Flocked Swabs and UTM-RT are Pre-analytical Tools Suitable for Rapid Antigen Kits, Direct Immunofluorescence, Culture and PCR Diagnostics Assay for Viral Infections.

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Abstract

Objective: Detection of antigens, nucleic acids, and isolation of microbes depend on pre-analytical devices used for specimen's collection. Diagnostic sensitivity varies with the number of cells and free organisms released in the transport system. It was reported that Flocked Swabs (FS) and UTM-RT (Copan, Brescia Italy) enhances analytical sensitivity of antigen detection, culture and nucleic acid amplification assays. To compare the Copan FS and UTM-RT to the Remel Dacron swabs (DS) and M4-RT for virus culture, epithelial cells recovery for direct immunofluorescence assay (DFA), antigens and nucleic acids stability for rapid kits and amplification assays from nasopharyngeal swabs (NPS) for the diagnose of respiratory viruses.

Methods: 5002 consecutive NPS, collected with FS and UTM-RT, submitted to the Virology Laboratory from Nov.1, 2004 to Apr. 30, 2006, were compared to 4288 NPS collected with DS and M4-RT from Nov.1, 2002 to Apr. 30 2004. Of the NPS collected with FS and UTM-RT, 261 were analyzed for Flu A/B and 375 for hMPV by PCR, 291 were tested with 4 rapid antigen kits for RSV, flu A and B. All NPS were tested by DFA, cells pellets were spotted on glass slides, fixed and stained with the para 1, 2, and 3, flu A, and B, RSV, adenovirus and hMPV FITC antibodies. NPS were inoculated into R-Mix shell vial cultures. After 48 hrs, cells were fixed, and stained with the Pool and hMPV reagents. Pool positives were typed. For PCR, nucleic acids were extracted with the miniMAG system. 5 ul purified nucleic acid was test with the RealArt™ kit for Influenza A/B or with an hMPV specific RT-PCR.

Results: 1318/4288 were positive in the NPS collected with DS and M4-RT: DFA/culture had 538 flu A, 635 RSV, 10 para 1-3, 35 adenovirus; 2099/5002 were positive in the NPS collected with FS and UTM-RT: DFA/culture had 663 flu A, 277 flu B, 879 RSV, 171 para 1-3, 109 adenovirus; 101 positive out of 868 tested for hMPV. Antigen tests had 121 RSV, 33 flu A, 37 flu B and 100 negatives. The PCR detected 102/261 Flu, 66/375 hMPV. NPS collected with FS and UTM-RT detected 2009/5002 (42%) positive compared to1318/2288 (31%) positive detected in NPS collected with DS and M4-RT.

Conclusions: NPS collected with flocked swabs in UTM-RT detected a higher number positive than NPS collected with DS and M4-RT. The Copan flocked swabs and UTM-RT collection and transport system is a universal system compatible with rapid antigen kit, DFA, culture and PCR and supports the detection and growth of hMPV.

Objectives

Detection of antigens, nucleic acids, and isolation of microbes depend on pre-analytical devices used for specimen's collection. Diagnostic sensitivity varies with the number of cells and free organisms released in the transport system. It was reported that Flocked Swabs (FS) and UTM-RT (Copan, Brescia, Italy) enhances analytical sensitivity of antigen detection, culture and nucleic acid amplification assays.

To compare the Copan FS and UTM-RT to the Remel Dacron swabs (DS) and M4-RT for virus culture, epithelial cells recovery for direct immunofluorescence assay (DFA), antigens and nucleic acids stability for rapid kits and amplification assays from nasopharyngeal swabs (NPS) for the diagnose of respiratory viruses.

Methods

•The 5002 consecutive NPS, collected with flocked swabs (a plastic shaft with a tip covered with nylon fibers), and UTM-RT from Nov.1, 2004 to Apr. 30, 2006 were compared to 4288 NPS collected with Dacron swabs and M4-RT during the previous years (November 1,2002 to April 30 2004).

•An aliquot of all original specimen was stored at -70°C for PCR testing. 261 NPS were analyzed for Flu A/B and 375 NPS for hMPV by PCR.

•The 291 of the original NPS specimens were tested as listed , 45 with the BinaxNow RSV, 50 with Clearview RSV 30 with X/pect RSV,36 with Coris RSV Respstrip, 25 with the BinaxNow Influenza A, 25 with the BinaxNow Influenza B, 45 Clearview Flu A/B, and 35 X/pect Flu A/B rapid antigen testing kits as per each manufacturer specifications.

•The remainder of each 5002 NPS samples was centrifuged to pellet the cells, a 10 smears were spotted on glass slides. After fixing, the cell smears were stained with the Parainfluenza 1, 2, and 3, Influenza A, influenza B, RSV, Adenovirus and hMPV FITC conjugated monoclonal antibodies. (Diagnostic Hybrids Inc).

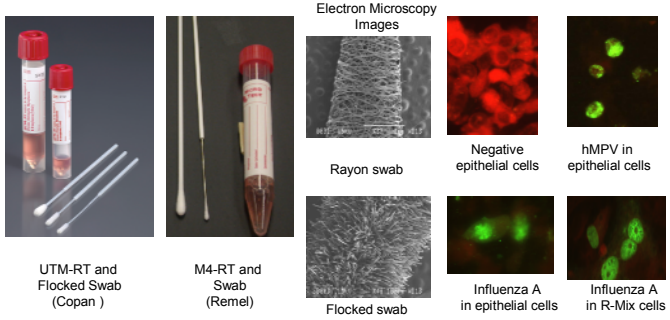
•The supernatant was inoculated into 3 R-Mix shell vial cultures (DHI).

•After 48 hours incubation the 2 shell vial cultures were fixed, 1 vial stained with the D3 Respiratory Screening Pool and one vial stained with the hMPV monoclonal antibody.

•The respiratory screening pool positive cultures , the remaining shell vials were washed with PBS, scraped and the cells were resuspended in PBS. A 9 smears slide was prepared with the suspended cells, fixed and stained with the individual monoclonal antibodies for identification. Only 698 NPS were tested for hMPV by DFA and culture

•For PCR testing the nucleic acid was extracted using the miniMAG system (bioMérieux). Five mL of 261 purified nucleic acid was tested with the real time RealArt™ kit for Influenza A and B; Five mL of 375 purified nucleic acid was amplified with an hMPV specific RT-PCR (Maertzdorf et al., 2004).

•All testing was performed by the bench technologist at the time of rotation.



Results

Influenza PCR Results

NPS (n=261)	DFA/ Culture	PCR
71	+	+
31	-	+
159	-	-

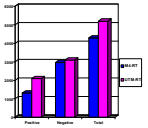
hMPV PCR Results

NPS (n=375)	DFA/ Culture	RT-PCR
47	+	+
19	-	+
308	-	-

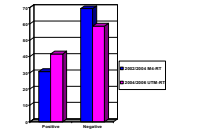
Rapid Antigen Results

Rapid kits	Binax/ DFA		Clearview /DFA		X/pect/DFA		Coris/DFA		Total	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
RSV	32	13	40	10	23	7	26	10	121	40
Flu A	16	9	8	12	9	11	NT	NT	33	32
Flu B	15	10	13	12	9	6	NT	NT	37	28
Total									191	100

FS/UTM-RT versus DS/M4-RT



FS/UTM-RT Positive versus DS/M4-RT



•Of the 5002 NPS, collected with Flocked Swabs and UTM-RT (November 1, 2004 to April 30 2006), DFA/culture had 663 [13.23 %] Flu A, 277 [5.53%] Flu B, 879 [17.57%] RSV, 171 [3.41%] Para 1-3, 109 [2.17%] Adenovirus, and 101/868 hMPV [15%].

•The 291 NPS tested with the rapid antigen tests had 121 RSV, 33 Flu A, 37 Flu B and 100 negatives.

•The PCR had 102 positive (71 DFA/culture positive and 31 PCR positive DFA culture negative, confirmed by another RT-PCR) and 159 negative for Flu A/B

•The 375 NPS tested for hMPV, 66 were positive (47 DFA/culture/RT-PCR, 19RT-PCR positive/DFA/culture negative confirmed by another set of primers) and 308 negative.

•The 4288 (November 1,2002 to April 30 2004). NPS, collected with M4-RT and swabs, DFA/culture had 538 [12.54 %] Flu A, 635 [14.8%] RSV, 110 [2.56%] Para 1-3, 35 [0.81%] Adenovirus; hMPV was not tested at that time, therefore was not included in the calculation of the positivity rate.

•The positive rates for each virus of the NPS collected with Flocked swabs and UTM-RT were higher than the NPS collected with the M4-RT; the overall positive rate of NPS collected with Flocked swabs was 41.94% compared to 30.73% for the M4-RT.

Conclusions

•NPS collected with flocked swabs in UTM-RT detected a higher number positive than NPS collected with DS and M4-RT. It increased the positivity by 11%, 41.94% compared to 30.73% for the M4-RT.

•NPS collected with FS in UTM-RT are compatible with the Binax, Coris, Clearview and Xpect rapid antigen tests detecting the same number of positive than DFA for the rapid detection of respiratory viruses

•NPS collected with flocked swabs and UTM-RT are compatible with amplification methods detecting additional influenza A/ and B and hMPV positive that were missed by DFA/culture.

•The Copan flocked swabs and UTM-RT collection and transport system is a universal system compatible with rapid antigen kit, DFA, culture and PCR and supports the detection and growth of hMPV.