 1. Directigen™ Flu A & B vs. Mnk Lung Culture

| | Directigen™ Flu A & B | Mnk Lung |
|---|---|---|
| Influenza A | 24 | 25 |
| Influenza B | 10 | 10 |
| Negative | 51 | 50 |

n = 85

**Conclusion:** The Directigen™ Flu A & B test demonstrated a sensitivity of 97% and specificity of 100% when testing clinical specimens submitted in the SMS transport media. This performance demonstrates the ability to use transport media to maintain specimen integrity, when immediate testing is not available for the rapid detection and differentiation of Influenza A and Influenza B viruses. The use of transport media also allows for additional culture testing, if required, without recollection of the sample.


### Objective

Annually, Influenza A and B viruses cause significant morbidity and mortality with symptoms ranging from malaise, fever and sore throat to potentially fatal pneumonia. Numerous other respiratory virus infections can also produce similar, flu-like symptoms. The rapid, differential diagnosis of Influenza virus infection is important in early treatment and patient management. Our laboratory wanted to find an accurate, rapid diagnostic system that would also allow for the availability of reflex culture testing on Flu A/B negative specimens, if requested.

### Methods

Eighty-five (85) respiratory specimens that had been submitted in Starswab Multitrans transport media were used for this retrospective evaluation. Thirty-five (35) specimens were collected from patients whom the physician had a high degree of suspicion for Influenza virus infection and were culture – positive as part of a clinical trial. Fifty (50) negative specimens were all from respiratory sources that had been submitted for routine viral culture.

Tissue Culture

Mink Lung cells were stored at room temperature and pre-incubated for at least 1 hour prior to inoculation. 0.8mL of refeed media with trypsin (Diagnostic Hybrids, Inc.) was used to replace shipping media. Two wells of Mink Lung cells were inoculated with 0.2mL specimen per well. The plates were centrifuged at 700xg for 60 minutes in a centrifuge equipped with biohazard containment plate carriers. Plates were incubated at 35 – 37ºC in 5% $CO_2$. Cultures were stained for Influenza A and B with monoclonal antibodies after 24 hours incubation. Remaining specimens were frozen at –70ºC for storage.

Directigen™ FluA & B Test

All specimens were vortexed; 0.2mL of each specimen was added to 8 drops of Extraction Reagent E; all mixtures were re-vortexed; Dispenstube tips were inserted in each dilution tube; 4 drops specimen dilution were dispensed into each A and B test wells. 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 and 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 figure 1 – 4).

Flu A/B Neg.


Fig 1

Flu A Pos.


Fig 2

Flu B Pos.

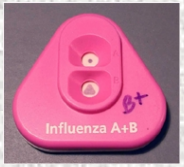


Fig 3

Flu A/B Pos.


Fig 4

### Results

The staining of the Mink Ling tissue culture with monoclonal antibodies resulted in twenty-five (25) specimens that were positive for Influenza A, ten (10) that were positive for Influenza B and fifty (50) that were negative for both Influenza A and B. The Directigen™ Flu A & B Immunoassay detected 24 of the 25 specimens which were culture positive for Influenza A, 10 of 10 that were culture positive for Influenza B and 50/50 which were culture negative for both Influenza A and B. One specimen was culture positive for Influenza A and negative by the Flu A & B Immunoassay even after repeat testing was performed. The quantity of specimen was not sufficient to repeat the culture. (Table 2)

Table 2. Directigen™ Flu A & B vs. Mink Lung Culture

| | Directigen™ Flu A & B | Mink Lung |
|---|---|---|
| Influenza A | 24 | 25 |
| Influenza B | 10 | 10 |
| Negative | 51 | 50 |

n = 85

Note: When performing the Directigen™ Flu A & B Immunoassay, the collar must be securely fastened to the testing device, failure to do so may result in erroneous results, usually false negatives. We discovered this when a customer using the kit and referring specimens to us called regarding discrepant results. We noticed on some kits the devices were completely separated. For this reason, checking the collars became part of our standard operating procedure. A call to the manufacturer acknowledged that there was a manufacturing problem but a recall had not been issued.

### Conclusion

This study was designed to assess the performance of the SMS transport with the Directigen™ Flu A & B Immunoassay using well-characterized specimens. The results obtained demonstrate that Starswab Multitrans transport media is an appropriate transport medium for specimens to be tested by the Directigen™ Flu A & B Immunoassay. The use of culture transport media provides the opportunity to perform culture on specimens that were Immunoassay negative.