Evaluation of a Commercial Direct Fluorescent-Antibody Assay for Human Metapneumovirus in Respiratory Specimens[▽]

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The performance of a commercially manufactured direct fluorescent-antibody assay kit for human metapneumovirus was compared to that of reverse transcriptase PCR, the current “gold standard.” The kit demonstrated a sensitivity of 95.2%, a specificity of 100%, and an accuracy of 98.9%.

Human metapneumovirus (hMPV) is a recently identified member of the *Paramyxoviridae* family, subfamily *Pneumovirinae*. It has been demonstrated previously to be a significant viral etiology of respiratory tract infections, particularly among infants and children (3) but also among the elderly and immunocompromised patients (2, 6). Although isolation in cell culture is the “gold standard” for the detection of many other respiratory viruses, this methodology for hMPV is not feasible in most clinical laboratories since the introduction and standardization of methods for the recovery of hMPV (e.g., optimal permissive cell lines and the duration of incubation) have not become widespread. Currently, the gold standard for the diagnosis of hMPV infection is reverse transcriptase (9). PCR (RT-PCR), which is superior to virus isolation in cell culture (5). However, RT-PCR for the detection of hMPV may be beyond the capacity of many clinical laboratories and thus performed only in reference laboratories. Therefore, the detection of this relatively new virus is hampered by the absence of a routinely available commercial detection test with sufficient sensitivity and specificity. Direct fluorescent-antibody (DFA) staining of cells from nasopharyngeal aspirate samples is a rapid detection method for conventional respiratory viruses and is currently performed in many clinical virology laboratories (8). We evaluated the performance of a commercially manufactured DFA kit for the detection of hMPV (D³ DFA metapneumovirus; Diagnostic Hybrids Inc., Athens, OH) in respiratory specimens submitted to the clinical virology laboratory and compared the test characteristics to those of RT-PCR.

From November 2006 to mid-January 2007, respiratory specimens were collected from patients who presented at or were admitted to the hospitals of the McGill University Health Centre with respiratory illness. Specimens for which routine DFA testing for conventional respiratory viruses (see below) yielded negative results were subsequently tested by the DFA assay for hMPV. As well, all specimens submitted for respiratory virus testing over two arbitrarily selected consecutive days

were concomitantly tested for conventional respiratory viruses and hMPV by the DFA assay. In total, 454 respiratory specimens were analyzed by the DFA assay for hMPV. Of these, 401 (88.3%) were nasopharyngeal aspirates from children and 53 (11.7%) were respiratory specimens (sputum or bronchoalveolar lavage specimens) from adults admitted to the hospital (median age, 61 years; mean age, 58.5 years), including nine elderly patients (i.e., ≥65 years of age). All samples were collected, after informed consent was obtained from the patients or appropriate representatives, upon or shortly after patient presentation and were submitted in viral transport medium. After gentle trituration in Hank's salt solution (1×) to remove mucoid material, each specimen was divided into two aliquots, one for DFA testing and one for cell culture.

The specimen for DFA testing underwent two cycles of washing with phosphate-buffered saline, followed by centrifugation at 600 × *g* for 8 min at 4°C. The cell pellet was then resuspended in 50 to 80 μl of phosphate-buffered saline, and a sample was applied to a glass slide for the testing of each virus and then fixed in cold acetone for 10 min at 4°C. After the evaporation of the acetone, the appropriate commercial fluorescence-labeled monoclonal antibody was applied and the sample was processed according to the manufacturer's instructions. During the winter season, DFA testing for the following respiratory viruses is routinely performed (the routine assay kits and/or kit manufacturers are listed in parentheses): human respiratory syncytial virus (Chemicon; Chemicon International, Temecula, CA), adenovirus (Dako Imagen, Cambridgeshire, United Kingdom), influenza viruses A and B (Diagnostic Hybrids Inc., Athens, OH), and human parainfluenza viruses 1, 2, and 3 (ViraStat; ZymeTx Inc., Oklahoma City, OK). DFA testing for hMPV was performed according to the instructions of the assay kit manufacturer.

The specimen for cell culture was inoculated onto the routine CMK (cynomolgus monkey kidney), A549 (human lung carcinoma), and HEL (human embryonic lung fibroblast) cell lines, permitting the isolation of common respiratory viruses (i.e., human respiratory syncytial virus, adenovirus, influenza viruses A and B, and parainfluenza viruses 1, 2, and 3). If cytomegalovirus culture was requested or if the sample was from an immunocompromised patient, shell vial cultures using MRC-5 (normal human fetal lung fibroblast) and Mink cell

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lines were performed as well. Cell cultures using lines permissive specifically for hMPV isolation (e.g., LLC-MK2 and Vero monkey kidney cells) were not performed, as these cultures are not routinely done in our laboratory and the objective of this study was to compare DFA testing to RT-PCR analysis. Bronchoalveolar lavage specimens that were >5 ml in volume were centrifuged at $600 \times g$ for 10 min and resuspended in 2.0 ml of Hank's salt solution (1×) prior to undergoing processing as described above. The confirmation of DFA assay results was performed by RT-PCR at the Laboratoire de Santé Publique du Québec (the provincial reference laboratory), as previously described (4).

The sensitivity and specificity were calculated with RT-PCR as the gold-standard detection test. Test efficiency, used to assess the accuracy of the DFA kit, was calculated as the number of correctly identified positive and negative results divided by the total number of specimens tested (1).

Of the 454 respiratory specimens analyzed, 17 (3.7%) were of insufficient quality, precluding adequate DFA testing, and were thus excluded; all 17 samples were from pediatric patients. Of the remaining 437 respiratory specimens, 20 (4.6%) were positive for hMPV by the DFA assay. Of these, all 20 (100%) were confirmed to be positive by RT-PCR. All of the 20 positive samples were from children (median age, 11 months; mean age, 15 months; range, 2 to 61 months; male-to-female ratio, 1.2:1). The coisolation of another virus from the respiratory tract, as detected by the corresponding DFA testing or by cell culture, occurred with specimens from four patients; the concomitant viruses were enterovirus, cytomegalovirus, and adenovirus (twice).

There were 53 respiratory specimens from adults. None were positive for hMPV by DFA testing. Of the 417 specimens that were negative for hMPV by the DFA assay, 66 randomly selected specimens from children were referred for confirmation. Since hMPV has also been reported to be an important cause of viral respiratory infections among elderly patients (2), samples from the most elderly patients in our study ($n = 9$) were also referred for confirmation. Thus, 75 samples negative by the DFA assay were subsequently tested by RT-PCR. Of these, 74 (98.6%) were negative by RT-PCR. The one specimen with discrepant results was weakly positive by RT-PCR, possibly reflecting a low number of virion particles, below the analytical sensitivity of the DFA assay. Alternatively, this discrepancy (DFA assay negative and RT-PCR positive) may reflect differential capacities of recognition of the hMPV strains by the two methods, as has been described previously (7). Less likely, it may also represent a false-positive RT-PCR result. With RT-PCR as the gold standard, the test characteristics of the DFA kit are shown in Table 1. Based on these data, the kit performed very well in a clinical diagnostic setting, with >95% sensitivity, 100% specificity, and 98.9% efficiency.

TABLE 1. Test characteristics of DFA assay for hMPV relative to those of RT-PCR^a

hMPV DFA assay result	No. of samples with PCR result of:	
	Positive	Negative
Positive	20	0
Negative	1	74

^a Sensitivity is calculated as the number of DFA assay-positive samples divided by the number of RT-PCR-positive samples (20/21; 95.2%). Specificity is calculated as the number of RT-PCR-negative samples divided by the number of samples that also were negative by the DFA assay (74/74; 100%). The test efficiency is determined by the number of DFA assay-positive results confirmed by RT-PCR plus the number of DFA assay-negative results confirmed by RT-PCR, divided by the total number of RT-PCR results [(20 + 74)/95; 98.9%].

hMPV is a recently described virus whose clinical significance and epidemiology are being defined. The availability of a commercial DFA detection kit (D³ DFA metapneumovirus; Diagnostic Hybrids Inc., Athens, OH) with acceptable performance characteristics may be useful, not only for diagnostic purposes, but also to help elucidate the epidemiology of hMPV infection, particularly in specific groups (e.g., elderly and immunocompromised populations). Further studies assessing the cost-effectiveness of the implementation of this test in routine clinical virology laboratories are warranted.

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