

Performance of Binax NOW[®] and ImmunoCard STAT![®] Influenza Assays with Specimens Collected in Starplex Multitrans[™] S160 Transport Medium

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Abstract

Starplex Multitrans[™] S160 transport medium (Starplex Scientific Inc., Etobicoke, ON) has not been formally evaluated for use with either the Binax NOW[®] Influenza A&B (NOW[®]; Binax Inc., Portland, ME) or ImmunoCard STAT![®] Flu A&B Plus (ICStat[®]; Meridian Bioscience Inc., Cincinnati, OH) assays. We evaluate the performance of these two assays using nasopharyngeal swabs (NPS) collected and transported with the Starswab[™] Multitrans[™] System to determine if S160 has any effect on test performance.

Methods: NPS from adults (20-93 yrs) presenting (2/15/06-4/18/06) with influenza symptoms to local EDs, outpatient offices and clinics, admitted to regional hospitals, or resident in long term care facilities were collected and transported with the Starswab[™] Multitrans[™] System. Upon receipt in the laboratory, specimens were processed and examined by DFA (Light Diagnostics[™] Influenza A and B monoclonal antibodies (mAbs), Milpore Corp., Temecula CA), and were inoculated into cell culture (RMK (DHI Inc., Athens OH), HFL (CHEO)). Specimens were tested by NOW and ICStat assays concurrently. According to product inserts, sensitivity and specificity (vs. DFA and/or culture) of the NOW assay are 75% and 100% respectively (Flu A), and 50% and 100% respectively (Flu B). For ICStat, sensitivity and specificity (vs. culture) are 72.5% and 99.1% (Flu A), and 76% and 100% (Flu B).

Results: *Relative to DFA* (n=86), overall sensitivity and specificity of NOW were 65% (32/49) and 100% (37/37); overall sensitivity and specificity of ICStat, were 67.3% (33/49) and 100% (37/37). *Relative to culture* (n=103), overall sensitivity and specificity of NOW were 69.8% (30/43) and 95% (57/60); of ICStat, were 74.4% (32/43) and 95% (57/60). *Relative to DFA and/or culture*, Flu A sensitivity and specificity of NOW were 59% and 100%, and for Flu B, 70% and 100%. Flu A sensitivity and specificity of ICStat were 61% and 100%, and for Flu B, 80% and 100%.

Conclusion: In this study, performances of NOW and ICStat influenza assays were comparable, demonstrating high specificity but poor sensitivity. These findings are consistent with published data using other approved transport media. Thus, S160 medium included in the Starswab[™] Multitrans[™] System is suitable for use with either of these test kits.

Introduction

Influenza infections are a major cause of acute respiratory illness in all age groups worldwide.

The Eastern Ontario Regional Virology Laboratory (RVL) located at the Children's Hospital of Eastern Ontario (CHEO) routinely detects influenza by DFA testing of patient specimens, followed by conventional cell culture. At RVL, the most frequently submitted specimen type for adult patients is a nasopharyngeal swab (NPS). These are collected and transported with the Starswab[™] Multitrans System (Starplex Scientific Inc., Etobicoke, ON), containing S160, a proprietary transport medium.

During peak respiratory season, urgent requests for influenza laboratory diagnosis are accommodated by rapid antigen test. Among the new generation of immunochromatographic (lateral flow) rapid antigen tests are the Binax NOW[®] Influenza A&B (NOW[®]; Binax Inc., Portland, ME) and ImmunoCard STAT![®] Influenza A&B Plus (ICStat[®]; Meridian Bioscience Inc., Cincinnati, OH) assays. Transport medium S160 has not been formally evaluated for use with either of these rapid tests.

Objectives

- To evaluate and compare the performance of Binax NOW[®] Influenza A&B and ImmunoCard STAT![®] Flu A&B Plus assays relative to DFA and to cell culture.
- To determine if Starplex transport medium S160 has any effect on the performance of either of these rapid antigen tests.

Methods

Specimens and specimen processing

- NP swabs (NPS) collected using the Starswab[™] Multitrans[™] System (S160-100) were tested.
- DFA and cell culture were performed upon specimen arrival to RVL.
- Binax and ImmunoCard tests were performed concurrently.

DFA

On arrival at RVL, specimens were vortexed and transferred to centrifuge tubes. After centrifugation (2600 rpm, 5 min.), cell pellets were resuspended in S160 transport medium (0.2 – 0.4 mL). Cell suspensions (10 µL) were applied and fixed to microscope slide test wells, and stained with 10 µL of Light Diagnostics[™] Influenza A and B monoclonal antibodies (Milpore Corp., Temecula CA). After 30 minutes, slides were washed, dried, coverslipped and examined at 100 and 400X (Nikon Eclipse PF 100/F microscope, 450-490nm).

DFA interpretation:

Positive: ≥2 columnar epithelial cells (c.e.) exhibiting specific fluorescence.

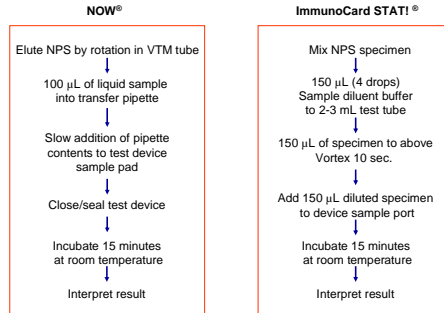
Indeterminate: < 2 c.e. exhibiting specific fluorescence.

Unsuitable: < 5 c.e. per low power field; no specific fluorescence.

Cell culture

An aliquot (150 µL) of each specimen was added to 1 tube of RMK (Diagnostic Hybrids, Inc., Athens OH) and 1 tube of HFL (CHEO) cells. Cells were incubated (33.5°C, 8 days) and examined for cpe. Upon cpe development or on day 8, cells were trypsinized and processed for DFA.

Rapid antigen tests



Results

Specimen description

- Patient age range: 20-93 years.
- Number of specimens submitted for testing: 103 (All NPS)
- Analyzed by rapid test and DFA: 86
- Analyzed by rapid test and culture: 103

Summary of virus isolations

N= 44
(44/103=42.7%)

Virus	Number
Influenza A	33
Influenza B	10
HSV 1	1

Results

Binax NOW overall performance

NOW	Versus DFA (n= 86)		NOW	Versus cell culture (n= 103)	
	Positive	Negative		Positive	Negative
Positive	32	0	Positive	30	3
Negative	17	37	Negative	13	57

Sensitivity = 65%
Specificity = 100%

Sensitivity = 69.8%
Specificity = 95%

Binax NOW Influenza A and Influenza B - specific performance

Virus	Sensitivity	Specificity
Influenza A	59%	100%
Influenza B	70%	100%

ImmunoCard STAT overall performance

ICStat	Versus DFA (n= 86)		ICStat	Versus cell culture (n= 103)	
	Positive	Negative		Positive	Negative
Positive	33	0	Positive	32	3
Negative	16	37	Negative	11	57

Sensitivity = 67.3%
Specificity = 100%

Sensitivity = 74.4%
Specificity = 95%

ImmunoCard STAT Influenza A and Influenza B - specific performance

Virus	Sensitivity	Specificity
Influenza A	61%	100%
Influenza B	80%	100%

Summary of discordant specimens

DFA Positive Rapid Antigen Test Negative (n=17)	Number
BINAX and ICStat Both Negative	16
BINAX Only Negative	1
ICStat Only Negative	0

Culture Positive Rapid Antigen Test Negative (n=13)	Number
BINAX and ICStat Both Negative	11
BINAX Only Negative	2
ICStat Only Negative	0

Results

Culture Negative Rapid Antigen Test Positive (n=3)	Number
BINAX and ICStat Both Positive	3
BINAX Only Positive	0
ICStat Only Positive	0

Summary / Conclusions

- There was no statistically significant difference between the performance of Binax NOW[®] Influenza A&B and ImmunoCard STAT![®] Flu A&B Plus assays when testing NPS specimens from adults. Specificity of these assays was high, in keeping with the manufacturers' claims. Sensitivity of both was lower than the manufacturers' claims, though not significantly so (1,2), and were consistent with published ranges for this class of test (3).
- Relative to DFA*, overall sensitivity and specificity of the Binax assay were 65% and 100%; of the ImmunoCard assay, 67.3% and 100%. *Relative to cell culture*, overall sensitivity and specificity of the Binax assay were 69.8% and 95%; of the ImmunoCard assay, 74.4% and 95%.
- Type-specific performances *relative to DFA and/or cell culture* were: Binax sensitivity and specificity influenza A : 59% and 100%; influenza B: 70% and 100%. ImmunoCard sensitivity and specificity influenza A: 61% and 100%; influenza B: 80% and 100%.
- Both assays were easy to perform, and neither experienced any testing failures. Interpretation of weakly reactive specimens was more challenging with the Binax assay, because of glare from the test device result window. Two specimens gave false results with the Binax assay only.
- Starplex S160 transport medium included in the Starswab[™] Multitrans System did not affect the performance of either Binax NOW[®] Influenza A&B and ImmunoCard STAT![®] Flu A&B Plus assays, thus is suitable for use with both of these test kits.

References

- Product insert: Binax NOW Influenza A&B test kit. Binax, Inc., ME.
- Product insert: ImmunoCard STAT Flu A&B PLUS. Meridian Bioscience, Inc., OH.
- Leland and Ginochio. (2007). Clin. Microbiol. Reviews 20 (1): 49-78.

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