

VIRUS ISOLATION by CELL CULTURE

Background

Every year during the winter season the UK population is under attack from respiratory viruses such as Influenza and Respiratory Syncytial Virus (RSV). These viruses infect our respiratory tract and cause mild to severe illness. They characteristically change from year to year, occasionally producing pandemics.

To enable us to track these viruses, clinical virology laboratories have traditionally used tissue culture cell lines. The cells used have to be readily infected by these viruses to isolate them from patient specimens. The cell lines amplify the amount of virus present, express the viral antigens and in many cases die as a consequence of the viral infection producing characteristic cytopathic effects in the cell monolayer. The amplified viruses are then available for further identification by molecular techniques to determine whether they are common or new strains of the virus.

This approach is used by the Centre for Disease Statistic and Control (CDSC) in London to track the epidemiology of respiratory viruses in England and Wales. This monitoring and surveillance service has been a function of the Public Health Laboratory Service (PHLS), now the Health Protection Agency (HPA) for many years.

The Current Dilemma

For many years the main cell culture method used for this work in the UK has been primary kidney cells which are readily infected by a wide range of respiratory and enteric viruses. Due to ethical concerns over the use of primary tissue culture a great deal of research has been undertaken to produce immortalised cells which retain the ability to be infected by a wide range

of viruses. Unfortunately this work has not produced a suitable alternative so the use of less

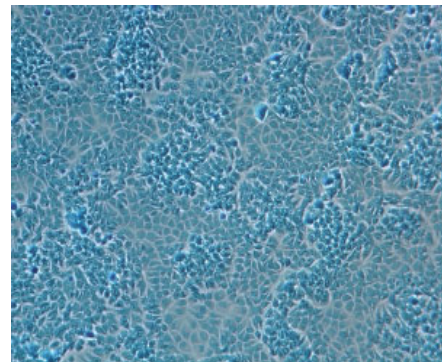


Figure 1: R-Mix a mixed cell monolayer

R-Mix is a mix of two established cell lines Mink Lung Cells (Mv1Lu) and Human Lung Carcinoma Cells (A549). Use for the routine isolation and detection of respiratory viruses Influenza A&B, Parainfluenza 1, 2 & 3, RSV and Adenovirus.

suitable continuous cell lines has been advocated. Alternatively, to reduce the need for primary cells, cultured primary cells to second and third passage have been employed.

This shift in methodology has profound implications for the monitoring and surveillance of emerging viruses in the UK and should be a great cause for concern within the health service given the recent emergence of new viruses such as SARS and Meta-pneumovirus.

The Potential Solution

In the USA a company (Diagnostic Hybrids Inc., Athens, Ohio) has developed alternative cell culture systems which have enabled clinical virology laboratories to switch from primary cell culture to their alternative mixed cell cultures with great success. The use of multiple cell lines in the same culture, has enabled DHI to produce tissue culture systems capable of routinely

isolating a wide range of viruses including the seven leading respiratory virus types (R-Mix – see Fig 1). A second culture system that is excellent for the isolation of enteric viruses (Super E-mix) has also been developed.

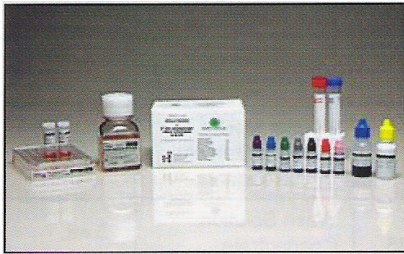


Figure 2 – R-Mix and D³

Using these two culture systems clinical virology laboratories in the USA and Canada have been able to eliminate the use of primary cell culture methods from their laboratories. These new products are therefore providing the alternative isolation technique that has long been sought in the UK. Ethically they provide a much more acceptable solution for the monitoring and surveillance of emerging respiratory and enteric viruses in the UK.



Figure 3 An example of a shell vial

Furthermore, the R-Mix and Super E-Mix products are supplied either as growing cultures ready to use or as ready cells which can be stored frozen for many months and only used when required.

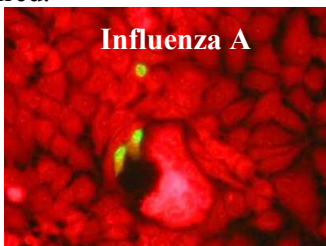


Figure 4a – R Mix infected with influenza A as revealed by staining with D³ kit

One of the major advantages to this methodology is that the cell culture technique being used in every laboratory can be standardised to ensure that every region is using the same method for virus isolation that should improve the monitoring and surveillance infrastructure in the

UK. At present there are a range of techniques being used throughout the country with varying levels of success.

In this newsletter we intend to describe the new cell culture systems and compare them with the cell culture virus isolation systems currently in use within the UK.



Figure 4b – R Mix infected with influenza B as revealed by staining with D³ kit

R-Mix for respiratory virus isolation

R-Mix can be supplied in two main formats. R-Mix freshcells™ are supplied as living cultures in shell vials containing coverslips (Figure 3). These shell vial cultures must be used within 10 days for virus infectivity. Alternatively, R-Mix can be supplied as Readycells™ these are frozen shell vial cultures that can be stored at –70°C for up to six months and used when required. Both of these formats are CE marked for use in detection of respiratory viruses from human samples.

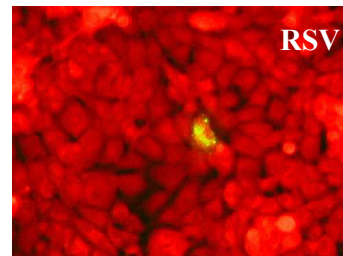


Figure 4c – R Mix infected with RSV as revealed by staining with D³ kit

After 24 hours, following the addition of the patient's sample to the R-Mix shell vial the coverslip can be stained with a screening reagent supplied in the respiratory virus detection kit D³ (Figure 2). At this stage in the procedure Influenza A&B and Parainfluenza 1,2 & 3 can be detected. After 48 hours RSV and Adenovirus can be detected (Figure 4a-c show typical staining for Influenza A, Influenza B and RSV). D³ kit contains a general DFA screening reagent containing specific antibodies to Influenza A, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, RSV and Adenovirus plus vials containing the individual DFA reagents for virus identification.

R-Mix Performance Data

To assess the potential for R-Mix to replace primary MKC cell culture for the isolation and detection of respiratory viruses in the UK a study was performed at the Department of Clinical Microbiology, St. Mary's Hospital, Portsmouth over the 2003-2004 winter season testing 200 routine specimens. The culture systems used were R-Mix, primary Rhesus monkey kidney (PRhMK) glass tube cultures from DHI and secondary monkey kidney cells (MKC) from ECACC/CAMR. The viruses were detected using the D³ Direct fluorescent screening and identification kit. The results are summarised in the following tables:

Table 1: Respiratory virus detection in cell culture systems winter season 2003-2004

Respiratory Virus	R-Mix (DHI)	PRhMK (DHI)	MKC (CAMR)
Negative	129	168	191
Influenza A	9	7	0
Influenza B	0	0	0
Parainfluenza 1	0	0	0
Parainfluenza 2	1	0	0
Parainfluenza 3	3	2	0
RSV	55	19	4
Adenovirus	2	2	1
Contaminated	1	2	4

Table 2: Isolation rates for Influenza A in the three culture systems

Influenza A Isolate	R-Mix (DHI)	PRhMKC (DHI)	MKC (CAMR)
Patient 007	+	+	-
Patient 009	+	+	-
Patient 076	+	+	-
Patient 108	+	-	-
Patient 112	+	-	-
Patient 117	+	-	-
A/PortChalmers/1/73	+	+	-
A/Mal/302/54	+	+	-
A/Victoria/3/75	+	+	-
A/WS/33	-	+	-

Conclusions:

- The results show that the use of MKC cells from ECCAC/CAMR, in the period under investigation, resulted in poor to no respiratory virus detection and isolation
- Whilst true PRhMK cells can be used effectively to detect and isolate the vast majority of detectable respiratory viruses they are not as effective or efficient as the R-Mix culture system
- In this study it is clear that the optimal culture system for respiratory virus detection and isolation is R-Mix
- R-Mix used in combination with the D³ DFA detection kit delivers a complete isolation and detection system for laboratories monitoring respiratory virus activity in the community
- On ethical and quality grounds, there would appear to be no reasonable justification for continuing to use the ECCAC/CAMR cells for respiratory virus isolation
- By adopting this system laboratories would be adopting a consistent, fully quality controlled, CE marked cell culture and fluorescence detection / identification system that **avoids the need for primary cell culture and therefore removes ethical concerns.**

New from DHI - Super E-Mix

To broaden the use of mixed cell cultures DHI developed a set of enteric mixed cell culture systems in the late 1990s which gave good enteric virus detection in comparison with standard culture techniques such as primary cell culture, see Tables 3 and 4 below.

Table 3: E-Mix Performance

	E-Mix	Shell Vials			
		PMK, BGMK, A549, MRC-5			
Vials (+)	20	18	16	7	0
Total (+)	20	22			
Total (-)	14	12			

These mixed cultures have been used routinely in North America for enteric virus isolation over the past few years. It was clear, however, that no culture system was optimal for enteric virus isolation and detection so DHI used genetic cell engineering to enhance their product and make it superior to any other cell culture system currently available. They achieved this goal by developing a new mixed cell culture system which they have named Super E-Mix. Super E-Mix has been designed to enable laboratories to isolate enteric viruses from clinical specimens. This mixed cell culture system combines the Human Lung Carcinoma cell line A549 and a genetically modified Buffalo Green Monkey Cell line *SuperBGMK*. *SuperBGMK* have been transformed by the addition of Decay Acceleration factor (DFA). Validation studies have confirmed that viral infectivity is enhanced with these DFA transformed cells. The combination of *SuperBGMK* and A549 provides the capability to detect all known culturable enteroviruses in a single shell vial.

Table 4 Time to visible Cytopathic Effect (CPE)

	Time to Detection					
	24h	48h	72h	96h	120h	>120h
HFF	16	9	6	4	4	5
PrMK	23	10	9	7	3	3
RMix	16	10	5	4	2	0
Super Emix	29	21	17	7	1	0

Based on 75 positive enterovirus samples

New from DHI Ready Cells

One of the major issues in running an efficient virus cell culture laboratory is the need to ensure that cultures are available to cope with the influx of specimens during fluctuating levels of demand. For primary cell culture this is all a question of guess work and experience as these cells need to be made and used within two weeks. Freshcells from DHI are similarly seeded and used in a two week cycle so there is some potential for wastage. To overcome this DHI have been further developing their cell culture systems to produce cell culture systems ready to use at a few minutes notice called Readycells.

These shell vial cultures are stored at -70°C and are thawed out just prior to use. The shell vials can be stored for up to 4 months so it is now possible to buy in bulk and use as required in a monthly or quarterly cycle.

Readycells can be summarised thus:

- Cells delivered frozen (dry ice)
- Cells stored frozen (-70°C)
- Vial thawed at 37°C for 4 minutes
- Replace medium with re-feed
- Inoculate sample
- Incubate inoculated cells at 37°C

The result is no waste, cell culture on demand and all the laboratory requires is access to a -70°C freezer.

Clinical Sensitivity

	ReadyCells™	
FreshCells™	Neg	Pos
Neg	22	0
Pos	0	26*

* includes 9 RSV, 15 Influenza A, 8 Influenza B, 2 Parainfluenza 1, 2 Parainfluenza 2, 3 Parainfluenza 3, 5 Adenovirus.

R-Mix Readycells are already available and Super E-Mix Readycells are due to be launched in the autumn of 2004.

FOR MORE INFORMATION ON DHI PRODUCTS, PLEASE CONTACT YOUR LOCAL MICROGEN DISTRIBUTOR OR MICROGEN BIOPRODUCTS DIRECT

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