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Short communication

Detection of human metapneumovirus by direct antigen test and shell vial cultures using immunofluorescent antibody staining

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A B S T R A C T

Human metapneumovirus (hMPV) has emerged as an important etiologic agent of upper and lower respiratory tract infections, especially in young children. Although rapid and simple diagnostic methods for hMPV are needed in clinical laboratories, routine diagnostic tests are not readily available. The purpose of this study was to evaluate a commercial anti-hMPV monoclonal antibody for a direct antigen test and a shell vial culture of hMPV. In the pilot study, 15 nasopharyngeal aspirates from 15 children with acute respiratory tract infections positive for hMPV by reverse transcriptase polymerase chain reaction (RT-PCR) were tested. Both direct antigen test and shell vial culture detected hMPV in 14 of 15 (93.3%) nasopharyngeal aspirates at initial diagnosis. In the larger group prospective study, nasopharyngeal aspirates from 92 children with acute respiratory tract infections were tested for hMPV with RT-PCR, direct antigen test, and shell vial culture. Both direct antigen test and shell vial culture showed positivity for 4 out of 5 specimens positive by RT-PCR. These findings indicate that direct antigen test and shell vial culture would be reliable and timely methods for the diagnosis of hMPV infection in clinical laboratories.

Human metapneumovirus (hMPV) is a newly described RNA virus of the family *Paramyxoviridae*, and the subfamily *Pneumovirinae* along with respiratory syncytial virus (RSV) (Kahn, 2006). This virus is a respiratory viral pathogen that causes upper and lower respiratory tract infections in individuals of all ages, particularly in young children (Chung et al., 2006; Hamelin and Boivin, 2005; van den Hoogen et al., 2001), in both community and health care settings worldwide (Bastien et al., 2003; Falsey et al., 2003; Fouchier et al., 2005; van den Hoogen et al., 2004). The prevalence of hMPV in children with acute respiratory tract infections has been estimated to vary from 5% to 15% (Chung et al., 2006; Hamelin and Boivin, 2005; Kahn, 2006; Principi et al., 2006; van den Hoogen et al., 2001; Williams et al., 2004), making it the second or third most prevalent pathogen in children with acute respiratory tract infections, with only RSV and possibly rhinovirus being more prevalent (Garcia-Garcia et al., 2006; Koetz et al., 2006; Principi et al., 2006; Sarasini et al., 2006; Williams et al., 2004).

hMPV was first diagnosed by reverse transcriptase polymerase chain reaction (RT-PCR) (van den Hoogen et al., 2001), which is still a reference method for diagnosis, and the virus can also be isolated by conventional cell cultures using LLC-MK2 or Vero cells (Tang and Crowe, 2007). RT-PCR and conventional cell cultures, however, are not readily available in clinical laboratories. Due to the clinical importance of hMPV, rapid and simple diagnostic methods are required. Recently, FITC-conjugated monoclonal antibodies specific for hMPV have become commercially available. The purpose of this study was to evaluate the performance of these antibodies in the diagnosis of hMPV with a direct antigen test and a shell vial culture.

In the pilot study, 15 nasopharyngeal aspirates were obtained from 15 children positive for hMPV by RT-PCR. RT-PCR was performed using a Seeplex RV Detection Kit (Seegene Biotechnology Inc., Seoul, Korea) (Sung et al., 2008). The PCR products from each positive specimen were sequenced and were consistent with hMPV. The 15 hMPV strains detected in this study were classified as 11 strains of genogroup A2, one strain of genogroup B1, and 3 strains of genogroup B2. Follow-up nasopharyngeal aspirates were obtained from 2 patients on days 9 and 14, respectively, and from 1 patient on days 8 and 13 after initial diagnosis. All nasopharyngeal aspirates were stored at 4 °C for 2–6 days prior to direct antigen test and shell vial culture. Each RT-PCR positive specimen was centrifuged at 700 × *g* for 60 min, and each cell pellet was spotted onto a slide. After fixing, the slides were incubated with FITC-anti-hMPV monoclonal antibodies (D3 DFA metapneumovirus; Diagnostic Hybrids Inc., Athens, OH, USA) for 15 min at 37 °C. In addition, virus was cultured on R-Mix Too cells (Diagnostic Hybrids Inc.). One shell vial of cryopreserved R-mix Too cell monolayer was thawed for 4 min, and the medium was removed and new medium was added. Then, 200 μL of the patient specimen supernatant was inoculated and the

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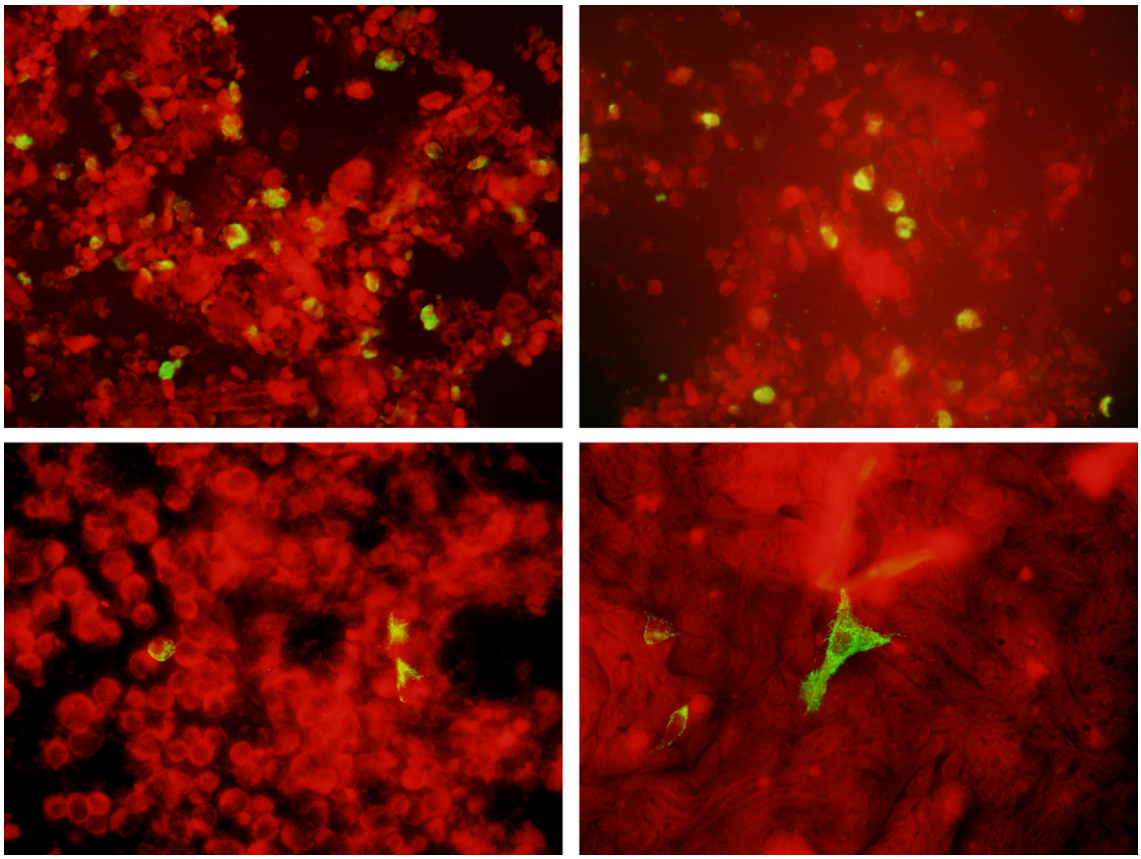

Fig. 1. Direct immunofluorescence staining of a nasopharyngeal aspirate with FITC-conjugated anti-hMPV monoclonal antibody. Green fluorescence was observed in the cytoplasm of hMPV-infected respiratory cells (upper left, upper right), and R-Mix Too cells (Diagnostic Hybrids Inc., OH, USA) showed a fine granular or reticular pattern of green fluorescence in the cytoplasm (lower left, lower right) (400×).

vials were centrifuged at 700 × *g* for 60 min at room temperature. After 48-h incubation at 36 °C in a CO_2 incubator, the coverslip containing the cells was fixed with acetone and stained with a D3 DFA metapneumovirus. The stained slide was examined using a fluorescence microscope. The patients' medical records were reviewed retrospectively for demographic findings and clinical diagnoses.

Of the 15 nasopharyngeal aspirates positive for hMPV by RT-PCR at initial diagnosis, 14 (93.3%) were positively stained with D3 DFA metapneumovirus on direct antigen test and shell vial culture. Using both methods, hMPV-infected human respiratory cells in nasopharyngeal aspirates and R-Mix Too cells showed fine granular or reticular fluorescence in the cytoplasm (Fig. 1). One nasopharyngeal aspirate from a pneumonic patient, which had been stored for 3 days before testing, was negative for hMPV by both direct antigen test and shell vial culture. Three follow-up nasopharyngeal aspirates, obtained from 1 patient on day 9 and from a second patient on days 8 and 13 after initial diagnosis, were positive by direct antigen test and shell vial culture, but one follow-up nasopharyngeal aspirates, obtained specimen taken on day 14 after initial diagnosis of pneumonia, was negative by both methods. Prior to testing, 2, 2, 5, 2, and 4 specimens had been stored for 2, 3, 4, 5, and 6 days, respectively. The median age of the 15 children with hMPV was 17 months (range, 4 months to 7 years), and 11 (73.3%) were male. Fourteen patients (93.3%) were less than 5 years old and 10 (66.7%) were less than 2 years old. Ten patients had been diagnosed with pneumonia, 4 with bronchiolitis, and 1 with clinical sepsis. All patients were negative for RSV, influenza virus, parainfluenza virus, and adenovirus by direct antigen test, shell vial test, and RT-PCR, and for coronavirus and rhinovirus by RT-PCR. Coinfection with bacteria was not observed.

In the larger group prospective study, nasopharyngeal aspirates from 92 children with acute respiratory tract infections were tested for hMPV with RT-PCR, direct antigen test, and shell vial culture according to established methods. All samples were negative for RSV, influenza virus, parainfluenza virus, and adenovirus at initial screening by direct antigen test with D3 Ultra DFA respiratory virus screening and ID kit (Diagnostic Hybrids Inc.). Both direct antigen test and shell vial culture showed positivity for 4 (80.0%) out of 5 specimens positive by RT-PCR. In 87 RT-PCR-negative samples, all were negative for hMPV by both direct antigen test and shell vial culture. The negative specimen in both direct antigen test and shell vial culture was a nasopharyngeal aspirate from a 34-month-old patient with pneumonia, whose antimycoplasmal antibody titer was 1:80 in particle agglutination test (reference range, <1:40). All 5 patients were less than 3 years, and three were male. Three patients had been diagnosed with pneumonia and two patients with bronchiolitis.

In this study, 18 (90.0%) out of 20 RT-PCR-positive specimens at initial diagnosis were positive for hMPV by both direct antigen test and shell vial culture. In previous reports, direct antigen test for hMPV with D3 DFA metapneumovirus (Diagnostic Hybrids Inc.) showed 90% sensitivity and 100% specificity compared with in-house RT-PCR (Zhang et al., 2007) and 74% sensitivity and 100% specificity compared with NucliSENS hMPV (bioMerieux, Durham, NC, USA) (Manji et al., 2007). Although the number of specimens was small, direct antigen test was at least equivalent to shell vial

culture in detecting hMPV-positive specimens. The high positivity rates of direct antigen test and shell vial culture may be due to the specimens all being nasopharyngeal aspirates collected at initial diagnosis, considered the best specimens for direct antigen test for respiratory viruses (Clinical and Laboratory Standard Institute, 2006). In addition, the use of D3 DFA metapneumovirus, a mixture of 3 hMPV-specific murine monoclonal antibodies that can detect both subtypes of hMPV, may have contributed to the high sensitivity of direct fluorescent antibodies. These antibodies were originally designed by Percivalle et al. (2005) and their sensitivity, specificity, positive predictive value, and negative predictive values were reported as 73.9%, 94.1%, 94.4%, and 72.7%, respectively (Percivalle et al., 2005; Gerna et al., 2006). Two specimens were negative by direct antigen test, and this specimen was also negative by shell vial culture. RT-PCR is sufficiently sensitive to detect viral RNA in respiratory specimens for several weeks or more after live virus shedding ceased (Tang and Crowe, 2007). The specimen that was RT-PCR positive but direct antigen test- and shell vial culture-negative may signify active infection of low viral load or a recent acute infection. Shell vial culture and direct antigen test may therefore supplement RT-PCR in determining the clinical significance of hMPV positive results of the latter. Three of 4 follow-up specimens were direct antigen test-positive, but 1 specimen, collected 14 days after initial diagnosis, was negative. Since patients with pneumonia and bronchiolitis may excrete hMPV for 2–3 weeks (Sarasini et al., 2006), it was not surprising to find that this patient was hMPV negative 14 days after initial diagnosis. The addition of hMPV direct antigen test using D3 DFA metapneumovirus to the current viral screening panel targeting RSV, influenza virus, parainfluenza virus, and adenovirus will provide a more complete diagnosis of respiratory viral infections in young children.

Shell vial culture results using R-Mix Too and D3 DFA metapneumovirus showed complete agreement with the direct antigen test results for all specimens, both at initial diagnosis and follow-up. hMPV replicates poorly in most conventional cell cultures used for diagnosis of respiratory viruses, including HEp-2, mink lung, and Madin–Darby canine kidney (MDCK) cells (Tang and Crowe, 2007). In addition, primary isolation of hMPV is facilitated by a low concentration of trypsin, which is not appropriate for isolating other respiratory viruses, except for influenza virus (Clinical and Laboratory Standard Institute, 2006; Tang and Crowe, 2007). hMPV was first isolated after passages in primary monkey kidney cells (van den Hoogen et al., 2001) and can be isolated in conventional cultures using LLC-MK2 or Vero cells (Landry et al., 2005). These isolations, however, require long incubation periods with several blind passages to observe cytopathic effects and are positive in only about 50% of samples for which nasopharyngeal aspirates are positive for hMPV by RT-PCR. In contrast, shell vial culture using the R-Mix Too cell culture method supported the growth of hMPV and showed 97.1% (33/34) sensitivity with direct fluorescent antibody positive specimens (Zhang et al., 2007). Shell vial culture using R-mix Too would thus be a rapid and reliable diagnostic method for hMPV.

In conclusion, direct and shell vial culture using D3 DFA metapneumovirus would be useful for the routine and rapid diagnosis of hMPV infection in clinical laboratories.

Conflict of interest

We do not have any conflict of interest relating to this article.

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