DIRECT DETECTION OF INFLUENZA VIRUS TYPES A AND B IN CLINICAL SPECIMENS DURING THE 2007/2008 RESPIRATORY SEASON: ANTIGEN vs. PCR

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ABSTRACT

Background
The rapid and specific detection of influenza virus during the respiratory season is critical for optimal patient management. Antigen-based testing is often used at the point of care to obtain the best turn-around time. However, these screening tests vary in their test performance and the ability to detect both influenza type A and B efficiently. Clinical decisions are sometimes made on the results of antigen screening tests alone without adherence to the recommendation of confirmation by cell culture or a molecular method. Many detection methods are available, including cell culture, shell vial, DFA, and molecular tests. Cell culture is considered the "gold standard", but has the distinct disadvantage of longer turn-around times compared to molecular methods. Likewise, molecular amplification has sometimes been referred to as the "platinum standard". This study compared antigen screening to a real-time PCR-based commercial assay in order to determine antigen test efficiency during the 2007/2008 respiratory season. Statistics were also provided by the Department of Health from to further support the efficiency of antigen testing as compared to molecular amplification.

Materials and Methods
Specimens (n = 190) consisted of throat and nasopharyngeal swabs submitted during the 2007/2008 respiratory season in Hawaii for influenza virus antigen testing (BD Directigen EZ Flu A+B). Additonal data (n = 1,071) from the Hawaii Department of Health, State Laboratory Division was included in this work. All specimens were further tested using a real-time RT-PCR method. Nucleic acid extraction was accomplished using the MagNA Pure LC Total NA Isolation Kit (Roche). Real-time RT-PCR amplification and detection was performed using the Cepheid real-time PCR influenza A/B assay in conjunction with the SmartCycler II real-time PCR instrument (Cepheid).

Results
The incidence of influenza virus type A and type B virus infection was 17.9% (34/190) and 11.0% (21/190) respectively in the patient sampling. Influenza virus was detected using RT-PCR in 28.9% (55/190) of the specimens. Alternatively, antigen testing (BD Directigen EZ Flu A+B) detected 7.9% (15/190) influenza type A and 1.0% (2/190) influenza type B. Influenza virus was detected using antigen testing in 8.9%(17/190) of the specimens. Compared to RT-PCR testing, the antigen screening assay missed detecting influenza type A and influenza type B in 55.9% (19/34) and 90.5% (19/21) of the cases respectively. Testing for combined influenza type A and type B virus from the Hawaii Department of Health revealed an influenza antigen sensitivity and specificity of 34.7 % and 100% respectively compared to RT-PCR.

Conclusions
These results indicate a much lower test performance for the antigen assays (e.g. BD Directigen EZ Flu A+B) than expected. This conclusion takes into account the specimen type, patient age and influenza virus type as specified by the manufacturer's test performance characteristics. Since sensitivity and not specificity was the least favorable, this study emphasizes the need for confirmatory testing (i.e. Cell culture or molecular) on antigen negative screening tests for optimal patient care.

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Table – 1 RT-PCR and Directigen Flu Antigen Results

ID No.	Ag (BD)	RT-PCR Result		Source	
		Flu A	Flu B	Directigen (Dry Swab)	PCR (M4)
1	B	Negative	Positive	Nasal swab	Nasal swab
2	A	Positive	Negative	Tracheal aspirate	Tracheal aspirate
3	A	Positive	Negative	Nasal aspirate	Nasal aspirate
4	A	Positive	Negative	Throat	NP
5	N/N	Positive	Negative	Tracheal aspirate	Tracheal aspirate
6	N/N	Negative	Positive	Nasal swab	Nasal swab
7	N/N	Positive	Negative	Throat	NP
8	N/N	Positive	Negative	Nasal swab	Nasal swab
9	N/N	Positive	Negative	Throat	Not specified
10	A	Positive	Negative	Throat	Nasal swab
11	N/N	Negative	Positive	Nasal swab	Nasal swab
12	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
13	A	Positive	Negative	N/P	NP
14	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
15	A	Positive	Negative	Nasal aspirate	Nasal aspirate
16	A	Positive	Negative	N/P	NP
17	A	Positive	Negative	Nasal swab	Nasal swab
18	A	Positive	Negative	N/P	NP
19	A	Positive	Negative	Nasal aspirate	Nasal aspirate
20	A	Positive	Negative	Nasal swab	Nasal swab
21	N/N (A)	Positive	Negative	Throat (NP)	NP
22	N/N (A)	Positive	Negative	Throat (NP)	NP
23	N/N (A)	Positive	Negative	Throat (NP)	NP
24	A	Positive	Negative	N/P	NP
25	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
26	N/N	Positive	Negative	Throat	Not specified
27	N/N	Positive	Negative	Throat	Not specified
28	N/N	Positive	Negative	Throat	NP
29	N/N	Positive	Negative	Nasal aspirate	Nasal aspirate
30	N/N	Negative	Positive	Tracheal aspirate	Tracheal aspirate
31	B	Negative	Positive	Nasal aspirate	Nasal aspirate
32	N/N	Positive	Negative	DOH form only	Throat
33	N/N	Positive	Negative	Nasal aspirate	Nasal aspirate
34	N/N	Positive	Negative	Nasal aspirate	Nasal aspirate
35	N/N	Negative	Positive	DOH form only	NP
36	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
37	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
38	N/N	Positive	Negative	Nasal aspirate	Nasal aspirate
39	N/N	Positive	Negative	DOH form only	NP
40	N/N	Positive	Negative	Throat	Not specified
41	N/N	Positive	Negative	Throat	Nasal swab
42	N/N	Negative	Positive	Nasal swab	Nasal swab
43	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
44	N/N	Negative	Positive	Nasal swab	Nasal swab
45	N/N	Negative	Positive	Nasal swab	Nasal swab
46	N/N	Positive	Negative	Nasal swab	Nasal swab
47	N/N	Negative	Positive	Bronch wash	Bronch wash
48	N/N	Positive	Negative	Throat	NP
49	N/N	Positive	Negative	Nasal swab	Nasal swab
50	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
51	N/N	Positive	Negative	Nasal aspirate	Nasal aspirate
52	N/N	Negative	Positive	Nasal aspirate	Nasal aspirate
53	N/N	Negative	Positive	Nasal swab	Nasal swab
54	N/N	Negative	Positive	Nasal swab	Nasal swab
55	N/N	Negative	Positive	Throat	Throat

Initially tested as negative from the dry swab with repeat antigen testing being positive from the M4.

Table – 2 Patient Demographics and Molecular Details

Average Age	48 yr
Age Range	6 w - 98 yr
Male	42.1%
Female	57.9%
RT-PCR (Ct) – Ag Neg	29.0 (Flu A) [Ave.]
RT-PCR (Ct) – Ag Pos	26.6 (Flu A) [Ave.]

Table - 3 Rapid Antigen Testing vs. RT-PCR

Oct 07-Jan 08

Rapid Influenza	RT-PCR +	RT-PCR -	Total
+	35	0	35
-	66	970	1036
Total	101	970	1,071

Sensitivity = **34.7%** (35/101)
Specificity = **100%** (970/970)
PPV = **100%** (35/35)
NPV = **93.6%** (970/1036)

DISCUSSION AND CONCLUSIONS

1. These results show a much lower test performance for the antigen assay (e.g. BD Directigen EZ Flu A+B) than expected (e.g. Sensitivity for Flu A in adults 63- 90% depending upon the specimen source). Additional antigen test performance is cited in the references.
2. As expected, in the respiratory season the specificity is favorable, but considering the low sensitivity of antigen testing the test should be used with an awareness of the usefulness in the particular clinical setting.
3. Antigen testing is a screen and a negative test should be confirmed by either cell culture or RT-PCR (i.e. data supported by this work.)

MATERIALS AND METHODS

Specimens and Controls
Specimens consisted of a nasopharyngeal swab (M4 media) and a throat swab (BBL™ CultureSwab™ EZ II Collection and Transport Systems) collected from symptomatic patients. Collection and handling was strictly followed according to the manufactures package insert instructions.

Specimen Processing and Nucleic Acid Extraction
Nucleic acid was extracted using the MagNA Pure LC RNA Isolation Kit – High Performance (#03542394001, Roche Applied Science, Indianapolis, IN). Lysis/Binding Buffer was added to the sample. A diluted internal control supplied by the ASR manufacturer was added to each sample prior to extraction. Purified nucleic acid was eluted in 50 uL of Elution Buffer and tested immediately or frozen at –70°C until testing was performed.

Influenza A and B Viral Antigen Detection
The Directigen™ EZ Flu A+B test (Becton, Dickinson and Co.) was used in most cases for the qualitative detection of influenza A and B antigens from nasopharyngeal or throat swabs of symptomatic patients. This rapid chromatographic immunoassay was performed according to the package insert instructions.

In a minority of the cases (n=16, no data presented) flu antigen detection was accomplished using the QuickVue Influenza Test (Quidel). The test used was the CLIA waived version and the intended use is for the rapid, qualitative detection of influenza type A and type B antigens directly from nasal swab, nasal aspirate, and nasal wash specimens. The test is "an aid in the rapid diagnosis of acute influenza virus infection." The package insert recommendation is that negative test results should be followed up by viral culture.

Nucleic Acid Amplification and Detection
Detection of influenza virus types A and B was performed using the Cepheid Flu A/B ASR assay (Cepheid), which includes an internal control. Reverse transcription to generate cDNA and real-time PCR amplification and detection was performed using the SmartCycler II (Cepheid) instrument.

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