



Cat No: V6HMPV
Format: 12x8 well EIA
hMPV-408-03



Human Metapneumovirus Enzyme Immunoassay

An enzyme immunoassay for the qualitative detection of human metapneumovirus antigen in human respiratory specimens.

English

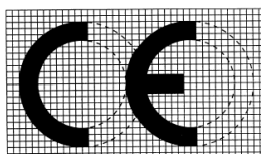




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Intended Use

The Human Metapneumovirus Enzyme Immunoassay is intended for the qualitative detection of human metapneumovirus antigen in human respiratory specimens.

Introduction

Human metapneumovirus (hMPV) was first reported as a cause of respiratory tract disease in June 2001 in Dutch children, and has subsequently been shown to be the causative agent of acute respiratory tract illness (ARTI) worldwide, with almost 100% of individuals seropositive by the age of 5 years^(1,2,3). An increase of antibody titre with age in children has been observed, which suggests boosting of antibody response by re-infection⁽¹⁾, and studies have shown that hMPV causes predominantly mild respiratory tract infections in healthy adults^(5,6). Most severe cases are found in paediatric patients, the elderly, or the immunocompromised, which suggests that naturally acquired infection produces partial protection against the disease. However, there is no indication of cross-protection between the different strains of hMPV⁽⁴⁾. A case has been described of the death of an immunocompromised child following infection by two genetically distinct hMPV strains during two consecutive seasons⁽⁸⁾.

The clinical manifestations of hMPV resemble those of human Respiratory Syncytial Virus (RSV), with symptoms ranging from mild influenza-like illnesses to severe lower respiratory tract infections such as bronchiolitis, croup, and pneumonitis^(1,7). Exacerbation of existing asthma and wheezing has also been observed⁽⁴⁾. Co-infections with other respiratory pathogens have been documented, although the contribution of the co-infection to the severity of disease has not been clarified^(9,10).

hMPV is the first human pathogen assigned to the genus *Metapneumovirus*, of the family Paramyxoviridae, subfamily Pneumovirinae⁽¹⁾. The morphology of the virus is typical of the Pneumovirinae; an enveloped virus approximately 150-200nm in diameter, 1000-10000nm long, with a helical nucleocapsid, appearing pleomorphic or spherical in electron micrographs. RSV is also a member of the Pneumovirinae, but hMPV is most closely related to avian pneumovirus serotype C, the type species of the metapneumovirus genus. The hMPV genome is a single strand of negative RNA of approximately 13kb coding for nine proteins⁽¹⁾. Two major lineages of hMPV have been identified, A and B, with sublineages A1 and A2, and B1 and B2, based on sequence analysis of the fusion (F) and attachment (G) genes^(5,11,12). Nucleotide and amino acid sequence identities between two strains representing the two major hMPV groups have been found to be in the region of 80 and 90% respectively⁽¹³⁾.

hMPV infection is community-acquired, and has been widely reported in countries such as the Netherlands⁽¹⁾, the United Kingdom⁽⁶⁾, Ireland⁽¹⁸⁾, France⁽¹⁴⁾, Canada⁽⁷⁾, the United States⁽¹⁵⁾, Australia⁽³⁾, South America⁽¹⁷⁾ and Hong Kong⁽¹⁶⁾ indicating global distribution. hMPV infection has been generally observed to occur during the colder months of winter and spring, similar to influenza and RSV^(6, 10, 16, 17).

Detection of the virus in respiratory specimens by reverse transcription-polymerase chain reaction (RT-PCR) has been reported in the literature^(2, 6, 16, 17, 18). Shell vial culture has also been documented, utilising monoclonal antibodies directed against virus antigens^(19, 20). Evaluation of several cell lines for the culture of hMPV has demonstrated poor applicability for the timely detection of the pathogen in the clinical setting⁽²¹⁾.



Assay Principle

The Biotrin hMPV EIA is an antigen capture assay for the detection of hMPV antigens in human respiratory specimens. The assay is designed to detect both cell-associated and free hMPV antigen in the specimen. Specific metapneumovirus antigen, if present, will bind to monoclonal antibodies coated onto the surface of the microtitre plate. Following a wash step, peroxidase-labelled anti-hMPV monoclonal antibodies are added. The whole complex is then detected by addition of tetramethylbenzidine substrate (TMB) which turns blue in the presence of peroxidase. A stable yellow end product is achieved by the addition of a stopping reagent.

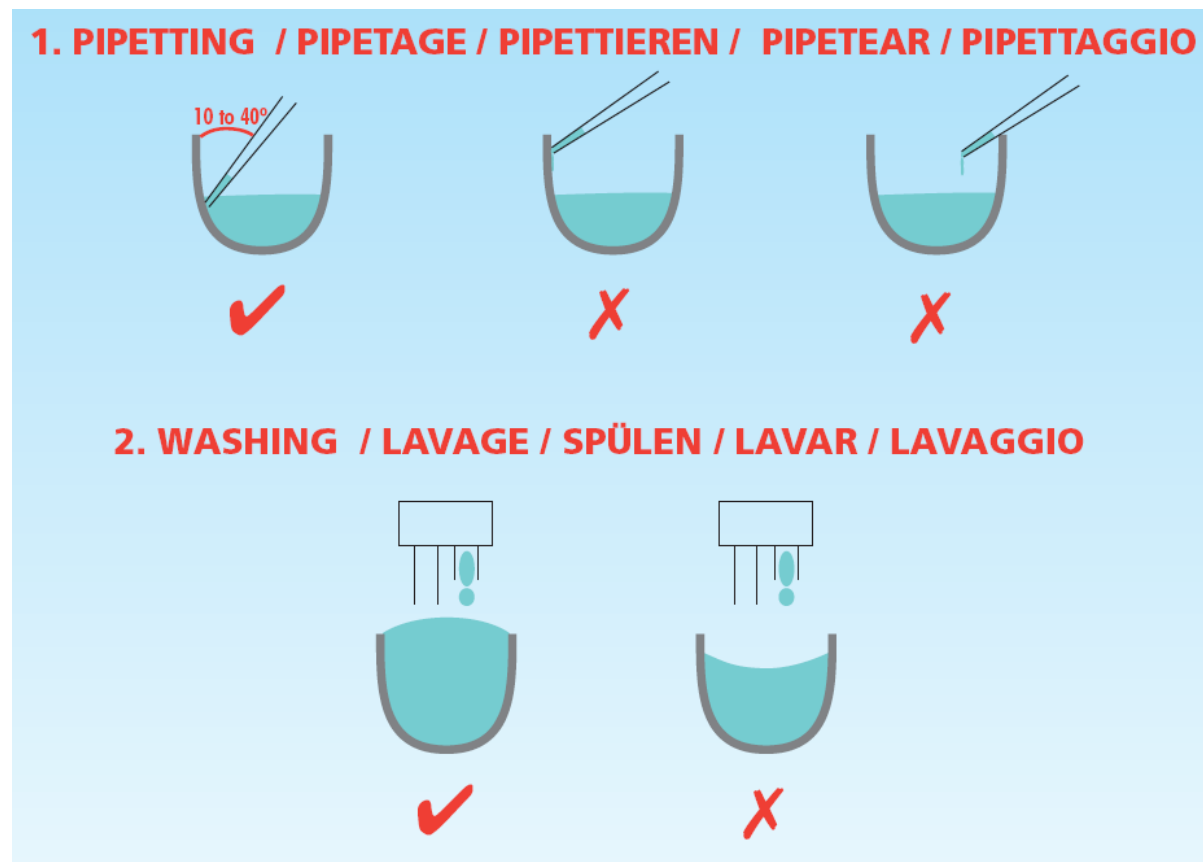
Precautions

Safety

- For *in vitro* diagnostic use only.
- Some reagents contain ProClin 950, Bronidox L and Thimerosal which may be toxic if ingested.
- Stop Solution contains sulphuric acid, which is corrosive. Avoid contact with the skin and eyes. If contact occurs rinse off immediately with water and seek medical advice.
- The substrate contains TMB, which may irritate the skin and mucous membranes. Any substrate that comes in contact with the skin should be rinsed off with water.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of as though potentially infectious.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by the mouth and never eat or drink at the laboratory workbench.
- Residues of chemicals, preparations and kit components are generally considered as hazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- The kit is intended for use by qualified laboratory staff only.

Procedural

- Due to the concentrated nature of the conjugate, high background may occur where pipetting and washing are not optimal – conjugate may remain on the upper surface of the well and cause high background signal. The method of pipetting into the wells



and of washing should be as indicated in figure 1:

Figure 1. Pipetting and washing methods.

- Reagent delivery should be aimed at midpoint of the side of the wells, taking care not to scratch the side with the pipette tip. The tip should be removed directly from the well without contacting the sides or top of the well.
- Adequate washing is essential for good performance of the assay. When using automatic plate washing devices an excess of wash buffer is added to the wells and aspirated as it is filled to create a positive meniscus.
- If negative control values are repeatedly outside of specification, or poor duplicate values are seen for patient specimens or controls, the assay should be washed manually i.e. after removal of the conjugate from the wells, the wells should be completely filled with wash buffer. The plate should be inverted to flick out buffer and tapped firmly on an absorbent surface. This wash should be repeated for a total of 4 washes.



- **Note:** Do not use glass or polystyrene tubes for storing the positive control.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers, with the exception of the Wash Concentrate and Stop Solution.
- Do not mix strips from different EIA plates of the same lot number.
- The presence of whole blood contamination of the respiratory specimen may lead to an elevation in background signals. Positive specimens with whole blood contamination should be confirmed by follow up sampling and/or alternative confirmatory methods.
- Deviation from the protocol provided may cause erroneous results.
- Allow all reagents to reach Room Temperature (20-25°C) in an incubator and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- High quality distilled or deionised water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background colour in the assay.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Remove only the volume of conjugate required for the assay. To reduce the risk of contamination, do not pour unused reagent back into the bottle or pipette directly from the bottle.
- Adequate agitation (shaking) is essential for optimal performance of the assay. The recommended shaking speed of the assay is 300 – 400rpm using an orbital diameter of 3mm.
- Do not allow the wells to dry at any stage during the assay procedure.
- Always keep the upper surface of the wells free of droplets. Drops should be gently blotted dry on completion of the procedural step.
- Ensure that the bottom surface of the plate is clean and dry before reading.



- Before commencing the assay an identification and distribution plan should be established.
- If using an automatic or semi-automatic EIA processor, it is essential to demonstrate equivalence with the manual test method for the Biotrin hMPV product.



Kit Components

Materials Provided

1. Coated ELISA plate

PLA	ANTIGEN
------------	----------------

12 x 8 wells coated with a combination of anti-hMPV monoclonal antibodies contained in a resealable pouch.

Clear rim

2. Positive Control

CONTROL	+	hMPV
----------------	----------	-------------

1 x 3mL of recombinant hMPV antigen in a stabilising buffer containing ProClin 950 and Bronidox L.

Red Cap

3. Negative Control

CONTROL	-	hMPV
----------------	----------	-------------

1 x 3mL of hMPV negative reagent containing ProClin 950 and Bronidox L.

White Cap

4. Enzyme Conjugate – Ready-to-use

CONJ	ENZ	1X
-------------	------------	-----------

1 x 17mL of anti-hMPV-HRP conjugate in a stabilising buffer containing Thimerosol.

Green Cap

5. Extraction Buffer – Ready to Use

SOLN	EX	1X
-------------	-----------	-----------

1 x 6mL of extraction solution containing 0.05% sodium azide

Blue Cap

6. Wash Concentrate

BUF	WASH	25X
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1 x 55mL of concentrated (25X) Tris buffered saline with Tween 20 and ProClin 950

Clear Cap



7. Substrate

SUBS	TMB
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1 x 17mL of tetramethylbenzidine (TMB) solution.

Brown Cap

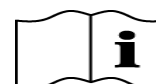
8. Stop solution

SOLN	STP
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1 x 17mL of 0.5mol/L H₂SO₄.

Clear Cap

9. Instructions for use.



Instructions for use.

Additional Materials Required

- Respiratory specimen collection equipment.
- Transport Media (optional).
- High quality distilled or deionised water.
- Accurate pipettes, micropipettes and disposable tips to deliver 10uL, 100uL 1mL and 5mL volumes.
- Test tubes or equivalent for sample preparation.
- Clean volumetric labware.
- Graduated cylinders.
- Plastic lid or sealing tape for microwell plate.
- Paper towels or absorbent paper.
- Timer.
- Room temperature incubator.
- Manual or Automatic washing device.



- ELISA plate shaker.
- ELISA plate reader with 450nm filter (additional 630 – 650nm filter is optional).

Storage and Stability

The kit is stable until the expiry date indicated on the outer box label provided it is stored under the recommended storage conditions.

- All unused components should be returned to 2-8°C storage immediately after use.
- **Note:** Do not use glass or polystyrene tubes for storing the positive control.
- Reconstituted Wash Solution is stable for 1 month when stored at 2-8°C.
- 8-well Strips should be stored in the resealable pouch along with the sachet of desiccant.

Specimen Collection and Storage

Fresh respiratory specimens are recommended for optimal assay performance.

Nasopharyngeal aspirates/nasal washes/bronchoalveolar lavage:

Use procedures appropriate for the age of the patient, and standard materials. Run samples on the assay as soon as possible after collection. For long term storage, samples can be diluted into an equal volume of transport media and stored at less than -20°C. Increased dilution will result in lower test sensitivity.

Nasopharyngeal swabs (Please refer to Limitations of Use):

Add swab specimens to 0.5–1mL of a suitable liquid transport system immediately after collection. Run samples on the assay as soon as possible after collection. If immediate testing is not possible, eluted liquid swab samples should be stored at less than -20°C. Repeated freeze-thaw cycles should be avoided.

Note: Results should be interpreted with caution due to the limited availability of performance data for swab-collected specimens. The user should independently assess the performance of the specific swabbing protocol in routine use at the collection site. The performance of conventional swabs (wound cotton or rayon), was compared to the newer flocced swabs (COPAN type) using cultured hMPV isolates and it was found that the flocced swabs yielded significantly higher recoveries.



Transport media:

The following transport media were found suitable for use in the Biotrin hMPV EIA.

- M4 Media
- M4-RT Media
- M5 Media
- Phosphate Buffer Saline (PBS)
- Hank's Balanced Salt Solution

Reagent and Specimen Preparation

Reagent Preparation

Reagent volumes are based on duplicate sample testing

Wash Solution

For each 8-well Strip add 4mL of Wash Concentrate to 96mL of deionised water. Prepared reagent is stable for 1 month if stored at 2-8°C.

All remaining reagents are supplied ready to use and are at working dilution.

Specimen Preparation

For each sample dispense 55uL of extraction buffer into a labelled test tube or equivalent. Add 165uL of the respiratory sample and mix. Samples diluted in transport media should be treated the same as undiluted samples, but a reduced level of sensitivity should be anticipated.

Note: Samples treated with extraction buffer should not be stored, if a repeat test is required, a fresh preparation should be used.



Assay Procedure

1. Allow all reagents and specimens to equilibrate to room temperature (20-25°C) in an incubator before use.
2. Determine the number of 8-well strips required. Establish an identification and distribution plan for controls and samples as indicated in Figure 1 (below). The first strip is suitable for testing two patient specimens, each additional strip allows for testing of a further 4 patient specimens.

Figure 1: Strip 1

A		Negative Control
B		Negative Control
C		Positive Control
D		Positive Control
E		Patient No. 1
F		Patient No. 1
G		Patient No. 2
H		Patient No. 2

Reagent volumes are based on duplicate sample testing. It is recommended that users familiarise themselves with the performance characteristics and validate the assay in their own laboratory before attempting singleton testing.

3. Remove the desired number of 8-well Strips, place in a plastic frame and cover with a plastic lid/sealant tape. Return the remaining strips to the pouch and reseal along with desiccant.
4. Prepare Wash Solution (Please refer to Reagent and Specimen Preparation section).
5. Prepare patient specimen (Please refer to Reagent and Specimen Preparation section).
6. Remove cover from strips and pipette 100uL, in duplicate, of the ready to use Negative Control, ready to use Positive Control, and prepared patient specimens to the wells.
7. Cover the wells with a plastic lid/sealing tape and incubate for 60 minutes (+/-2 minutes) at room temperature (20-25°C) with shaking in an incubator.



- 8.** Remove cover and wash each well 4 times with Wash Solution (completely fill the well if washing manually - see procedural precautions for manual washing instructions) or 500uL volume if washing with an automated plate washer – an excess of wash buffer is added to the well and aspirated as it is filled to create a positive meniscus). After washing firmly tap the plate against an absorbent paper towel.
- 9.** Pipette 100uL of the Enzyme Conjugate into all wells immediately after the wash step is completed.
- 10.** Cover the wells with a plastic lid/sealing tape and incubate for 60 minutes (+/-2 minutes) at room temperature (20-25°C) with shaking in an incubator.
- 11.** Remove cover and wash each well 4 times with Wash Solution (completely fill the well if washing manually - see procedural precautions for manual washing instructions) or 500uL volume if washing with an automated plate washer – an excess of wash buffer is added to the well and aspirated as it is filled to create a positive meniscus). After washing firmly tap the plate against an absorbent paper towel.
- 12.** Pipette 100uL of Substrate into all wells immediately after the wash step is completed.
- 13.** Incubate for 15 minutes (+/-1minute) at room temperature (20-25°C) in an incubator. Note, shaking is not required for this step.
- 14.** Pipette 100uL of Stop Solution into all wells and mix. Ensure that each addition is in the same sequence and time interval as the addition of Substrate.
- 15.** Read immediately with an ELISA plate reader.

Note: Dual wavelength reading is recommended at 450nm with 630nm as the reference wavelength. If this function is not available on the ELISA plate reader use a single wavelength reading at 450nm.



Interpretation of Results

The presence or absence of hMPV is determined in relation to a calculated Cut Off Value (COV).

Calculation of COV

$$\text{COV} = \text{Mean Negative Control (NC) OD} + 0.1$$

Interpretation (1): Absorbance

Samples with a mean absorbance reading greater than the COV x 1.1 are considered reactive (positive) for hMPV.

Samples with a mean absorbance reading less than the COV x 0.9 are considered non-reactive (negative) for hMPV.

Samples with a mean absorbance reading greater than or equal to COV x 0.9 and less than or equal to COV x 1.1 are equivocal.

Interpretation (2): Index Value

Data comparison between different assay runs is facilitated by using an index value whereby sample absorbance is expressed relative to the assay cut-off value. In this case, an index value <0.9 or >1.1 indicates sample negativity or positivity, respectively. Equivocality is indicated if the index value is in the range 0.9-1.1 inclusive.

$$\text{Index} = \frac{\text{Sample absorbance}}{\text{Cut-off Value (COV)}}$$

Samples which are neither reactive (positive) or non-reactive (negative) are considered equivocal and should be re-tested. If the re-test result is equivocal then a second sample should be taken and tested. If the follow up sample is equivocal, then the results should be confirmed by alternative methods such as PCR and viral culture.



Quality Control Criteria

The Positive Control and Negative Control must always be included to determine the validity of test results. Results of an assay are considered valid if the following criteria are met.

1. The mean absorbance of the Positive Control is greater than or equal to 0.8 Optical Density Units (450nm).
2. The mean absorbance of the Negative Control is less than or equal to 0.15 Optical Density Units (450nm).

If the above criteria are not met the assay is considered invalid and must be repeated. See procedural precautions for wash method in the case of repeated out-of-specification negative control results.

Limitations of Use

- Results must be correlated with the patient's clinical and epidemiological profile and other clinical laboratory results in making the diagnosis of human metapneumovirus infection.
- Excessive dilution of samples in viral transport media is not recommended (a preferred dilution of 1:1, if dilution is required e.g. 2mL of sample and 2mL of transport media). Consideration must be given to the means of sample collection - there is considerable variation between clinics in the protocols used for collection and handling of respiratory specimens.
- We recommend that the positive control is stored in polypropylene or high density polyethylene tubes. Storage in other materials such as glass or polystyrene may cause rapid deterioration of the positive control.
- Nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage sample types are the preferred sample type for use in the Biotrin hMPV EIA.
- The performance of nasal swab sample types was evaluated on the Biotrin hMPV EIA and found to have adequate performance. However results should be interpreted with caution due to the nature of the sample and the laboratory should verify the performance of the swab in routine use independently.
- A non-reactive (negative) result does not exclude the possibility of hMPV infection, patients with symptoms consistent with hMPV infection should be tested by some other method for hMPV status confirmation.
- Insufficient data is available to support the interpretation of results of tests performed on other body fluids.



- The presence of whole blood contamination of the respiratory specimen may lead to an elevation in background signals. Positive specimens with whole blood contamination should be confirmed by follow up sampling and/or alternative confirmatory methods.
- Test performance may be affected by deviation from the procedure, interpretation or recommended precautions.
- Assay performance has been validated based on testing duplicate samples. It is recommended that laboratory staff familiarise themselves with the performance characteristics as well as validating the assay in their own laboratory before assaying patient samples in singleton.
- Adequate washing is essential for good performance of the assay – adhere to the instructions provided.
- Avoid excessive freeze-thaw cycles of patient samples, as this may affect their reactivity on the assay.

Performance Characteristics

Clinical Sensitivity and Specificity

The performance of the Biotrin hMPV EIA was compared against panels of hMPV PCR positive and hMPV PCR negative specimens at three independent sites (Table 1). The clinical specimens included nasal wash, nasopharyngeal aspirate and bronchoalveolar lavage.

Table 1: Comparison of Biotrin hMPV EIA against hMPV PCR at 3 independent sites*

	Site 1		Site 2		Site 3		Combined 3 sites	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Biotrin hMPV EIA / PCR	20/21	20/21	17/21	17/21	11/17	16/22	48/59	53/64
EIA / PCR Agreement (Sensitivity/ Specificity)	95%	95%	81%	81%	65%	73%	81%	83%

* Note: Caution is advised in the interpretation of the sensitivity and specificity performance data presented above due to the following important considerations:

- Different, in-house specimen panels were used at each evaluation site.



- Various reverse transcribed PCR methods were used to establish hMPV status according to the evaluation site. Due to the lack of a certified international standard reference calibrator it was not possible to compare the relative performance of the PCR methods used.
- PCR methods are inherently of higher analytical sensitivity than immuno-based methods but can miss minor variants within a genotypic sub-group or strain.

Specimen recovery and handling techniques vary from site to site; we have determined that excessive dilution of the specimen in particular can reduce sensitivity.

The clinical sensitivity of the Biotrin hMPV EIA compared to cell culture was determined by testing 20 culture positive specimens on the EIA. Results are shown in Table 2.

Table 2: Comparison of Biotrin hMPV EIA against cell culture positive specimens

	Positive
Biotrin hMPV EIA / Cell Culture	20/20
EIA / Cell Culture Agreement (% Sensitivity)	100%

A panel of 44 hMPV negative respiratory specimens were tested to assess the clinical specificity of the Biotrin hMPV EIA (Table 3). The specimens were determined to be hMPV negative by both PCR and cell culture. The panel consisted of 16 specimens that were negative for common known respiratory viruses and 28 specimens that were positive for a variety of potentially cross-reactive respiratory viruses. Background reactivity was not greater in the potentially cross-reactive group.

Table 3: Clinical Specificity Study of the Biotrin hMPV EIA

Specimen Group	Assay 1	Assay 2	Mean Specificity
Negative for hMPV and all common RTI viruses	14/16	12/16	81%
Negative for hMPV and Positive for Flu A (n=6), ParaFlu (n=6), Adeno (n=6), RSV (n=10)	26/28	25/28	91%
Overall Specificity			86%



Analytical Sensitivity and Specificity

Sensitivity / hMPV sub-type reactivity:

hMPV genotypes A and B (including subgroups 1 and 2) grown in vero cell culture were all detected by the Biotrin hMPV EIA at concentrations $>1 \times 10^4$ TCID₅₀ / mL.

Specificity:

A range of microorganisms and other interfering substances were tested on the Biotrin hMPV EIA. Results are shown in Table 4, 5 and 6.

Table 4: Microorganism cross-reactivity analysis of the Biotrin hMPV EIA

Micro-organism	ATCC Number
<i>Escherichia coli</i>	25922
<i>Klebsiella pneumoniae</i>	33657
<i>Listeria grayi</i>	700545
<i>Streptococcus pneumoniae</i>	49619
<i>Haemophilus influenzae</i>	49766
<i>Candida albicans</i>	10231
<i>Neisseria gonorrhoeae</i>	31426
<i>Staphylococcus aureus</i>	25923

None of the above were found to interfere with the performance of the assay at a concentration of approximately 10^5 cfu/mL

Table 5: Virus cross-reactivity analysis of the Biotrin hMPV EIA

Virus	TCID50/ml
Parainfluenza Type 1	1.58E+05
Parainfluenza Type 2	8.90E+05
Parainfluenza Type 3	1.58E+08
Influenza A	1.58E+06
Influenza B	2.81E+05
Coronavirus 229E	1.58E+04
Adenovirus 3	8.90E+06
Adenovirus 4	8.90E+04
Rhinovirus 4	2.00E+04
RSV A2	2.80E+04
RSV B	8.90E+03

None of the above were found to interfere with the performance of the assay at the stated concentrations.



Table 6: Potential Interfering substance analysis of the Biotrin hMPV EIA

Substance	Concentration
Proprietary aspirin medication	5mg/mL
Proprietary paracetamol medication	5mg/mL
Proprietary cough drops	5%
Proprietary mouth wash	10%
Proprietary nasal spray	10%
Proprietary cough medicine	5%
Whole blood*	Visible whole blood in respiratory specimen*

All of the above substances were tested and found not to interfere with the hMPV assay, with the exception of whole blood* which may cause elevated background signals; see Limitations of Use Section for precautionary measures.

Intra-assay Reproducibility

A series of hMPV virus culture material and recombinant control material ranging from weakly to strongly reactive were each assayed a total of 30 times. Replicates were tested on a single ELISA plate. The resultant Optical Density (OD) values were summarised and the mean OD, standard deviation (SD) and percentage coefficient of variation (%CV) was calculated (Table 7). These results are also presented in terms of index values in Table 8.

Table 7: Intra-assay reproducibility expressed in terms of OD on 30 or more replicates of 3 different dilutions of hMPV culture material from weak to strongly reactive, and positive and negative controls.

Assay material	Mean OD	SD	% CV	N
Negative control	0.071	0.006	8.332	32
Positive control	1.694	0.075	4.403	32
High QC	2.012	0.089	4.411	30
Medium QC	1.201	0.051	4.222	30
Low QC	0.707	0.031	4.359	30

Table 8: Intra-assay reproducibility expressed in terms of Index Value on 30 or more replicates of 3 different dilutions of hMPV culture material from weak to strongly reactive, and positive and negative controls.

Assay material	Mean Index	SD	% CV	N
Negative control	0.413	0.027	8.332	32
Positive control	9.904	0.337	4.403	32
High QC	12.050	0.532	4.411	30
Medium QC	7.193	0.304	4.222	30
Low QC	4.236	0.185	4.359	30



Inter-assay Reproducibility

Inter-assay reproducibility was investigated using 2 batches of the Biotrin hMPV EIA. A range of dilutions of hMPV viral culture material of strongly reactive, moderately reactive, weakly reactive and non-reactive were tested ten times on each batch. This resulted in an overall sample size (n) of 20 for each dilution.

When this data is analysed in terms of inter-assay and inter-batch reproducibility, the Biotrin hMPV EIA demonstrates very good correlation in test results between different assays and different batches. Reproducibility data for all specimens is given in Table 9.

Table 9: Overall inter-assay reproducibility of the Biotrin hMPV EIA

Viral material range	Dilution of hMPV viral material**	Mean OD	SD	% CV	n
Non-reactive	0	0.074	0.010	13.671	20
Non-reactive	0	0.076	0.021	27.689	20
Weakly reactive	1/960	0.384	0.054	14.116	20
Weakly reactive	1/480	0.649	0.064	9.822	20
Moderately Reactive	1/240	1.176	0.083	7.081	20
Strongly reactive	1/120	2.021	0.108	5.336	20

***hMPV viral material = cell culture-grown virus*



Summary of hMPV EIA Procedure

Please read the entire product instruction leaflet before starting the assay. This summary is for quick reference only.

Prepare Wash Buffer



Dilute samples 3:1 in extraction buffer (e.g. 165uL of sample and 55uL of extraction buffer)



Pipette 100uL of (ready to use) Negative Control in duplicate,
100uL (ready to use) Positive Control in duplicate,
and 100uL of prepared samples in duplicate, into wells



Incubate for 60 minutes @ room temperature (20-25)°C shaking in an incubator



Wash strips 4 times



Add 100uL of Enzyme Conjugate



Incubate for 60 minutes @ room temperature 20-25°C shaking in an incubator



Wash strips 4 times



Add 100uL of TMB Substrate



Incubate for 15 min @ room temperature 20-25°C in an incubator without shaking










Add 100uL of Stop Solution



Read at 450nm
With or without 630nm reference



Interpretation of Symbols

 <i>In vitro</i> diagnostic medical device	 Use by
 Temperature limitation	 Batch code
 Manufacturer	 Catalogue Number
 Instructions for use	



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