Use of Antigen Detection for the Direct Analysis of Influenza A (pH1N1) in Respiratory Specimens

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Abstract

Background
In 2009, the global spread of influenza A (pH1N1) created a public health emergency. While realtime RT-PCR (RTPCR) is the gold standard laboratory test for pH1N1, DFA is an important tool for the rapid, sensitive direct detection of influenza antigen in patient samples. MAbs used for influenza DFA are known to target preserved type-specific epitopes, but the sensitivity of this method for pH1N1 was not known. Here, the performance of influenza A DFA for detection of pH1N1 is evaluated, relative to RTPCR/culture. One client hospital of our laboratory was considering the use of the BD Directigen™ EZ Flu A+B test to screen patients for pH1N1. Here, the performance of this test is also analyzed on a subset of respiratory specimens.
Methods
Respiratory specimens recovered from adult and pediatric patients and submitted to the Regional Virology Laboratory (RVL) between 04/23/09-07/03/09, were prospectively and concurrently analyzed by DFA (Light Diagnostics Influenza A and B; Millipore, Temecula CA), and by realtime RT-PCR (influenza A H1, H3, pH1N1). A subset of adult patient specimens was also analyzed by BD Directigen™ EZ Flu A+B test (BD Biosciences, Mississauga ON). Specimens testing DFA and RTPCR -negative were inoculated for standard respiratory virus culture (RMK [DHI Inc., Athens, OH] and HFL [RVL] cells).
Results
209 NP aspirates from pediatric patients and 405 NP swabs (S160-Naso, Starplex Scientific Inc., Etobicoke, ON) from adult patients were suitable for analysis. Relative to RTPCR and culture, respective sensitivities and specificities of DFA were: 74.7% (65/87) and 100% (318/318) for adults; 81.9% (59/72) and 100% (137/137) for pediatrics. Six DFA negative / RTPCR negative specimens yielded pH1N1 in culture; one on culture day 6, and five on day 8. 52 adult patient NP swab specimens were tested by Directigen™ EZ Flu A+B. Sensitivity versus DFA/PCR was 64.7% (11/17), while specificity was 86% (31/35).
Conclusions
Influenza A DFA is a useful test to rapidly identify patients with pH1N1. There were false negative DFA results (25.3% adult; 18.1% pediatric), but no false positive results. These data are consistent with the performance of DFA versus "conventional" influenza A, and validate the algorithm of reporting influenza A DFA positive samples to expedite patient care, and of confirming pH1N1 status with RTPCR. Based on the performance characteristics of the BD Directigen™ assay, this test was not endorsed as an option to extend the pH1N1 testing algorithm in place at RVL.

Introduction

The emergence and widespread transmission of a novel strain of influenza A (H1N1) in 2009 (pH1N1) caused a global public health emergency.

Molecular detection (real time RT-PCR) is the gold standard method for detecting and identifying pH1N1. However, DFA is a very powerful method of rapidly examining patient samples for the presence of influenza virus. Anti-influenza monoclonal antibodies target type-preserved NP antigen. While these reagents bind pH1N1, the sensitivity of DFA for this virus strain was unknown in the early stages of the pandemic.

At the Eastern Ontario Regional Virology Laboratory (RVL), we wished to use DFA to provide rapid positive pH1N1 results to members of the health care team. In the proposed algorithm, positive results would be provided in a preliminary report. Final results would be issued following pH1N1 detection and subtyping RT-PCRs performed on all specimens regardless of DFA result.

One hospital in our region strongly advocated for the use of the BD Directigen™ EZ Flu A+B test for the identification of influenza-infected patients. RVL performed parallel testing of samples using this test, DFA, and realtime RT-PCR.

Objectives

1. To evaluate the performance of Light Diagnostics™ Influenza A and B DFA relative to RT-PCR for the detection of influenza A(pH1N1)
2. To evaluate the performance of the BD Directigen™ EZ Flu A+B test relative to DFA / RT-PCR.

Methods

Specimens and specimen processing

- NP aspirates (NPA) were received for pediatric patient testing.
- NP swabs (NPS) were collected and transported using Starswab™ Multitrans™ System (S160 Naso; Starplex Scientific Inc., Etobicoke, ON).
- DFA, nucleic acid extraction and amplification, and inoculation of cell culture tubes were performed upon specimen arrival.

DFA

NPA specimens were treated with 0.5% N-acetyl cysteine, and cells were pelleted and resuspended in IMDM (Sigma, St. Louis, MO) – based buffer. NPS specimens in S160 were vortexed and transferred to centrifuge tubes. After 5 min. at 2600 rpm, cell pellets were resuspended in transport medium (0.2 – 0.4 mL).
Cell suspensions (10 µL) were applied and fixed to microscope slide test wells, and stained with 10µL of Light Diagnostics™ Influenza A and B monoclonal antibodies (Millipore Corp., Temecula CA). After 30 min., slides were washed, dried, coverslipped, and examined at 100 and 400X (Nikon Eclipse PF 100/F microscope, 450-490nm).

DFA Interpretation

DFA Result	Observation
Positive	≥2 columnar epithelial cells exhibiting specific fluorescence
Negative	< 2 columnar epithelial cells exhibiting specific fluorescence
Indeterminate	≥5 columnar epithelial cells per low power field; no specific fluorescence
Specimen unsuitable	< 5 columnar epithelial cells per low power field; no specific fluorescence

Nucleic acid extraction

200 µL of specimen were added to 300 µL of lysis buffer (MagNA Pure LC Total Nucleic Acid Isolation Kit), and mixed (30 min., room temperature). Total nucleic acid was extracted from lysed samples (500 µL input; 100 µL eluate) using the MagNA Pure Compact instrument and MPC Total Nucleic Acid Isolation Kit I (Roche Diagnostics, Laval, QC).

Real-time RT-PCR

Influenza A detection and pH1N1 typing were performed using the CDC Protocol of Realtime RT-PCR for Influenza A (H1N1) (1), using the AgPath-ID 1-step RT-PCR Kit (AM1005; Applied Biosystems, Foster City, CA) on the ABI 7500Fast platform (Applied Biosystems).

Primers and probe were from Applied Biosystems and Integrated DNA Technologies (Coralville, IA), respectively.

Cell culture

RT-PCR negative specimens were inoculated into cell culture tubes. 150 µL of specimen were added to 1 tube of RMK (Diagnostic Hybrids, Inc., Athens OH) and 1 tube of HFL (RVL) cells. Cells were incubated (33.5ºC, 8 days) and examined for cpe. Upon cpe development or on day 8, cells were trypsinized and processed for DFA. Cultures testing positive for influenza A by DFA were processed and analyzed by RT-PCR, as described above.

Lateral flow assay

A subset of specimens was analyzed using the BD Directigen™ EZ Flu A+B test (BD Biosciences, Mississauga ON). Testing was performed as per the manufacturer's instructions, and preceded DFA and RT-PCR.

Results

Specimen description I: Light Diagnostics™ Influenza A and B DFA

April 23 – July 3, 2009	Adult	Pediatric
Specimen type	NP swab	NP aspirate
Number specimens submitted	495	225
DFA unsuitable	74	8
DFA inconclusive	16	8
Number specimens used in analysis	405	209

Light Diagnostics™ Influenza A and B DFA performance

Adult (n= 405)

vs. PCR/Culture

	Positive	Negative
Positive	65	0
Negative	22	318

Sensitivity = 74.7%
Specificity = 100%

Pediatric (n= 209)

vs. PCR/Culture

	Positive	Negative
Positive	59	0
Negative	13	137

Sensitivity = 81.9%
Specificity = 100%

DFA and PCR negative / culture positive specimens

Patients	Number	Culture recovery	Method of detection	Detection and typing PCRs of culture supernatants
Adult	2	Day 8	cpe followed by DFA	pH1N1
Pediatric	4	Day 6 (1 specimen) Day 8 (3 specimens)		

DFA Unsuitable specimens

Patients	Number	PCR (+)	Result	PCR (-)	Culture	Result
Adult	74	6	pH1N1	68	67 Negative 1 Positive (day 8)	67 Negative 1 pH1N1
Pediatric	8	1	pH1N1	7	Negative	Negative

DFA Inconclusive specimens

Patients	Number	PCR (+)	Result	PCR (-)	Culture	Result
Adult	16	6	pH1N1	10	Negative	Negative
Pediatric	8	4	pH1N1	4	Negative	Negative

Specimen description II: BD Directigen™ EZ Flu A+B

June 1 – September 1, 2009	Adult
Specimen type	NP swab
Number specimens used in analysis	52

Results

BD Directigen™ EZ Flu A+B performance

(n= 52)

vs. PCR/Culture

	Positive	Negative
Positive	11	4
Negative	6	31

Sensitivity = 64.7%
Specificity = 86%

Summary / Conclusions

- There were no false positive pH1N1 DFA results using the Light Diagnostics™ Influenza A and B test. False negative DFA results were observed in 25.3% and 18.1% of adult and pediatric specimens, respectively. These findings are consistent with the performance of DFA in the detection of seasonal influenza A.
- In this study period, 18% (90/495) of adult NP swabs and 7% (16/225) of pediatric NP aspirates were of insufficient quality for DFA analysis and reporting. Of these 106 specimens, 17 (16%) were PCR positive, and one was initially PCR negative but pH1N1 positive on the final day of culture (day 8).
- Of the 614 DFA-acceptable specimens analyzed, 6 (1%) were DFA and PCR negative, but culture positive for pH1N1. This represented 3.5% (6/165) of all positive specimens.

  CPE developed on culture day 6 for one specimen and on day 8 for the remaining five. Thus, little virus was present in these original samples; biological amplification was required prior to detection. This is a reminder that while RT-PCR is the gold-standard method for pH1N1 detection, it is not an infallible technique.
- The performance characteristics of Light Diagnostics™ Influenza A and B DFA validated the strategy of performing influenza A DFA and reporting positive results in order to expedite patient care.
- The poor specificity of BD Directigen™ EZ Flu A+B precluded this assay from being endorsed as an option to extend the testing algorithm in place at RVL.

➢ Influenza A DFA is a valuable component of a testing algorithm that includes downstream RT-PCR detection and identification of pH1N1.

References

1. CDC Protocol of Realtime RTPCR for Influenza A (H1N1). CDC Reference #I-007-05

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