NOTES

Prospective Study of Human Metapneumovirus Detection in Clinical Samples by Use of Light Diagnostics Direct Immunofluorescence Reagent and Real-Time PCR

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Human metapneumovirus (HMPV) has been recognized as a common cause of respiratory infections, especially in infants and young children, and may be second only to respiratory syncytial virus (RSV) as a serious respiratory pathogen in this age group (4, 5, 14, 16). HMPV has also been found to be a significant pathogen in compromised hosts and the elderly (2, 3).

Laboratory diagnosis of HMPV infection has been limited because of the difficulty in growing the virus and the lack of readily available diagnostic reagents. Isolation in conventional cell cultures can take 2 weeks or more, and cytopathic effects can be difficult to recognize. Shell vial centrifugation cultures can provide results within 2 days, but high-quality antibodies have not been commercially available (11). Thus, reverse transcription-PCR (RT-PCR) is the most widely reported test (9, 15).

Direct immunofluorescence (DFA) staining of clinical specimens, with results available within 2 to 4 h, is commonly used in clinical virology laboratories for the rapid diagnosis of respiratory viruses (9, 10, 15). In this study, we evaluated a commercial monoclonal antibody reagent to HMPV for its utility in the rapid diagnosis of HMPV infection (Light Diagnostics, Chemicon International [now part of Millipore], Temecula, CA).

Respiratory samples submitted to the Clinical Virology Laboratory for respiratory virus testing from February through May 2007, the peak HMPV season in Connecticut, were used (4). Cytospin-prepared slides were fixed in acetone and stained with SimulFluor respiratory screen reagent (Chemicon International, Temecula, CA) as previously described (10). Excess samples from children <5 years of age testing negative by the respiratory screen were selected. An extra slide was stained for HMPV on the day of receipt, prior to RT-PCR testing. Additional samples from older patients were included when HMPV testing was requested. HMPV DFA results were not reported, since the reagent was considered a developmental device at the time of the study. For RT-PCR, 200 µl was placed in lysis buffer and stored at −70°C until tested, usually within 1 to 7 days. RNA was extracted using the NuclSens EasyMag extraction system (bioMérieux, Durham, NC). The real-time TaqMan RT-PCR assay targeted the HMPV fusion protein gene as previously described (11).

Two hundred nasopharyngeal (NP) swabs (MicroTest M4 medium; Remel, Lenexa, KA) and 2 bronchoalveolar lavage samples were tested; 190 samples were from children less than 5 years old, 5 were from older children, and 7 were from adults. Forty-eight (23.8%) were positive for HMPV by RT-PCR, and 41 of these were positive for HMPV by cytospin-enhanced DFA (Table 1). Forty-two (87.5%) of the 48 positives were from children <2 years old. One adult on steroid therapy was positive by RT-PCR and DFA. One PCR-negative sample was read as showing one DFA-positive cell. On rereading, one DFA-positive cell was again observed; however, staining of a second slide from this sample was negative. For the purposes of the study, the RT-PCR result was considered the true result and the DFA result was considered false positive. Thus, DFA had a sensitivity of 85.4%, a specificity of 99.4%, a positive predictive value of 97.6%, and a negative predictive value of 95.7%. The differences between the results for cytospin-enhanced DFA and RT-PCR were not statistically significant (McNemar’s test; P = 0.0771). DFA staining of respiratory samples from children was performed on February 7, 2021 by guest http://jcm.asm.org/ Downloaded from

### TABLE 1. Comparison of Light Diagnostics HMPV direct immunofluorescence reagent and HMPV real-time TaqMan RT-PCR

<table>
<thead>
<tr>
<th>HMPV DFA result</th>
<th>No. of samples with indicated result for HMPV TaqMan RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>41</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
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epithelial cells was bright, speckled, and predominantly cytoplasmic, with essentially no background staining (Fig. 1). Due to the selection of samples that were respiratory screen DFA negative, the specificity of the Light Diagnostics HMPV reagent was not fully evaluated. However, three samples that were RSV positive and one that was influenza virus A positive by DFA were tested for HMPV, because HMPV was requested. All four were negative with the HMPV DFA reagent, and no nonspecific staining was observed.

Although the PCR was not performed as a quantitative assay, the cycle threshold \( (C_T) \) value in real-time PCR is an indicator of relative viral load, with lower \( C_T \) values indicating higher virus titers. The 41 DFA-positive samples had TaqMan RT-PCR results with \( C_T \) values of 19.13 to 35.81, with a median of 26.53. The seven RT-PCR positive but DFA-negative samples had \( C_T \) values of 31.55 to 39.23, with a median of 36.18 (Fig. 2).

Reports of immunofluorescence for detecting HMPV in clinical samples are limited. Our laboratory previously tested an anti-HMPV monoclonal antibody (MAb-8) developed at the CDC in

FIG. 1. Examples of ciliated columnar respiratory epithelial cells from patients’ samples stained with Light Diagnostics HMPV DFA reagent. Staining is bright, apple green, speckled, and predominantly cytoplasmic. Nonspecific background staining is negligible.

FIG. 2. \( C_T \) values according to HMPV DFA results for 48 samples positive by TaqMan RT-PCR. The 41 samples positive by HMPV DFA had a median (—) \( C_T \) value of 26.53 (range, 19.13 to 35.81), whereas the 7 samples negative by HMPV DFA had a median (—) \( C_T \) value of 36.18 (range, 31.55 to 39.23).
an indirect-immunofluorescence format (an unlabeled HMPV monoclonal antibody detected by a fluorescein-labeled antimonials conjugate) (11). Although MAb-8 performed acceptably in shell vial assays, it was not suitable for direct testing of clinical samples, due to nonspecific background staining, which made finding true positive cells difficult. In a retrospective study, Perivaller et al. used a pool of three monoclonal antibodies developed in their own laboratory in a DFA assay and detected 17 of 23 RT-PCR-positive samples (73.9%) (13). In a recent prospective study, the same laboratory detected 46 HMPV-positive samples in 871 patients (6). DFA detected only 26 (56.5%), and surprisingly, RT-PCR detected only 39 (84.8%) of the 46 positives. Ingram et al. (8) reported the use of a polyclonal antiserum provided by A. D. M. E. Osterhaus in 162 samples. Indirect immunofluorescence detected 24 (96%) and RT-PCR detected 24 (96%) of 25 true positive samples. These studies used NP aspirates and multiwell slides spotted with cell suspensions. In our study, NP swabs and cytospin-prepared slides were used.

Our study is important because the reagent will be marketed and thus available to any laboratory. The Light Diagnostics HMPV DFA reagent detected 41 (85.4%) of 48 RT-PCR-positive samples. The staff found the HMPV DFA reagent superior in readability to any of the respiratory DFA stains currently used in our laboratory. It is possible that the one DFA-positive/RT-PCR-negative sample may have been a true positive, since the other studies of DFA have found DFA-positive/PCR-negative samples (7, 8). True HMPV positives have been missed by RT-PCR when only one HMPV gene was targeted (1), as with our assay, or when amplification was inhibited. Although inhibition following EasyMag extraction is rare in our experience, nevertheless the presence of inhibitors was not excluded.

PCR assays are generally more sensitive than other methods for detection of viruses, can be automated, and are suitable for high-volume testing in reference laboratories. However, in the average hospital laboratory, molecular amplification tests are done at most once a day and usually only on weekdays or are sent to a reference laboratory. Similarly, in our laboratory, PCR for respiratory viruses is reserved for DFA-negative hospitalized patients, and if the sample is not received by 7:30 a.m., the PCR result is not reported until the following afternoon.

DFA is best suited for in-house testing, where testing can be performed on demand or batched and tested several times a day. In our experience, the use of cytospin-enhanced slides provides many fewer inadequate samples, better readability, and more-sensitive results than standard slide preparation, in which the cell suspension is pipetted onto a multiwelled slide (12). During the peak winter respiratory season, our laboratory performs 40 to 120 respiratory DFA screens a day, with a result reported within 2 h of sample receipt, 16 h a day, 7 days a week. Respiratory screen DFA in a single cell spot detects RSV, influenza viruses A and B, and parainfluenza viruses 1 to 3 with sensitivities of 95 to >100% of cell cultures. For adenovirus, however, DFA detects only about 70% of culture-positive samples (10). Even if used on a limited basis, with its high sensitivity and specificity, ability to multiplex, and rapid turnaround time, DFA still retains a clinical advantage over PCR as a first-line test. Nevertheless, DFA testing is manual and requires a committed, well-trained, expert staff and results are highly laboratory dependent.

In summary, 6 years after the first report of HMPV by van den Hoogen et al. (16), a high-quality DFA reagent has become commercially available. Incorporating these HMPV antibodies into antibody pools used to screen for multiple respiratory viruses will further facilitate detection of this common virus. The availability of a high-quality DFA antibody for rapid diagnosis of HMPV infection should be of significant benefit to clinical laboratories and the patients that they serve.

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REFERENCES