Clinical Performance of Foam vs. Flocked Swabs Collected from the Anterior Nares in a Rapid Antigen Test for Influenza A & B

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ABSTRACT

Background: Posterior nasopharynngeal (NP) secretions collected by aspiration, wash or swab are preferred for laboratory testing to diagnose respiratory viral infections including influenza. However, anterior nares (AN) swabs are easier to collect and better tolerated by patients, and some rapid influenza antigen tests are FDA-cleared for this specimen type. There are limited data on the performance of such tests with respect to the effects of swab composition.

Objective: This study compared the clinical sensitivity and specificity of a polyurethane foam swab versus a nylon flocked swab collected from the AN for detecting influenza antigen.

Methods: For this prospective study, 100 children with symptoms suggesting influenza were recruited with informed consent from a large academic pediatric Emergency Department during the 2006-07 and 2007-08 influenza seasons. For each subject, a high absorbency foam (Puritan Medical) and high surface area flocked nylon fiber (Copan USA) swab specimen was obtained from left and right AN and placed in a transport tube (no transport medium). A polyester swab specimen was also collected from the posterior NP on each subject and placed in M4 transport medium. The AN specimens were tested for influenza antigen in the main hospital laboratory using the Quidel QuickVue® Influenza A+B Test. The posterior NP specimens in M4 were tested by culture, DFA, and RT-PCR (Prodesse). The results of the latter tests were used to establish the clinical performance of the Quidel test performed on the two AN swab types.

Results: Influenza was diagnosed by culture and/or DFA in 49 subjects- 34 influenza A and 15 B. Influenza was diagnosed by RT-PCR in 56 subjects- 37 influenza A and 19 B.

Standard Method	Swab Type	Antigen Test Sensitivity (%)	Antigen Test Specificity (%)
Culture and/or DFA	Foam	78 (38/49)	94 (48/51)
	Flocked	61 (30/49)	98 (50/51)
RT-PCR	Foam	71 (40/56)	98 (43/44)
	Flocked	54 (30/56)	98 (43/44)

Conclusions: High absorbency polyurethane foam swabs are preferable to high surface area nylon flocked fiber swabs for detection of influenza virus in the Quidel QuickVue® Influenza A+B Test.

BACKGROUND

Rapid and accurate point of care testing for diagnosis of influenza virus infection is desirable for outpatient management of children. There are several laboratory methods for detection of influenza virus in respiratory secretions including antigen detection by fluorescent microscopy or enzyme immunoassay, viral culture by traditional tube or rapid "shell vial" culture, and nucleic acid amplification. Currently, only antigen detection provides the potential for point-of-care testing with rapid turnaround-time allowing for management decisions while the patient is being seen by the primary care provider.

In order to facilitate the collection of respiratory secretions for rapid testing in various outpatient clinical settings, a relatively simple collection method is preferred. Among the specimen types that can be collected are swabs of the throat, anterior nares (NS), or posterior nasopharynx (NPS), and aspirates or washes (NPW) of the posterior nasopharynx. The anterior nares swab is the easiest to perform and is generally preferred by the clinical staff.

Little data exists for the performance of swab composition in rapid tests with this specimen collection.

MATERIALS & METHODS

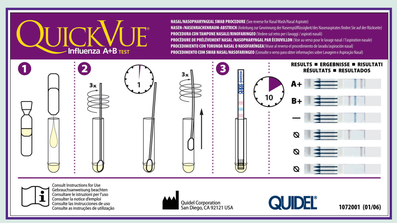
SUBJECTS AND SPECIMEN COLLECTION.

This study was approved by Children's Hospital IRB and informed consent was obtained for all subjects. Children presenting to the ED with symptoms suggestive of influenza infection during the annual winter outbreaks of 2007 and 2008 were eligible subjects. All specimen collections were performed by a research nurse or physician using standard methods and 2 techniques on each subject in sequential order as follows: using

Anterior nares swab (NS) collection with an absorbent foam swab on plastic shaft (Quidel Corp. Cat # 20171) manufactured by Puritan Medical and with a flocked swab (Copan Cat # 552C). In 2007 the foam swab was collected first and the flocked swab was collected second from the other naris. In 2008 the flocked swab was collected first and the foam second from the other naris.

Posterior nasopharyngeal swab (NPS) collection using a Dacron swab on flexible aluminum shaft (Puritan Medical Cat # 25-800 D) in 2 ml of M4 viral transport media. (Remel Inc. cat# 12520)

QUICKVUE INFLUENZA A + B TEST (Quidel Cat #20183). All rapid testing was performed in Nationwide Children's Hospital Diagnostic Virology Laboratory by one of several technologists. The kit reagents can be stored at room temperature. Testing requires 10-15 min with no more than 5 min hands-on-time.



VIRAL CULTURE

- ~0.2mL of posterior nasopharyngeal swab (NPS) specimen was inoculated into each of 2 R-Mix vials (Diagnostic Hybrids, Athena, OH; Cat # 96-0102) and incubated at 36°C
- One vial was stained ~42 hr later with Chemicon (Temecula,CA) SimulFluor Respiratory Screening reagent (Cat # 3296) and if positive for something other than RSV, the companion was scraped and stained with Chemicon Flu A/B direct FA reagent (Cat # 3121) and Chemicon Para 123/Adeno reagent (Cat # 3299).

DIRECT FLUORESCENT ANTIBODY PROCEDURE

- After removal of ~0.8ml for culture and PCR testing, the remaining volume of NPS specimen was washed with 10mL of phosphate buffered saline (PBS) in a 15mL sterile centrifuge tube, centrifuged and the cells resuspended in ~1.5mL of PBS.
- Two double-well cytospin slides were prepared using 200µL of the cell suspension per well, fixed with acetone and stained.
- One well was stained with Chemicon SimulFluor Respiratory Screening reagent, one with Chemicon Flu A/B direct FA reagent, one with Chemicon Para 123/Adeno reagent and one with Diagnostic Hybrids Human Metapneumovirus ASR (Cat # 01-035005-ASR)

NUCLEIC ACID EXTRACTION

- Total nucleic acids were extracted from 200µL of the NPS (in M4) using the bioMerieux easyMag Extractor and eluted into 55µL. For this study, the internal RNA control supplied by Prodesse (Waukesha, WI) was not used.

RT-PCR

- PCR was performed with the Prodesse ProFlu-1 ASR. (Cat # HSM58).
- Amplification was performed in an Applied Biosystems 7500 Sequence Detection System.
- Results of each run were determined by comparison of amplification curves with the positive and negative control curves and the cycle threshold (Ct).

Flocked Swab used in the study:

Foam Swab used in the study:

RESULTS

CLINICAL PERFORMANCE OF QUIDEL INFLUENZA A + B ANTIGEN TEST USING FOAM AND FLOCKED SWABS

Table 1.	Effectiveness of Swab Type for the detection of Influenza A & B using the QuickVue Influenza A+B Test			
n = 100		Flocked		
		A+	B+	neg
Foam	A+	21	—	10
	B+	—	7	3
	neg	2	1	56

Table 2.	Effect of Swab Type on Sensitivity and Specificity of the QuickVue Influenza A+B Test Relative to Culture and/or DFA or Rt-PCR or Expanded Gold Standard (Culture, DFA or PCR)		
Standard Methods	Swab Type	Antigen Test Sensitivity %	Antigen Test Specificity %
Culture and/or DFA	Foam	78 (38/49)	94 (48/51)
	Flocked	61 (30/49)	98 (50/51)
Rt-PCR	Foam	71 (40/56)	98 (43/44)
	Flocked	54 (30/56)	98 (43/44)
Expanded Gold Standard (Culture and/ or DFA and/or PCR)	Foam	71 (41/58)	100 (42/42)
	Flocked	53 (31/58)	100 (42/42)

Table 3.	Data Collected by Method				
	Foam	Flocked	DFA	Culture	ProFlu-1 PCR
A+	31	23	31	33	37
B+	10	8	11	15	19
neg	59	69	58	52	63
Total	100	100	100	100	100

- The intensity of the test band on most of the positive tests was greater with the foam swab.

Table 4.	Agreement with culture (%)			
	Foam	Flocked	DFA	Rt-PCR
A+	29	22	30	31
B+	9	8	11	15
neg	49	51	46	42
total	87	81	87	88

Table 5.	Agreement with PCR (%)			
	Foam	Flocked	DFA	Culture
A+	30	22	31	31
B+	10	8	11	15
neg	43	43	44	42
total	83	73	86	88

Table 6.	Agreement with Culture and/or PCR: Modified Gold Standard (%)		
	Foam	Flocked	DFA
A+	31	23	31
B+	10	8	11
neg	42	42	42
total	83	73	84

- Using **foam** swabs the QuickVue Influenza A+B test detected 38 of 49 specimens (78%) positive by culture and/or DFA and 40 of 56 specimens (71%) positive by RT-PCR (table 2).
- Using **flocked** swabs the QuickVue Influenza A+B test detected 30 of 49 specimens (61%) positive by culture and/or DFA and 30 of 56 specimens (54%) positive by RT-PCR (table 2).
- Both Prodesse ProFlu-1 ASR and culture detected 4 cases of RSV infection, 2 of which were detected by DFA. Additionally, DFA and home-brew Rt-PCR detected 2 cases of human metapneumovirus infection. Culture detected 3 cases of adenovirus infection of which 1 was also detected by DFA (data not shown).

OBJECTIVE

To compare the performance characteristics of two different types of anterior nares swabs collected in a pediatric Emergency Department (ED) using the Quidel (San Diego, CA) QuickVue Influenza Test A + B.

- We evaluated the sensitivity and specificity of anterior nares specimens collected with a polyurethane foam NS (manufactured by Puritan Medical, Guilford, Maine for Quidel Corp.) and a nylon flocked swab (manufactured by Copan Italia S.p.A., Bescia, Italy).

CONCLUSIONS

- High absorbency polyurethane foam swabs are preferable to high surface area nylon flocked fiber swabs for detection of influenza virus in the Quidel QuickVue Influenza A+B Test on anterior nares collections.
- Using the expanded gold standard there were no false positives.
- Feedback from medical personal that performed the collections, as well as patients/parents indicated a preference for the anterior nares collection due to its ease and comfort.
- The significant increase in sensitivity of the PCR testing indicates reflex testing in select patient populations is warranted.

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