

# Comparison of nasopharyngeal nylon flocked swabs with universal transport medium and rayon-bud swabs with a sponge reservoir of viral transport medium in the diagnosis of paediatric influenza

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This study compared a kit containing a nasopharyngeal nylon flocked swab and a tube with a liquid universal transport medium (UTM) with a kit containing a plastic-shafted rayon-budded swab with a sponge reservoir of viral transport medium for the molecular detection of influenza viruses in children. Respiratory samples were collected from 314 children aged <5 years with influenza-like illness (186 males; mean age  $2.32 \pm 2.27$  years) using both swabs in a randomized sequence for each patient. The flocked swabs permitted the detection of 28 influenza A (8.9%) and 45 influenza B (14.3%) cases, and the rayon-bud swabs 26 influenza A (8.3%) and 43 influenza B (13.7%) cases, with detection rates of 23.2 and 22.0%, respectively, and similar cycle threshold values. Paediatricians and laboratory staff were significantly more satisfied with both the simplicity ( $P < 0.0001$ ) and rapidity ( $P < 0.0001$ ) of the nasopharyngeal flocked swabs with UTM. These findings show that the flocked swabs with UTM and the rayon-bud swabs with a sponge transport medium are similarly efficient in preserving influenza virus nucleic acid, but that the kit containing a flocked swab with a UTM allows easier and more rapid collection and processing of specimens.

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## INTRODUCTION

Respiratory infections are the most common diseases of infants and children (Bourgeois *et al.*, 2006), and most are viral in origin (Esposito *et al.*, 2008a). The rapid identification of the virus causing each disease makes it possible to optimize clinical care, reduce antibiotic consumption and implement all of the procedures useful for limiting viral spread (Esposito *et al.*, 2003; Vega, 2005).

Antigen detection tests and PCR-based methods are both currently used to detect viruses in respiratory secretions (Esposito *et al.*, 2008b; Harper *et al.*, 2009), but the correct collection, preservation and transport of respiratory specimens are the first essential steps for the adequate identification of infectious agents. Although nasopharyngeal aspirates are usually considered the best specimens for identifying respiratory viruses, nasopharyngeal swabs are significantly more widely used because they are more pleasant for the patient, do not require any specific equipment or an experienced operator, and consequently can be performed routinely not only in hospitals, but also

in the community or at home (Heikkinen *et al.*, 2002; Lambert *et al.*, 2008). Moreover, the recently marketed nasopharyngeal swabs seem to be as efficient at collecting respiratory cells and detecting respiratory viruses as nasopharyngeal aspirates (Abu-Diab *et al.*, 2008; Spyridaki *et al.*, 2009; Walsh *et al.*, 2008).

There are various kits containing a nasopharyngeal swab and a tube with transport medium on the market, but only a few studies, mainly of adults, have compared their efficiency in collecting respiratory cells and preserving influenza virus nucleic acid (Daley *et al.*, 2006; Moore *et al.*, 2008). The aim of this study was to compare the efficiency of a kit containing a nasopharyngeal nylon flocked swab and a tube with a liquid transport medium with a kit containing a nasopharyngeal plastic-shafted rayon-budded swab and a sponge reservoir of viral transport medium for molecular detection of influenza virus nucleic acid in children.

## METHODS

**Patients.** Respiratory samples were collected between 1 January 2008 and 31 March 2008 in the outpatient clinic of the Department of Maternal and Pediatric Sciences of the University of Milan, Italy.

**Abbreviations:**  $C_t$ , cycle threshold; PDV, phocine distemper virus; UTM, universal transport medium.

Participation in the study was offered to children aged under 5 years with symptoms of influenza-like illness. In accordance with the indications given by the Italian Ministry of Health (<http://www.ministerosalute.it>), influenza-like illness was defined as an acute respiratory disease of sudden onset with fever (a temperature of  $>38^{\circ}\text{C}$ ), accompanied by at least one of the general symptoms of headache, generalized malaise, a feverish sensation (sweating and chills) and asthenia, and at least one of the respiratory symptoms of cough, pharyngodynia and nasal congestion. The study design was approved by the Ethics Committee of the University of Milan, and written informed consent was obtained from a parent or legal guardian of each child.

**Sample collection.** Two samples were collected from each patient and transported by means of two kits: one containing a flexible nasopharyngeal nylon flocked swab and a mini-tube with 1 ml liquid universal transport medium (UTM; Copan Italia), and the other a rayon-budded swab with a tube containing a sponge pre-impregnated with transport medium (Virocult; Medical Wire & Equipment). Using the swabs in a randomized sequence, two nasopharyngeal samples were collected from each child (one from each nostril) by trained paediatricians (L. C., L. G. and S. B.). The distance between the patient's nares and ear lobe was measured to estimate the length of insertion, after which the swabs were gently inserted towards the pharynx until resistance was felt and then rotated three times to obtain epithelial cells. They were then withdrawn and put into the tube containing the specific transport medium. All of the specimens were kept cool and delivered to the laboratory within 3 h of collection.

**Sample processing.** In the laboratory, each swab was processed in triplicate by three researchers (C. G. M., C. D. and A. V.) as indicated by the manufacturers: 190  $\mu\text{l}$  of the liquid transport medium for the flocked swabs was used directly, whereas the rayon-budded swabs were placed in a tube containing 1 ml liquid lysis buffer (the same amount as that contained in the mini-UTM), the tube was vortexed and incubated for 10 min at room temperature, and 190  $\mu\text{l}$  of the solution was used for extraction.

**PCR.** Viral RNA was extracted from all of the samples by means of a NucliSENS EasyMAG automated extraction system (bioMérieux), using phocine distemper virus (PDV) as an extraction/PCR inhibition control as described previously (Bosis *et al.*, 2005; Clancy *et al.*, 2008; Esposito *et al.*, 2008a). A total of 20  $\mu\text{l}$  eluate was reverse transcribed using a TaqMan Reverse Transcription Reagents kit (Applied Biosystems) in a reaction mixture containing 4.5 mM  $\text{MgCl}_2$ , 0.5 mM each dNTP, 2.5  $\mu\text{M}$  random hexamers, 20 U RNase inhibitor, 62.5 U reverse transcriptase and 0.1 mg BSA  $\text{ml}^{-1}$ . All of the reactions for real-time PCR were set up as singleplex PCRs in a total volume of 25  $\mu\text{l}$ , using TaqMan Universal Master Mix (Applied Biosystems), 200–800 nM primers, 100 nM TaqMan probe and 10  $\mu\text{l}$  cDNA template, and the products were amplified using an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems) with standard cycling parameters. The primer/probe sets (with the primer concentrations used in the mix) for influenza A were sense primer 5'-AAGACCAATCCTGTACCTCTGA-3' (800 nM), antisense primer 5'-CAAAGCGTCTACGCTGCAGTCC-3' (600 nM) and probe 5'-FAM-TTTGTGTTTACGCTCACCGTGCC-BHQ1-3'; those for influenza B were sense primer 5'-GAGACACAATTGCCTACCTGCTT-3' (200 nM), antisense primer 5'-TTCTTTCCACCGAACCAAC-3' (800 nM) and probe 5'-TET-AGAAGATGGAGAAGGCAAA-GCAGAACTAGC-Eclipse-3'; and those for PDV were sense primer 5'-CGGGTGCCTTTTACAAGAAC-3' (600 nM), antisense primer TTCTTCTCAACCTCGTCC-3' (800 nM) and probe 5'-VIC-ATGCAAGGGCCAATTCTTCCAAGTT-BHQ1-3'. The criterion for a positive reaction was a cycle threshold ( $C_t$ ) of  $<40$  cycles. Specimens with out-of-range PDV values (range=mean PDV

$C_t \pm 3 \times \text{SD}$ ; the mean PDV  $C_t$  was calculated on a reference set of 60 samples) were reprocessed, starting from RNA extraction, with no modifications to the protocol. After reprocessing, all specimens had in-range PDV values.

**Staff satisfaction.** Trained paediatricians and members of the laboratory staff were asked to record their satisfaction with the simplicity and rapidity using the swabs after the enrolment of each patient or the completion of the analysis of each pair of swabs by completing a 5-point scale (from 5 'very satisfied' to 1 'very dissatisfied').

**Statistical analysis.** The data relating to the paired specimens collected from 314 children (186 males, 59.2%), with a mean age of  $2.32 \pm 2.27$  years, were compared using SAS version 9.1 software (SAS Institute). Continuous variables were analysed using Wilcoxon's signed rank test or rank sum test as appropriate, and the categorical variables by means of contingency tables and a  $\chi^2$  or Fisher's test.

## RESULTS AND DISCUSSION

Table 1 shows the data regarding the detection of influenza viruses and the satisfaction of the study staff. The sensitivity of the two swabs was similar, with the flocked swabs detecting 28 influenza A (8.9%) and 45 influenza B (14.3%) cases, and the rayon-budded swabs detecting 26 influenza A (8.3%) and 43 influenza B (13.7%) cases. The detection rates were 23.2 and 22.0%, respectively. There were no discordant pairs and there were no instances where the rayon swab detected nucleic acid and the flocked swab did not. Specimens in which viral nucleic acid was detected only by flocked swabs displayed  $C_t$  values of 31.66 and

**Table 1.** Detection of influenza viruses using different nasopharyngeal kits, and study staff satisfaction

Satisfaction was based on a 5-point scale from 5 'very satisfied' to 1 'very dissatisfied'.

Variable	Nylon flocked swab with UTM	Rayon-budded swab with transport medium pre-impregnated sponge
Detection		
Influenza A ( <i>n</i> [%])	28 (8.9)	26 (8.2)
Influenza B ( <i>n</i> [%])	45 (14.3)	43 (13.7)
$C_t$ values		
Influenza A (mean $C_t \pm \text{SD}$ )	$28.71 \pm 4.61$	$29.95 \pm 5.16$
Influenza B (mean $C_t \pm \text{SD}$ )	$30.70 \pm 5.48$	$31.20 \pm 5.57$
Paediatricians' satisfaction		
Simplicity (mean $\pm \text{SD}$ )	$4.88 \pm 0.38^*$	$4.28 \pm 0.88$
Rapidity (mean $\pm \text{SD}$ )	$4.79 \pm 0.57^*$	$4.55 \pm 0.81$
Laboratory staff satisfaction		
Simplicity (mean $\pm \text{SD}$ )	$4.79 \pm 0.50^*$	$3.22 \pm 0.52$
Rapidity (mean $\pm \text{SD}$ )	$4.85 \pm 0.42^*$	$3.90 \pm 0.78$

\* $P < 0.0001$  versus rayon-budded swab with sponge transport medium.

27.88 (influenza A), and 31.04 and 35.72 (influenza B). For both methods, there was no co-detection of influenza A and influenza B viruses. The viral load data were also similar, with the flocked swabs giving viral load mean  $C_t$  values of 28.71 for influenza A virus and 30.70 for influenza B virus, and the rayon-bud swabs 29.95 for influenza A virus and 31.20 for influenza B virus. However, the paediatricians and laboratory staff were significantly more satisfied with both the simplicity ( $P < 0.0001$ ) and the rapidity ( $P < 0.0001$ ) of the nasopharyngeal flocked swabs with UTM.

Our study showed that the flocked swabs with UTM and the rayon-budded swabs with transport medium pre-impregnated sponge were similarly efficient in preserving influenza virus nucleic acid, but that the former were considered better in terms of the simplicity and rapidity of collection and laboratory testing.

Systematic evaluation of the aetiology of paediatric respiratory infections is increasingly being considered an important means of preventing their spread and rationalizing therapy (Harper *et al.*, 2009), which explains why it is useful to have validated kits that are appreciated by health-care staff and patients and that are supplied with an adequate transport medium for maintaining the vitality of the collected viral nucleic acid. A previous study has shown that flocked swabs collect significantly more epithelial cells than rayon swabs (Daley *et al.*, 2006). We found that there was no significant difference between the two kits in terms of viral detection rates and  $C_t$  values, although slightly better results were obtained using the flocked swabs with UTM.

Our main finding was that the paediatricians preferred the flocked swabs because they were more flexible and made it easier and quicker to collect the samples. In addition, the laboratory staff found that the kit containing a flocked swab and liquid transport medium was advantageous insofar as it allowed RNA extraction and PCR to be performed directly on the liquid without the need to add further buffer, whereas the kit containing a transport medium pre-impregnated sponge required an additional step that made the procedure more complicated, time-consuming and at risk of contamination.

One limitation of this study is represented by the fact that the interpretation of the results on simplicity and rapidity of collection and laboratory testing may be devalued by repeated scoring and clustering by the same staff members. This means that further studies that involve several swab collectors and laboratory researchers are required to confirm our results. Moreover, our aim was to compare the efficiency of the two kits in detecting influenza virus nucleic acid, but further studies are required to evaluate the sensitivity of the two transport systems with serial dilutions of positive samples of influenza A and B viruses. Finally, a complete comparison of the sensitivity and specificity of the two kits should also include detection of other respiratory viruses that are commonly found in respiratory

samples (e.g. respiratory syncytial virus, adenovirus, rhinovirus), and future research should address this aim.

In conclusion, both the flocked swabs with UTM and the rayon-bud swabs with a sponge reservoir of viral transport medium allow adequate collection, transport and preservation of nasal secretions for influenza detection. However, the kit containing a flocked swab with a liquid transport medium facilitated rapid specimen collection and processing. These factors should be considered together with local costs when choosing a product to use in clinical practice.

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