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MICROGEN BIOPRODUCTS

Microgen Bioproducts Newsletter N° 11 2004

The Improved Detection and Identification of Common Respiratory Viruses

Respiratory Viral Pathogens – An Overview

The introduction of new antiviral drugs for the treatment of Influenza(1) and Respiratory Syncytial Virus (2) combined with the need for clinicians to be more discriminating in the prescribing of antibiotics has increased the need for faster and more specific diagnostic testing procedures.

Historically, three types of laboratory procedures have been employed for the diagnosis of viral infections (direct detection, culture isolation/ confirmation and serology). Methods for direct detection of viral antigens include immunological (including immunochromatographic and Enzyme Immunoassay), and immunofluorescence. These methods although rapid lack sensitivity (3,4), see Table 1. Culture isolation with confirmation (normally using immunofluorescence) is still the standard method for the most sensitive detection of respiratory viruses. Serology, using methods such as

ANNOUNCEMENT:

Microgen Bioproducts are the pleased to announce the recent signing of a distribution agreement with Diagnostic Hybrids Inc, Ohio, USA for their innovative range of tissue culture and virus detection and identification reagents. Diagnostic Hybrids is a fast growing bioscience firm that develops, manufactures, and markets innovative cell culture products for multiple applications in diagnostic virology and endocrine disease. These products offer laboratory staff a rapid, cost-effective diagnostic solution to an otherwise lengthy and expensive process.

Products included in the Diagnostic Hybrids Range include:

Fresh Cells PMK – Tubes of guaranteed Primary Monkey Kidney cells, ready for use.

Mix Freshcells™ R-Mix™ – a mixed cell tissue culture for the rapid and improved isolation of the 7 major respiratory viruses.

D^3 – DFA Respiratory Virus Screening & ID Kit - a direct fluorescence screening kit for the direct specimen detection/ identification and culture confirmation of Adenovirus, Influenza A + B, Parainfluenza groups 1, 2 + 3 and Respiratory Syncytial Virus

ELVIS™ – Transgenic cell culture for the rapid (18 – 24 hours) detection and identification of Herspes simplex 1 + 2.

Coming Soon:

Mix Freshcells™ Super E-Mix™ - a superior system for the detection and identification of Enteroviruses.

AVAILABLE: ENGLAND, WALES, SCOTLAND AND IRELAND ONLY

haemagglutination inhibition or complement fixation may also be employed but provide delayed results which have no advantages in treatment.

R-Mix – Improved Culture

Although tissue culture isolation remains the "gold standard" for the detection of viruses and chlamydial agents, it is often beyond the capacity of many laboratories due to issues including cost, quality and manufacturing requirements. In addition, traditional viral tissue culture isolation has required that multiple cell lines be available. For example, laboratories isolating respiratory viruses should employ a combination of two or three cell lines including A549, Hep-2 and PMK cells to ensure the effective isolation of adenovirus, influenza, parainfluenza and respiratory syncytial viruses.
It has been reported that mink lung (Mv1Lu) cells exhibited a high level of sensitivity for the detection of influenza viruses (7,8). In separate studies (9,10), Mv1Lu cells were also demonstrated to be moderately sensitive for parainfluenza 1, 2 and 3 and respiratory syncytial virus but were not sensitive for adenovirus. To overcome this lower sensitivity towards parainfluenza 1, 2 and 3, respiratory syncytial virus and adenovirus, Diagnostic Hybrids have developed mixed cell cultures in which the Mv1Lu cells are co-cultures with the adenovirus sensitive A549 cell line. Using patented technologies Diagnostic Hybrids, have combined

Table 1: Reported Sensitivities of Direct Detection Methods for Influenza A Virus.

Method	% Positive	Reference
IF – Polyclonal	86%	3
IF – Monoclonal	80%	4
Immunochromatographic	55%	5
	68%	6

Mv1Lu and A549 cells in a single shell vial culture. This cell cocktail has been found to provide a highly sensitive culture system for all of the above mentioned respiratory viruses. This mixed cell culture, known as R-Mix™ has been found to be as sensitive as PMK for influenza B, parainfluenza 1,2 and 3, and Hep-2 cells for respiratory syncytial virus and A549 for adenoviruses. In addition, the cell mixture was found to be more sensitive than individual PMK and Mv1Lu cell lines for the detection of influenza A (9).

The above mixed cell culture, R-Mix™, is now available through Microgen Bioproducts as a ready to use fresh cell preparation in shell vials.

Advantages of R-Mix™ Over Conventional Culture

Apart from reducing the number of cell lines required for the isolation of the 7 most common respiratory viruses, R-Mix™ offers a number of additional advantages to laboratories including:

- Ease of use – R-Mix™ is provided as Mixed Freshcells™, a ready to use format which eliminates the need for cell line maintenance tube preparation and quality control.

- "Rapid Results" – using R-Mix™ influenza A + B is detectable after 24 hours incubation, whilst the other respiratory viruses are detectable after 48 hours. Conventional culture requires 10 – 14 days incubation.

- Improved Sensitivity – numerous reports are available that demonstrate clearly that the mixed cells are more sensitive than individual cell lines for the detection of influenza A in particular and as sensitive as individual cell lines for the detection of the other viruses (9, 11)

- Earlier Treatment – the combination of earlier detection and increased sensitivity allow the more effective prescribing of the new range of emerging antiviral agents.

D³ DFA Respiratory Virus Screening & ID Kit

The D³ DFA Respiratory Virus Screening & ID Kit from Diagnostic Hybrids employs viral antigen specific

murine monoclonal antibodies labeled with fluorescein for the rapid detection of the 7 common respiratory viruses directly in clinical specimens, or for cell screening or the confirmation of isolates grown in cell culture.

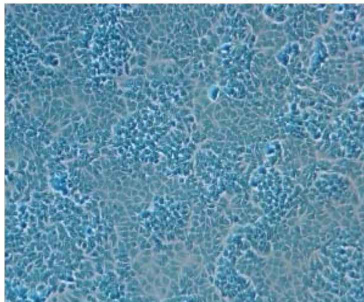
The kit includes a Screening Reagent containing a mixture of monoclonal antibodies for each of the 7 respiratory viruses as well as individual monoclonal for use in the specific identification of each of the individual viruses.

The cells to be examined , are transferred onto a multiwell fluorescence microscopy slide, air dried and fixed in acetone. It is suggested that cells initially be stained using the Screening Reagent by the application of a single drop of this reagent. After incubation for 15 minutes at 35 – 37°C, the stained cells are washed using the wash buffer supplied. A drop of mounting medium is added to the stained cells and a coverslip applied. The stained microwell is examined using a fluorescence microscope for the presence of infected cells indicated by the presence of bright apple green fluorescent staining (Table 2). Uninfected cells appear red due to the Evans Blue counterstain.

If positive fluorescence is observed, the remaining wells should be stained in a similar manner using each of the individual typing monoclonal antibodies.

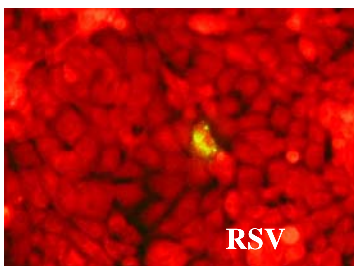
If no fluorescent-stained cells are found in direct specimen smears, specimens should be routinely cultured using R-Mix™ and blind stained using the Screening Reagent.

TABLE 2: EXAMPLES OF TYPICAL STAINING OF R-MIX™ USING D^3 DFA RESPIRATORY VIRUS SCREENING & ID KIT



R-Mix™ monolayer stained with methylene blue – note typical mountains and valleys appearance

Influenza A & B – fluorescence is cytoplasmic, nuclear or both, cytoplasmic staining is often punctuate with large inclusions while nuclear staining is uniformly bright



RSV – fluorescence is cytoplasmic and punctuate with small inclusions in the syncitia

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FOR MORE INFORMATION PLEASE CONTACT YOUR LOCAL MICROGEN DISTRIBUTOR, COMPLETE THE INFORMATION REQUEST FORM ENCLOSED AND RETURN IT TO MICROGEN BIOPRODUCTS LTD, UK OR VISIT THE DIAGNOSTIC HYBRIDS WEBSITE: www.dhiusa.com

MICROGEN BIOPRODUCTS LTD
1 Admiralty Way
Camberley
Surrey
United Kingdom GU15 3DT

Phone: +44 1276 600081
Fax: +44 1276 600151
E-mail: sales@microgenbioproducts.com
Web: www.microgenbioproducts.com