

RSV and hMPV: The Testing Solution

Worldwide, respiratory tract infections are the second-leading cause of deaths in children under the age of 5 years (1), whilst infections due to influenza, influenza-like illnesses and pneumoniae are the sixth-leading cause of death in the United States (2). Respiratory Syncytial Virus (RSV), parainfluenza viruses and the influenza viruses are responsible for the majority of cases of bronchiolitis and pneumonia in the paediatric population. However, in 15% -34% of cases of bronchiolitis and pneumonia in the general population, a specific cause of the illness cannot be determined (3). This suggests that there are cases caused by the presence of as yet unidentified pathogens or current methods are not adequate for the detection of known pathogens.

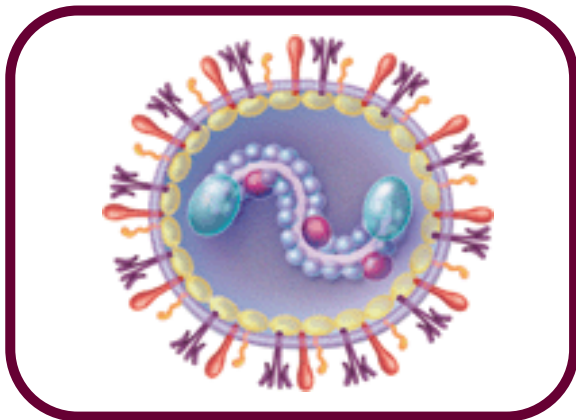


Figure 1. Respiratory Syncytial Virus (RSV)

A new pneumovirus, human *Metapneumovirus* (hMPV) was reported from the Netherlands in 2001 (4). hMPV is currently the only member of the genus *Metapneumovirus*, (family *Paramyxoviridae*, subfamily *Paramyxovirinae*). The family *Paramyxoviridae* is divided into 2 sub families, *Paramyxoviridae* and

Pneumovirinae which contains two genera, *Pneumovirus* (Avian *Pneumovirus*) and *Metapneumovirus*, (Human *Metapneumovirus*), with the Avian *Pneumovirus* and the Human *Metapneumovirus* highly related.

Respiratory syncytial virus (RSV) was first reported from infants in 1957 (5) and has since established itself as the most important virus causing acute respiratory-tract infections in infants and children.

The differential diagnosis of these two infections is difficult on the basis of clinical symptoms as both RSV and hMPV are very similar. RSV is often characterised by lower respiratory tract diseases (pneumonia, tracheobronchitis and bronchiolitis), or upper respiratory tract illness, often accompanied by a low grade fever. hMPV is often associated with a common cold like illness with lower respiratory tract illnesses (bronchiolitis, pneumonia and croup). The development of otitis media is also a common occurrence with both infections.

Laboratory Diagnosis

Methods for the detection, isolation and identification of infections caused by RSV have been well developed over the last few decades. Detection can easily be achieved using direct specimen examination using a range of DFA or IFA staining methods. More recently a range of rapid detection formats based on immunochromatographic test systems have become commercially available, but the sensitivity of these tests are often poor, 60 – 80%, whilst the DFA and IFA methods have reported sensitivities in the range of 90 -100%.

Real-Time Polymerase-Chain-Reaction (RT-PCR) methods with high sensitivity have been developed but the high cost of reagents and the need for expensive instrumentation has been an obstacle in the adoption of RT-PCR methods for routine use.

The isolation of the virus using cell culture still remains the gold standard method for the detection of RSV, exhibiting high levels of both sensitivity and specificity when performed using appropriate clinical samples and using suitable cell lines such as A549, HEP-2 and HeLa.

At this point in time, no rapid immunochromatographic tests are available for the detection of hMPV. Currently, the only methods available for the rapid detection are DFA based tests. To improve the sensitivity of detection, the inoculation of samples into LLC-MK2 or A549 cell lines is the best option available.

The effectiveness of the “conventional” isolation and identification methods, although still considered as the “gold standard” can however be subject to variation due to a number of factors including:

- The quality of the sample used.
- The viability of virus within the sample – methods of sample transport and storage are important.
- The cell lines used.
- Variability in the technical expertise of laboratory staff.

Many of these issues can be readily addressed through the use of shell vials for culture, combined with centrifugation and commercially available cell lines.

The Testing Solution

The simple, rapid, sensitive and specific means of isolating and identifying RSV and hMPV simultaneously has been made possible through the use of mixed cell monolayers R-Mix™ and R-Mix Too™ combined the D³ Double Duet™ DFA Respiratory Virus Screening and ID Kit. R-Mix™ and R-Mix Too™ both utilise a mixture of two cell lines in perfect ratio to produce a mixed cell culture in shell vials, for the culture and identification of 8 emerging and established respiratory viruses; hMPV (including

all 4 subtypes), RSV, Parainfluenza 1, 2 and 3, Adenovirus, Influenza A and B, within 24-48 hours.

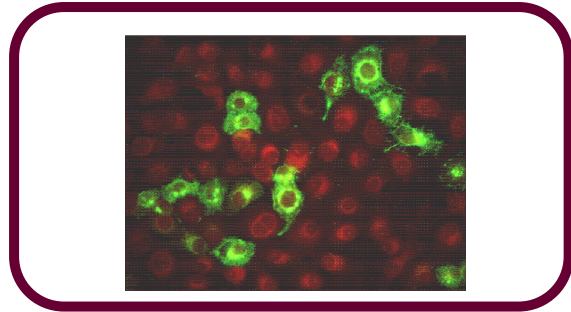


Figure 2. hMPV infected R-Mix Too™ cells.

R-Mix™ is a mixed cell monolayer consisting of human adenocarcinoma cells (A549) and mink lung cells (Mv1Lu). Similarly, R-Mix Too™ is a mixed cell monolayer consisting of human adenocarcinoma cells (A549) but in combination with Madin-Darby Canine Kidney cells (MDCK). Both R-Mix™ and R-Mix Too™ have excellent utility when looking for a combination of respiratory viruses, and have been shown to have superior sensitivity (100%) 1 day post-inoculation than conventional cell culture (67%) 3-8 days post-inoculation (8). The mixture of A549 and MDCK makes the R-Mix™ Too slightly superior for the isolation of Influenza, in particular, Influenza A, and is more sensitive at 20-24 hours than LLC-MK2 cultures for the isolation and amplification of hMPV (9). The speed and sensitivity of the mixed cell culture lines is a vast improvement on conventional culture and the result allows the implementation of correct anti-viral treatment whilst preventing the incorrect use of anti-viral drugs.



Figure 3. R-Mix ReadyCells

The patented mixed cell monolayer utilised by R-Mix™ and R-Mix Too™, has significant impact on the cost, ease-of-use and quality issues associated with conventional cell culture. As only 1 shell vial has to be inoculated per

specimen, for the isolation and identification of 8 respiratory viruses; the hands-on-time of laboratory staff is reduced, as is the amount of consumables and equipment required. The availability of R-Mix™ as frozen, ready-to-use, ReadyCell shell vials, eliminate storage issues, and manufacturing costs of conventional cell culture, as R-Mix™ ReadyCells have a shelf life of 6 months. Also, the ReadyCells format gives laboratories an inventory of shell vials which allows laboratories to manage an increase in testing as the shell vials can be thawed and ready-to-use in 4 minutes. Another advantage of the patented mixed cell monolayer format is unlike conventional cell culture lines, R-Mix™ and R-Mix Too™, have no history of containing mycoplasma, indigenous viruses or producing any viral components such as surface antigens.

- 91 RSV's,
- 51 Adenoviruses,
- 9 Parainfluenza 1's,
- 2 Parainfluenza 2's,
- 13 Parainfluenza 3's
- 2 MPV's.

The study highlights how the faster-turnaround offered by rapid cartridge testing is an illusion, as all negative results must be confirmed by rapid culture cell line R-Mix™ or R-Mix Too™ as clinically important respiratory viruses may be misdiagnosed or not detected at all. The lack of a rapid cartridge test for hMPV and the low sensitivity of commercially available rapid cartridge tests, confirms that R-Mix™ and R-Mix Too™, are the method of choice for rapid, isolation and identification of RSV and hMPV.

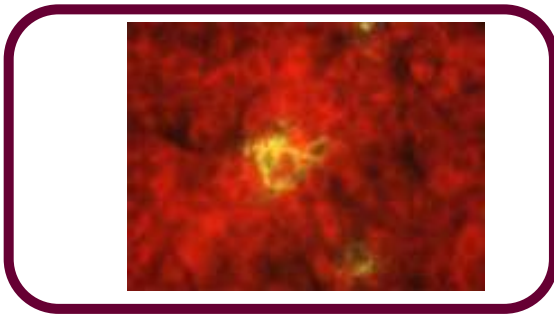


Figure 4. RSV infected R-Mix™ Too cells.

Health Network Laboratories, Pennsylvania, USA, performed a study with a rapid cartridge test for Influenza A and B (Binax NOW A & B™) and R-Mix™ (6), to determine if recommendations by CDC, FDA and WHO for the culture verification of negative cartridge tests led to statistically or clinically, significant results. Cultures were setup within 24 hours of the cartridge testing, with the shell vials being processed in triplicate as in the product insert, with the first shell vials stained at 17-24 hours, with the process repeated at 48 hours on all second shell vials for all culture negatives at 24 hours, using D³ staining reagents. Any positive screens were typed using the last shell vial. Out of 1224 requests for rapid influenza testing, 56 were positive for Influenza A and 3 for Influenza B by cartridge testing. The remaining 1165 negative specimens were reflexed to rapid culture using R-Mix™. A significant 33 false-negatives results for Influenza A and B were found through the testing by R-Mix™. Additional R-Mix™ positive results included:



Figure 5. D³ Double Duet™ DFA Respiratory Virus Screening and ID Kit.

R-Mix™ and R-Mix Too™ combined with the D³ Double Duet™ DFA provides the complete respiratory testing solution. D³ Double Duet™ permits efficient and cost-effective screening for respiratory viruses by utilising a combination of antibodies, allowing for the identification of key pathogens (RSV, MPV and Flu A) whilst permitting the screening of 5 other potential pathogens, with a single filter. The kit contains two staining reagents; one staining reagent will fluoresce golden-yellow to identify Influenza A and fluoresce apple-green to detect the other 5 viruses; and the second staining reagent will fluoresce golden-yellow to identify RSV and fluoresce apple-green to identify MPV. The use of R-Mix™ and R-Mix Too™ combined with the Double Duet™, allows for the rapid, cost-effective and sensitive isolation and identification of 8 respiratory viruses and the simultaneous identification of RSV, hMPV and Influenza A, offering a complete respiratory virus testing solution.

References:

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R-Mix, R-Mix Too and D³ Double Duet are all Trademarks of Diagnostics Hybrids, Athens, USA.

Also Available from Diagnostic Hybrids:

Fresh Cells:

R-Mix (96-0102) shell vials with cover slips for the detection of respiratory viruses.
R-Mix refeed Medium (10-330100)
Super E-Mix refeed Medium (10-380100)

Ready Cells:

R-Mix Ready Cells (F-96-0102-24) shell vials for the detection of respiratory viruses.
McCoy Ready Cells (F-54-0102-24) shell vials for the propagation of Chlamydia.
R-Mix Rinse Buffer for Ready Cells (05-360075)
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Super E-Mix Ready Cells

Frozen Fresh Cells:

R-Mix ampoule (96-00050)
R-Mix Too ampoule (97-00050)
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MDCK ampoule (83-00050)
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Respiratory Viruses:

D3 Ultra Kit (01-010000). Set of screening and typing antibodies for the detection of respiratory viruses
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fluid (01-030000).
Metapneumovirus Antigen Control Slides(01-00070).
D3 Duet Influenza A/Respiratory Screening Kit (01-200000)
D3 Duet RSV/Respiratory Screening Kit (01-210000)
D3 Ultra Respiratory Virus DFA Screening Reagent (01-013010)
D3 Ultra Influenza A DFA Typing Reagent (01-013102)
D3 Ultra Influenza B DFA Typing Reagent (01-013202)
D3 Ultra RSV DFA Typing Reagent (01-013302)
D3 Ultra Adenovirus DFA Typing Reagent (01-013402)
D3 Ultra Parainfluenza 1 DFA Typing Reagent (01-013502)
D3 Ultra Parainfluenza 2 DFA Typing Reagent (01-013602)
D3 Ultra Parainfluenza 3 DFA Typing Reagent (01-013702)
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