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Isolation of Viruses from Nasopharyngeal Secretions: Comparison of Aspiration and Swabbing as Means of Sample Collection

COLLEAGUES — Acute respiratory infections (ARI) are a worldwide, major health problem, especially among children [1, 2]. Although it has been possible to identify viral pathogens in only a small proportion of specimens collected from ill individuals in studies on the etiology of ARI, it seems that most of these illnesses are associated with viruses, such as influenza virus A and B, parainfluenza virus types 1 and 3, respiratory syncytial virus (RSV), adenovirus, and enterovirus [1–7].

The isolation rates may depend on sample transportation variables, such as the type of medium, temperature, and the time elapsed between specimen collection and processing. The rates may also be influenced by the susceptibility of the cell systems used for viral isolation or by the overall capacity of the laboratory to detect the agents. Another important variable may be the type of specimen collected for isolation: nasal and throat swabs, nasal washings, nasopharyngeal swabs, and nasopharyngeal aspirates are among the methods used to collect samples [3–9].

The present investigation was carried out to determine the adequacy of two of these techniques — nasopharyngeal swabbing and aspiration — for isolating virus in epidemiological studies of ARI in ambulatory children.

We studied 76 ambulatory children (0–5 years of age with mild-to-moderate ARI) who participated in a community study on the epidemiology of ARI. They attended the local clinic in March and April. During this period the minimum ambient temperature was 13 C, and the maximum was 34 C. The monthly rainfall was 0.32 and 0.41 mm^3, respectively.

Paired nasopharyngeal specimens were obtained from each child. First, swabbing was performed with a calcium alginate swab (Calgiswab® Type 1; Spectrum Diagnostics, Glenwood, Ill), which was inserted into the nasopharynx and maintained there for ~5 seconds with a slow backward and forward movement. The swab was then immersed and agitated in 2.1 ml of cold transport medium (veal infusion broth with 0.5% bovine serum albumin and antibiotics); the residual fluid in the swab was expressed against the side of the vial before being discarded. Then, the nasopharyngeal aspirate was obtained by inserting a feeding tube (#8 Bard; Vanguard International, Neptune, NJ), which was connected to a mucus trap (Nunc, Roskilde, Denmark), through the opposite nostril into the nasopharynx and by aspirating with the aid of an electric vacuum pump; cold transport medium (2.1 ml) was then sucked into the trap through the same tube used to obtain the specimen. Both samples were kept at 4 C until they were processed and inoculated into tissue cultures no later than 24 hr after collection.

The samples were centrifuged at 410 *g* rpm for 15 min at 4 C, and 0.1 ml of the supernatant was inoculated into duplicate tubes of Madin-Darby canine kidney (MDCK), rhesus monkey kidney ($LLC\text{-}MK_2$), Hep-2, and human fetal lung (MRC-5) cells. The tubes were then rotated in a roller drum for 1 hr at 33 C. The monolayers were examined daily for CPE. The kidney cells were kept for 14 days, fed with serum-free medium with trypsin (TPCK; Worthington Biochemical, Freehold, NJ), and were subjected to hemadsorption tests daily; the tests started on the third day postinoculation. The Hep-2 cells were discarded after seven days if they showed no CPE; the fibroblasts were monitored for 28 days. When hemadsorption occurred, indirect immunofluorescence (IF) tests were done on the cells by using monoclonal antibodies to influenza virus A and B, provided by Alan Kendal (Centers for Disease Control, Atlanta, Ga); the culture fluid was mixed with antisera against parainfluenza 1, 2, and 3 (Flow Laboratories, McLean, Va) for tests of hemadsorption neutralization. Adenoviruses and RSV were identified by IF, using antisera to adenovirus 6 (M.A. Bioproducts, Walkersville, Md) and bovine antisera to RSV (Wellcome Burroughs Diagnostics, England). Rhinoviruses and enteroviruses were differentiated by using acid lability tests at pH 3. Cytomegalovirus (CMV) was recognized by its characteristic CPE in fibroblasts.

Of the 76 samples studied, 36 (47%) swabs and 61 (80%) aspirates yielded viruses; 34 (94%) of 36 swab specimens positive for virus were also positive by aspiration, and 13 (33%) of 40 swab specimens negative for virus were also negative by aspiration. The difference in the isolation rates, as assessed by the McNemar test [10], was statistically significant ($P < .01$). Table 1 summarizes the findings. In only two cases were swab-collected specimens positive when their corresponding aspirate was negative. Despite this finding, more viruses of any type were isolated from material collected by aspiration than by swabbing, with the exception of RSV, which was isolated in only one instance from specimens obtained by both procedures. The mean time elapsed between inoculation of the 34 swab/aspirate-positive specimens and virus detection was 6.6 days for swabs and 5.9 days for aspirates.

Isolating viruses from individuals suffering from ARI is a difficult task. Conditions for collection, transportation, processing, and inoculation of specimens must be optimal, especially under field conditions when epidemiological studies are being performed. Our results clearly demonstrate the importance of the sampling procedure. More enterovirus, adenovirus, influenza virus, parain-

Table 1. Isolation of viruses from nasopharyngeal specimens obtained by swabbing and aspiration.

Virus	Isolation rates	
	No. isolated by swabbing (%)	No. isolated by aspiration (%)
Overall isolation (positive samples)	36* (47.0)	61† (80.0)
Enterovirus	19 (25.0)	35 (46.1)
Adenovirus	6 (7.9)	7 (9.2)
CMV	4 (5.3)	6 (7.9)
Parainfluenza virus 1–3	3 (3.9)	6 (7.9)
Influenza virus A, B	2 (2.6)	4 (5.3)
Rhinovirus	2 (2.6)	4 (5.3)
RSV	1 (1.3)	1 (1.3)
Total isolates	37	63

* One sample yielded two viruses.
† Two samples yielded two viruses.

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fluenza virus, rhinovirus, and CMV were isolated from aspirates than from swabs; additionally, their detection tended to occur earlier in cell cultures inoculated with material obtained by aspiration than in those exposed to secretions collected by the swab, a result strongly suggesting that more infectious material is collected with the former.

Reported viral isolation rates in cases of ARI from both community-and hospital-based studies in developing and developed nations range from 18% to 25.4% [3–8]. Even with our procedure with the lesser yield, nasal swabbing, we had an isolation rate of 47%, a result suggesting that our sample-handling procedures were appropriate. Moreover, although the majority of the viruses detected were enteroviruses (a phenomenon we consider to be specific to the ecological situation of ARI in Guatemala), our isolation rates for adenovirus, parainfluenza virus, influenza virus, and RSV are very similar to those reported from other series. With the aspiration technique, we isolated 28 respiratory viruses other than enterovirus. This represents an isolation rate for adenovirus, parainfluenza virus, influenza virus, RSV, rhinovirus, and CMV of 36.9%, which is still higher than the isolation rates reported by other centers around the world.

In summary, nasopharyngeal aspirates kept for <24 hr at 4 C in veal infusion broth enriched with bovine albumin, inoculated into MDCK, LLC-MK_2, Hep-2, and MRC-5 cells, and incubated at 33 C should provide satisfactory results in identifying viruses associated with ARI in community-based studies.

José Ramiro Cruz, Eduardo Quiñonez, América de Fernandez, Fiorián Peralta

Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

References

1. Anderson LJ, Patriarca PA, Hierholzer JC, Noble GR. Viral respiratory illnesses. Med Clin North Am 1983;**67**:1009–30
2. Bulla A, Hitze KL. Acute respiratory infections: a review. Bull WHO 1978;**56**:481–98
3. Monto AS, Ullman BM. Acute respiratory illness in an American community. The Tecumseh study. JAMA 1974;**227**:164–9
4. Soběslavský O, Sebikari SRK, Harland PSEG, Skrtić N, Fayinka OA, Soneji AD. The viral etiology of acute respiratory infections in children in Uganda. Bull WHO 1977;**55**:625–31
5. Belshe RB, Van Voris LP, Mufson MA. Impact of viral respiratory diseases on infants and young children in a rural and urban area of Southern West Virginia. Am J Epidemiol 1983;**117**:467–74
6. Sutmoller F, Nascimento JP, Chaves JRS, Ferreira V, Pereira MS. Viral etiology of acute respiratory diseases in Rio de Janeiro: first two years of a longitudinal study. Bull WHO 1983;**61**:845–52
7. Berman S, Duenas A, Bedoya A, Constain V, Leon S, Borrero I, Murphy J. Acute lower respiratory tract illnesses in Cali, Colombia: A two-year ambulatory study. Pediatrics 1983;**71**:210–8
8. Montanaro D, Ribera G, Attena F, Schioppa F, Romano F. Viruses associated with acute respiratory infections in children admitted to hospital in Naples, 1979–82. Bull WHO 1983;**61**:981–6
9. Hendry RM, Pierik LT, McIntosh K. Comparison of washed nasopharyngeal cells and whole nasal secretions for detection of respiratory syncytial virus antigens by enzyme-linked immunosorbent assay. J Clin Microbiol 1986;**23**:383–4
10. Conover WJ. Practical nonparametric statistics. 2nd ed. New York: John Wiley & Sons, 1980:130–3

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Minimal Infectious Dose of Rotavirus in Volunteers: Safety Issues

To the Editor—Recently, Ward et al. [1] reported the determination of the infectious dose of rotavirus in human volunteers. Their report addressed important questions but raised additional and possibly more-important concerns. The rotavirus preparation used to challenge the normal volunteers was filtrate of human stool obtained from an eight-month-old child hospitalized for acute gastroenteritis. The inoculum was "safety tested" by using procedures considered valid >20 years ago [2]. The preparation used was eventually shown to contain two rotavirus strains, and despite safety testing, the data presented suggest that other agents were present, i.e., three subjects experienced clinical illness without evidence of rotavirus shedding or seroconversion, and 13 subjects experienced temporary elevations of their serum transaminase activity.

Several questions emerge. (*1*) What safety testing should be required in the 1980s and 1990s before a filtrate of human stool can be used as an inoculum in volunteer experiments? (*2*) Should a fecal filtrate be used for experiments when the viruses are cultivatable? (*3*) How does one measure the importance of the question asked in relation to the risks accepted by the volunteers? (*4*) What is informed consent under such circumstances? (*5*) For how long and by what method should the volunteers be followed up after completion of the experiment?

The problem of defining guidelines for studies in which human stool filtrates are used is immense. The now-standard immunological tests, such as RIA or ELISA, require extremely large numbers of viral particles for a positive test and would thus be frequently scored as negative when a contaminating agent with a low minimal infectious dose was present. Such tests cannot be relied on to direct or prevent infection with known agents, such as hepatitis A virus; other investigators [3] have even administered gamma globulin after inoculation to prevent such infection. Even more-difficult problems are identifying still unrecognized or uncultivatable agents and the emergence of agents with which fatal outcomes are frequent (e.g., the human immunodeficiency virus).

There appear to be few questions sufficiently important to require using human stool as an inoculum when the virus is cultivatable. Human rotavirus, including the rotavirus used by Ward et al. [1], can be grown in vitro, be plaque purified, and be pre-