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Short communication

Detection of human metapneumovirus antigens in nasopharyngeal aspirates using an enzyme immunoassay

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ABSTRACT

Background: Human metapneumovirus (hMPV) is associated with acute respiratory tract infections (ARTI) in patients from all age groups.
Objective: To evaluate the performance of a rapid antigenic test for all hMPV genotypes.
Study design: Frozen nasopharyngeal aspirates from 93 individuals with ARTI were analyzed for the presence of hMPV antigens using an enzyme immunoassay (EIA, Biotrin Ltd.).
Results: The hMPV EIA showed a sensitivity of 81%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 77% compared to viral culture and RT-PCR.
Conclusion: The Biotrin hMPV EIA is a convenient alternative to cell culture for detection of hMPV with an excellent specificity.

1. Introduction

Human metapneumovirus (hMPV), a member of the *Pneumovirinae* subfamily in the *Paramyxoviridae* family, has been recently recognized as a leading cause of acute respiratory tract infections (ARTI) in children, elderly subjects and in those with comorbid conditions such as chronic obstructive pulmonary disease, asthma or immunosuppression.[1–3]

The most frequently used diagnostic methods for hMPV consist of a reverse transcription (RT)-PCR assay performed directly on clinical samples or isolation in cell culture followed by virus confirmation by RT-PCR.[1,4–6] However, RT-PCR assays are not commercially available and viral growth is fastidious and restricted to a limited number of cell lines such as LLC-MK2 cells. Incubation times of 2–3 weeks are typically required before hMPV cytopathic effect is seen in cell culture. Recently, direct fluorescent antibody staining of respiratory cells in nasopharyngeal aspirates (NPAs) has been reported using monoclonal antibodies,[7–9] which offers a rapid alternative to molecular assays for hMPV detection.

In this report, we compare the performance of conventional cell culture with that of a commercial enzyme immunoassay (EIA) for the detection of hMPV antigens in NPAs from subjects with ARTI. A specific RT-PCR assay was used to resolve discrepant results.

2. Methods

2.1. Specimens

For sensitivity analysis, a set of hMPV-positive NPAs collected between 2001 and 2007 were identified using viral culture on LLC-MK2 cells[1] and confirmed by RT-PCR of cell culture supernatants to contain hMPV nucleoprotein gene.[5] For specificity analysis, a set of hMPV-negative NPAs were selected from cell cultures that were negative for hMPV and contained or not other viruses detected by cell culture or RT-PCR.[1,5] The samples were randomly selected (only one per patient) based on available material and tested in a blinded manner.

2.2. hMPV EIA

All samples (93) were frozen at −80 °C for a period of up to 6 years before testing with the hMPV EIA as recommended by the manufacturer (Biotrin International Ltd., Dublin, Ireland); cell culture was done prior to freezing and not repeated as part of the current study. The hMPV EIA is an antigen capture assay for the qualitative detection of hMPV antigens in human respiratory specimens. The wells of the EIA plate were coated with a unique combination of monoclonal antibodies against the matrix and the fusion proteins of the virus. An extraction buffer was used to pre-treat samples before addition to the assay. Specific hMPV antigens, if present, bind to monoclonal antibodies coating the surface of the plate. A volume of 165 μl of respiratory

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Table 1
Human metapneumovirus (hMPV) RT-PCR cycle threshold (C_T) values and viral genotypes for selected concordant results (no. 1–5) and all discordant results (no. 6–23) between hMPV EIA and cell culture.

Sample	Date (sample)	Age/sex	hMPV EIA	Cell culture	hMPV PCR (C_T)	hMPV genotype
1.	03-26-2002	1 year/M	+	hMPV	41	B2
2.	04-10-2002	9 months/F	+	hMPV	16	A1
3.	03-14-2003	1 year/M	+	hMPV	33.5	ND[a]
4.	03-25-2003	5 months/M	+	hMPV	32.5	A1
5.	03-31-2003	1 year/F	+	hMPV	34.5	B2
6.	04-10-2002	5 months/M	Equivocal	hMPV	19	A2
7.	04-04-2003	ND[a]	Equivocal	hMPV	41.5	A2
8.	12-28-2005	5 months/F	Equivocal	hMPV	41	A2
9.	12-28-2005	3 months/F	Equivocal	hMPV	36	A2
10.	01-11-2006	72 years/M	Equivocal	hMPV	36	ND[a]
11.	05-06-2002	5 months/M	–	hMPV	30.5	A1
12.	02-05-2003	1 year/M	–	hMPV	38.5	A1
13.	02-19-2003	ND[a]	–	hMPV	35.5	A1
14.	04-16-2003	3 years/F	–	hMPV	41.7	A1
15.	04-17-2003	1 year/F	–	hMPV	41.5	A2
16.	04-22-2003	1 year/F	–	hMPV	42	A2
17.	04-30-2003	2 years/F	–	hMPV	40	B1
18.	01-04-2006	85 years/F	–	hMPV	41.5	A2
19.	01-19-2006	2 months/M	–	hMPV	39.5	ND[a]
20.	03-18-2003	1 year/F	–	hMPV	40	ND[a]
21.	04-15-2003	8 months/M	–	hMPV	–	ND[a]
22.	04-05-2002	7 months/M	–	hMPV	–	ND[a]
23.	04-03-2002	ND[a]	Equivocal	–	–[b]	ND[a]

[a] Not determined.
[b] Rhinovirus/enterovirus positive by RT-PCR.

secretions (for duplicate testing) was tested. Following a wash step, peroxidase-labelled anti-hMPV monoclonal antibodies were added. The bound antigen–antibody complex was then detected by addition of tetramethylbenzidine substrate (TMB), which turns blue in the presence of peroxidase. A stable yellow end product was achieved by the addition of a stopping reagent. The total incubation time for the assay was 2 h and 15 min. The presence or absence of hMPV was determined in relation to a calculated cut off value (COV). The latter was determined by adding a constant value of 0.1 optical density 450 units (as determined by the manufacturer during assay development) to the mean negative control value in each assay. Data comparison between different assay runs was facilitated by using an index value (IV) whereby sample absorbance is expressed relative to the assay COV. An IV <0.9 or >1.1 indicated sample negativity or positivity, respectively. Equivocality was indicated when the IV was in the range 0.9–1.1 inclusive.

2.3. RNA extraction, cDNA synthesis and real-time PCR

Discordant hMPV results between cell culture and the hMPV EIA were further resolved by the use of a real-time RT-PCR assay. Total RNA was extracted from NPAs using the QIAamp viral RNA minikit (Qiagen, Mississauga, Ontario, Canada) and complementary cDNA was synthesized using random primer and the Omniscript reverse transcriptase kit (Qiagen). cDNA was amplified using a previously described real-time PCR assay[5] with the SYBR^R Green Taq ReadyMix^{TM} (Sigma, St. Louis, MO) in a LightCycler instrument (Roche Diagnostics, Laval, Quebec, Canada). Cycling conditions were slightly modified and included an initial denaturation step of 30 s at 95 °C, followed by 50 cycles of 5 s at 95 °C, 5 s at 58 °C, and 25 s at 72 °C. Nucleotide sequences for selected hMPV nucleoprotein amplicons were determined by automated DNA sequencing (ABI Prism 3100; Applied Biosystems, Foster City, CA).

3. Results

Based on cell culture results, 60 NPAs were found to be positive for hMPV whereas 16 were negative (10 of the latter were positive for another virus). In addition, 17 NPAs found to be positive for other respiratory viruses by multiplex RT-PCR assay only[10] were also included in the study. Thus, in total, 27 viruses were used for specificity analysis including 10 human respiratory syncytial viruses, 4 parainfluenza viruses (1 PIV-1, 1 PIV-2 and 2 PIV-3), 2 adenoviruses, 1 coronavirus (NL-63), 1 influenza A virus, 1 rhinovirus, 1 herpes simplex virus, 1 measles virus and 6 co-infections with viruses other than hMPV.

Among the 60 NPAs that were positive for hMPV by cell culture, the hMPV EIA was positive in 43 (71.7%), negative in 12 (20%) and equivocal in 5 (8.3%). RT-PCR testing on discordant results revealed that all 5 EIA-equivocal as well as 10 of 12 EIA-negative NPAs were in fact true positive (Table 1). On the other hand, hMPV EIA was negative in 32 (97%) of 33 hMPV-negative NPAs by cell culture or RT-PCR. One rhinovirus/enterovirus RT-PCR-positive sample was found to be equivocal by hMPV EIA and was confirmed as hMPV-negative by RT-PCR (Table 1).

The performance of the Biotrin hMPV EIA was established with respect to cell culture complemented by the use of RT-PCR for discordant results (Table 2). According to this gold standard and counting EIA equivocal/RT-PCR-positive results as true positive, hMPV EIA had a sensitivity of 82.8%, a specificity of 97.1%, a positive predictive value of 98% and a negative predictive value of 77.3%. Because of the unavailability of specimens for repeat testing, exclusion of samples with equivocal results would result in a more valid estimate of test performance. In that case, the hMPV EIA had a sensitivity of 81.1% (95% CI: 68.0–96.6%), a specificity of 100%

Table 2
Performance of the Biotrin human metapneumovirus (hMPV) EIA with respect to cell culture and RT-PCR for discordant results.

hMPV	Cell culture/hMPV RT-PCR		
EIA	Positive	Negative	Total
Positive	43	0	43
Equivocal	5	1	6
Negative	10	34	44
Total	58	35	93

(89.7–100%), a positive predictive value of 100% (91.8–100%) and a negative predictive value of 77.3% (62.2–88.5%).

Ten hMPV samples identified as positive by cell culture and RT-PCR were not detected by hMPV EIA. This discrepancy was probably not related to a lack of detection of a specific hMPV genotype since we identified both hMPV types (A and B) with the EIA (Table 1). On the other hand, discrepant results correlated with viral load, and low viral load might limit detection by EIA. Thus, mean RT-PCR cycle threshold (C_T) values of 31.5, 34.7 and 39.1 were obtained for 5 selected EIA positive/RT-PCR positive samples, 5 EIA equivocal/RT-PCR positive samples and 10 EIA negative/RT-PCR positive samples, respectively (Table 1). We cannot rule out possible viral degradation in the two samples that were originally cell-culture positive in 2002 and 2003, but found to be negative by both EIA and RT-PCR on subsequent testing several years later.

4. Discussion

Development of sensitive and specific antigenic methods is important for rapid diagnosis of respiratory viral infections. RT-PCR has been considered the method of choice for hMPV detection due to the slow replication of this virus in very few cell lines[11] and, until recently, the unavailability of rapid antigenic detection methods.[7–9] However, "in-house" molecular methods require expensive laboratory equipment and carefully trained personnel. Despite its lower sensitivity compared to RT-PCR, the Biotrin hMPV EIA is a more rapid, less expensive and highly specific method for detection of hMPV in clinical samples that can be used in all microbiology laboratories. Importantly, the Biotrin hMPV EIA detected both major hMPV genotypes (A and B) from patients of different ages. One limitation of our study is the use of frozen NPA samples since cycles of freeze and thaw may lead to antigen degradation and potentially affect antigen detection; thus, we may have underestimated the true sensitivity of the EIA test. On the other hand, the frozen samples provided us with an opportunity to test different hMPV genotypes collected over several years. Another caveat is the generation of equivocal results with the EIA test, which implies the need for re-testing the same specimen with an alternative method, such as RT-PCR, or taking another sample for EIA testing (which was not feasible in our study). Additional prospective studies are needed for further evaluation of this assay using fresh respiratory samples and other types of specimens.

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