

## OBJECTIVE

Human Metapneumovirus (hMPV) is a causative agent of acute respiratory tract infections and is emerging as a significant cause of hospitalisation for such infections in young children and the immunocompromised worldwide. Here we describe the performance evaluation of an antigen detection enzyme immunoassay (EIA) system based on a unique combination of monoclonal antibodies directed to major hMPV proteins.

## METHODS

The diagnostic performance characteristics (sensitivity and specificity) of the hMPV EIA were evaluated using panels of confirmed hMPV positive or negative respiratory samples that were recovered from patients by a range of typical collection methods. Analytical performance parameters were estimated using viral cultures of known TCID<sub>50</sub>. Analytical specificity was evaluated by testing panels of potentially interfering viruses, bacteria and medications.

## RESULTS

The EIA exhibited close correlation to PCR in terms of diagnostic sensitivity. A high level of specificity was demonstrated against a panel of potentially cross-reactive, non-hMPV, specimens. The EIA was found to be reactive with hMPV subgroups 1 and 2 of genotypes A and B from viral culture.

## CONCLUSIONS

The hMPV antigen detection EIA is comparable to PCR in terms of key diagnostic performance characteristics, and it is an easy-to-use, cost-effective alternative to PCR suitable for use in the clinical laboratory.

## INTRODUCTION

Human metapneumovirus (hMPV) was discovered in 2001 by Van den Hoogen et al, and reported as a novel respiratory virus, causing acute respiratory tract illness (ARTI) in individuals of all ages, particularly children. Virtually all individuals have antibodies against the virus by the age of 5.<sup>(1)</sup> Serious infection has also been reported in the elderly and immunocompromised<sup>(2,3,4)</sup>. The virus has been detected in patients world-wide<sup>(4,5,6,7)</sup>.

Biotrin has developed a commercial assay for the detection of hMPV-specific antigens in human respiratory specimens. The test is specific for two major hMPV viral proteins, the matrix (M) and fusion (F) proteins. This assay has been assessed in multiple clinical centres, in comparison to reverse transcription – Polymerase Chain Reaction (RT-PCR) in most cases. Some of these assessments are summarised here.

## METHOD

The Biotrin hMPV assay is an antigen capture assay, which utilises monoclonal antibodies specific for the hMPV matrix and fusion proteins to detect hMPV-specific antigen in human respiratory specimens.

- 165µl of patient sample was mixed with 55µl of sample extraction buffer.
- 100µl of positive and negative controls and extracted patient specimens (in duplicate) were added to wells of the microtitre plate.
- The assay was performed as outlined in FIGURE 1.

Patient specimens are identified as positive/negative/equivocal by using the following formula:

$$\frac{\text{Mean patient OD450}}{\text{Mean negative control OD450} + \text{a constant of 0.1}} = \text{Index value}$$

| Index value | hMPV status |
|-------------|-------------|
| <0.9        | Negative    |
| ≥0.9 – ≤1.1 | Equivocal   |
| >1.1        | Positive    |

### Incubation times:

60 min shaking      60 min shaking      15 min static      Stop

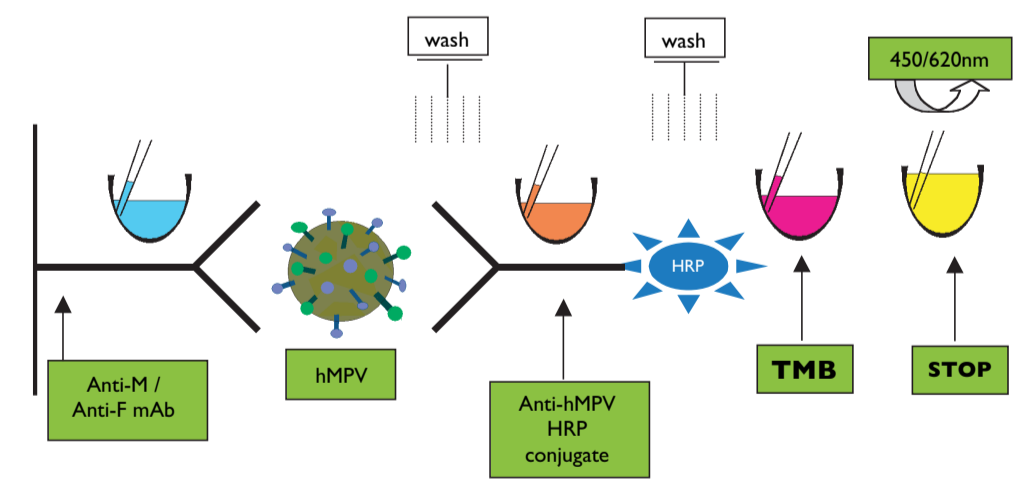


FIGURE 1: Biotrin hMPV antigen assay format.

## RESULTS

- hMPV-infected Vero cells were harvested and tested on the hMPV antigen assay. Results show reactivity on the assay of the four viral strains. (FIGURE 2). Differences in signal between the strains may be due to differences in virus titre.
- Assay of hMPV RT-PCR-positive specimens of known Ct value from clinical site 1 showed good correlation between Ct value and OD450 value on the Biotrin EIA (FIGURE 3). For primer sets and probes used at this site, refer to Table 1.
- Assay of serially diluted cultured hMPV of known TCID<sub>50</sub> value indicated excellent sensitivity of the assay for cultured virus; in this case, less than 100 TCID<sub>50</sub>/well (FIGURE 4).

- Assay of hMPV RT-PCR-negative nasopharyngeal aspirate specimens at clinical site 1 indicated assay specificity of 97% at this site in comparison to their RT-PCR. (TABLE 2)
- Assay of various viruses, micro-organisms, and potentially cross-reactive compounds at Biotrin demonstrated no cross-reactivity at the concentrations assayed. Whole blood may cause higher OD values at concentrations at which it is clearly visible in the sample; positive results with specimens that contain visible whole blood should be re-tested by an alternative method. (FIGURE 5, TABLE 3, TABLE 4).
- 18 RT-PCR positive and 8 RT-PCR negative specimens were assayed on the Biotrin EIA at clinical site 2; assay sensitivity of 89% and assay specificity of 100% were observed at this site, in comparison to their RT-PCR (Figure 6). For primer sets used at this site, refer to Table 5.

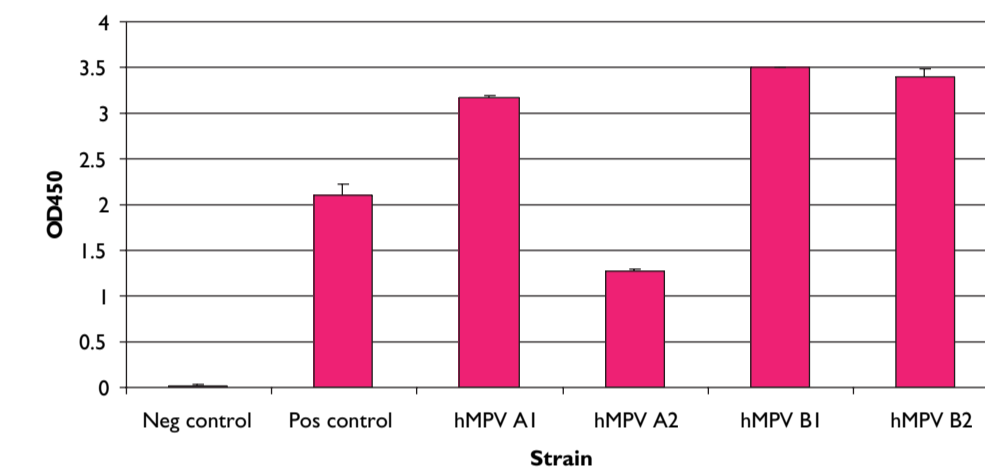


FIGURE 2: Detection of all four hMPV strains on the Biotrin EIA

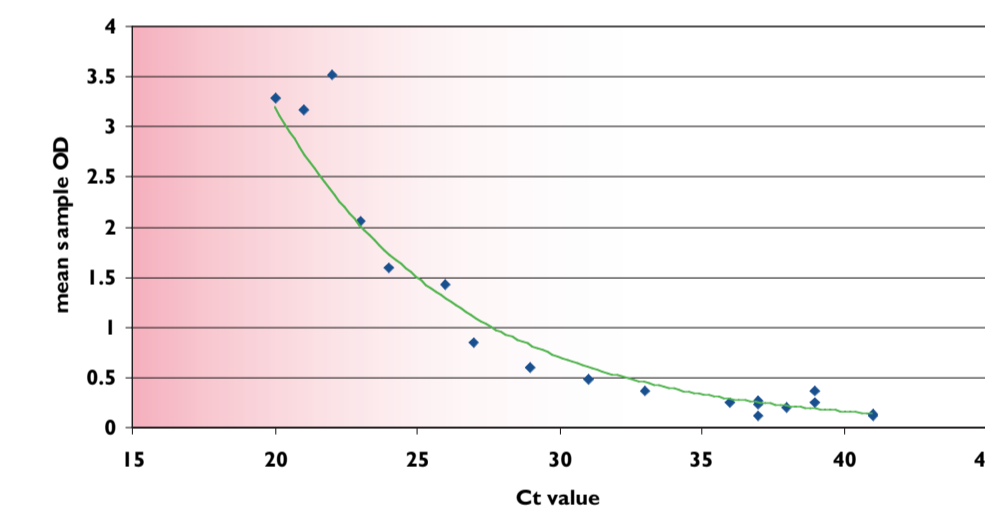


FIGURE 3: Clinical site 1: OD450 value vs Ct value for hMPV PCR-positive specimens

|  |
|--|
| Forward primer<br>(5'-CATATAAGCATGCTATATATAAAGAGTCTC-3')   |
| Reverse primer<br>(5'-CCTATTCTGCAGCATATTTGTAATCAG-3')  |
| probe<br>5'-FAM-TGYAATGATGAGGTTGCTCACTGCGGTTG-TAMRA-3'<br>(in which Y is either a C or a T residue). |

Table 1: Primer sets and Probes used in PCR assay at clinical site 1

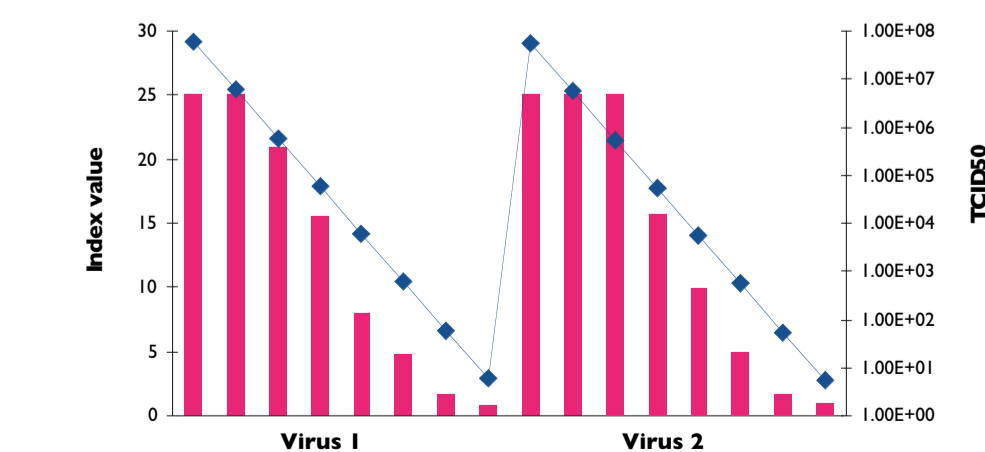


FIGURE 4: Clinical site 1: hMPV virus culture material (increasing dilutions) on the Biotrin hMPV antigen assay.

| Aetiological virus         | Sample type | n =        | Negative |
|----------------------------|-------------|------------|----------|
| Influenza A                | NPA         | 16         | 15       |
| Adenovirus                 | NPA         | 11         | 11       |
| RSV                        | NPA         | 3          | 3        |
| PIV-1                      | NPA         | 3          | 3        |
| PIV-2                      | NPA         | 1          | 1        |
| PIV-3                      | NPA         | 1          | 1        |
| Total                      | NPA         | 35         | 34       |
| <b>Overall specificity</b> |             | <b>97%</b> |          |

TABLE 2: Clinical site 1: hMPV RT-PCR-negative NPA specimens run on the Biotrin assay.

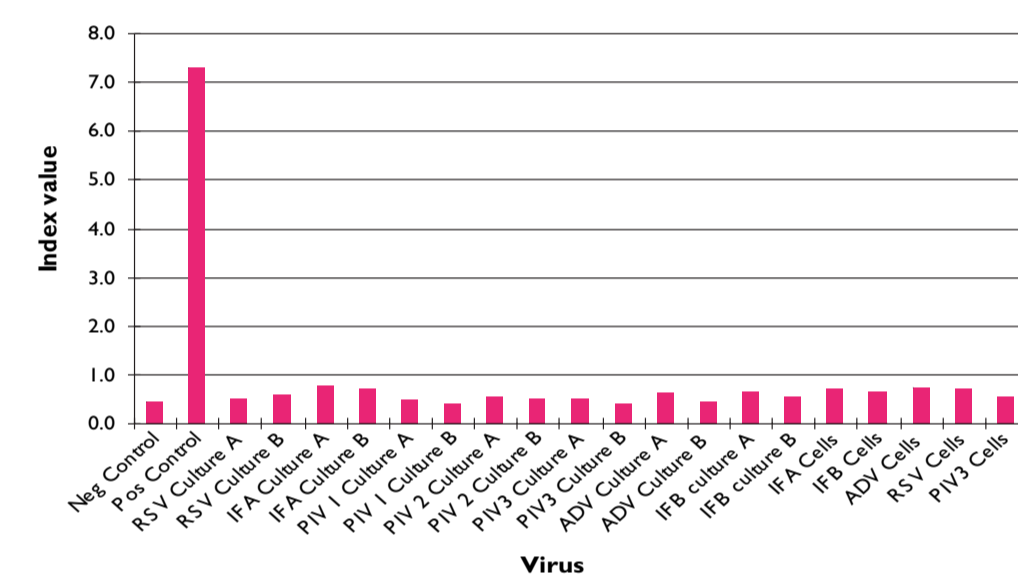


FIGURE 5: Clinical site 1: Potentially cross-reactive culture viruses on the Biotrin EIA; all with index <0.9

| ATCC Number | Micro-organism                  | Mean OD | INDEX |
|-------------|---------------------------------|---------|-------|
| 25922       | <i>Escherichia coli</i>         | 0.060   | 0.4   |
| 33657       | <i>Klebsiella pneumoniae</i>    | 0.057   | 0.4   |
| 700545      | <i>Listeria grayi</i>           | 0.065   | 0.4   |
| 49619       | <i>Streptococcus pneumoniae</i> | 0.063   | 0.4   |
| 49766       | <i>Haemophilus influenzae</i>   | 0.062   | 0.4   |
| 10231       | <i>Candida albicans</i>         | 0.061   | 0.4   |
| 31426       | <i>Neisseria gonorrhoeae</i>    | 0.068   | 0.4   |
| 25923       | <i>Staphylococcus aureus</i>    | 0.062   | 0.4   |

All organisms at approximately 10<sup>5</sup> cfu/ml in sample extraction buffer  
TABLE 3: Micro-organism cross-reactivity analysis of the Biotrin hMPV EIA

| Dilution   | Compound                           | OD450 | Index |
|--|------------------------------------|-------|-------|
| 1 part Extraction Buffer:<br>3 parts cross reactive material | Ext. Buffer + Extraction Buffer    | 0.080 | 0.5   |
|  | Ext. Buffer + Aspirin (5mg/ml)     | 0.067 | 0.4   |
|  | Ext. Buffer + Paracetamol (5mg/ml) | 0.073 | 0.4   |
|  | Ext. Buffer + Cough Drop (5%)      | 0.064 | 0.4   |
|  | Ext. Buffer + Mouth wash (10%)     | 0.036 | 0.2   |
|  | Ext. Buffer + Nasal spray (10%)    | 0.065 | 0.4   |
|  | Ext. Buffer + Cough medicine (5%)  | 0.069 | 0.4   |
|  | Ext. Buffer + PBS                  | 0.068 | 0.4   |
| Ext. Buffer + Whole blood not visible                        | 0.078                              | 0.5   |       |

TABLE 4: Potential Interfering substance analysis of the Biotrin hMPV EIA

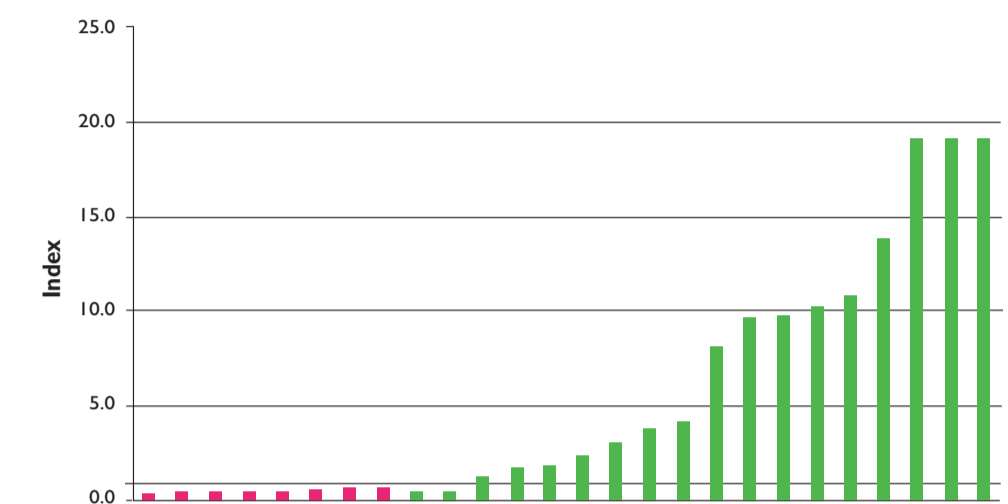


FIGURE 6: Clinical site 2: 8 RT-PCR hMPV negative and 18 RT-PCR hMPV positive specimens tested.

Sensitivity: 89% (16/18)  
Specificity: 100% (8/8)

### 347 bp from the fusion protein (F)

hMPV-F-FI-5' GAG CAA ATT GAA AAT CCC AGA CA 3'

hMPV-F-RI-5' GAA AAC TGC CGC ACA ACA TTT AG 3'

Table 5: Primer set used in PCR assay at clinical site 2

## CONCLUSION:

Overall, the Biotrin hMPV antigen assay has shown a high level of sensitivity and specificity with excellent correlation to PCR for clinical respiratory specimens at these clinical sites. The assay is potentially invaluable to the clinician in determining the hMPV profile of fresh or banked respiratory specimens and/or as a culture confirmation assay. With its fast and easy to use format, the Biotrin hMPV antigen EIA should be the assay of choice.

## REFERENCES:

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