Key Code TSMX7852A
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IMAGEN Respiratory Screen

REF K612011-2 ..... Σ 100 **EN**

1. INTENDED USE

The IMAGEN™ Respiratory Screen is a qualitative indirect immunofluorescence test for the presumptive detection of respiratory syncytial virus (RSV), influenza A and B virus, parainfluenza virus 1, 2 and 3 and adenovirus in respiratory specimens (nasopharyngeal aspirates) and in cell cultures. The assay cannot differentiate between the viruses. Individual viruses should be further identified and confirmed using monospecific FITC labelled monoclonal antibody reagents, such as the IMAGEN range, or other methods. The direct respiratory specimen results must be confirmed by cell culture.

2. SUMMARY

Respiratory virus infections are associated with major outbreaks of respiratory disease throughout the world which have significant impact on world health[1,2,3]. Viruses cause disease in all age groups but infections are most severe in infant, elderly and immunocompromised populations leading to hospitalisation of patients[4]. Nosocomial outbreaks can occur in paediatric and geriatric wards resulting in extended hospitalisation, prolonged patient management and increased morbidity and mortality[5,6]. Respiratory virus infections in infants may cause obstruction of airways and lead to respiratory distress.

The viruses mainly responsible for lower respiratory tract infection include RSV, influenza A and B viruses, parainfluenza virus 1, 2 and 3 and adenoviruses. Expected prevalence rates for individual viruses are detailed (with references) in Section 13, Expected Values.

RSV and parainfluenza viruses occur seasonally and are major causes of lower respiratory tract disease in infants and young children. They frequently cause bronchiolitis, croup, bronchitis and occasionally pneumonia[7,8,9].

Influenza A and B viruses cause world-wide seasonal epidemics of respiratory disease in adults and infants with a disease spectrum ranging from mild upper respiratory tract symptoms to severe pneumonia[10,11]. Acute pneumonia in elderly or immunocompromised patients can be life threatening particularly when associated with secondary microbial infections.

Adenovirus infections are associated with respiratory, ocular and enteric disease[12]. Adenoviruses are reported to be responsible for 5% of acute respiratory disease in children under the age of 4 years and are a common cause of pharyngitis in young children[13].

Rapid diagnosis of respiratory virus infections is an important aid in the management of patients, prevention and control of outbreaks and in influencing the use of antiviral therapy, particularly for influenza A and RSV viral infections[3,14,15,16].

The methods used for the diagnosis of acute respiratory virus infections include direct testing of specimens such as nasopharyngeal aspirates by immunofluorescence and/or enzyme immunoassays for detection of viral proteins, isolation and identification of the virus in cell culture monolayers or detection of immune response by serological techniques.

Immunofluorescence tests such as IMAGEN Respiratory Syncytial Virus and IMAGEN Influenza A and B virus are now widely used for direct detection of respiratory virus proteins in preparations of cells from nasopharyngeal aspirates, or from cell culture monolayers[17,18]. Immunofluorescence tests utilising specific monoclonal antibodies, are rapid and enable the quality of the specimen to be monitored[2,17]. Centrifugation enhanced culture systems (shell-vials) accelerate isolation of respiratory viruses in cell culture and when used in conjunction with immunofluorescence tests enable rapid diagnosis of respiratory infection by cell culture[19].

In any population a variety of respiratory viruses may circulate concurrently causing infections with similar clinical symptomology. The prevalence of specimens positive for respiratory viruses will vary according to population demographics, season, the type of specimens tested and the time at which specimens are collected during the course of infection. An immunofluorescence screening test for respiratory viruses provides a rapid and cost-effective method for detection of respiratory virus infection. Individual viruses can then be identified and confirmed using monospecific FITC labelled monoclonal antibodies.

IMAGEN Respiratory Screen is an indirect immunofluorescence test for the detection of respiratory viruses including RSV, influenza A and B viruses, parainfluenza virus types 1, 2 and 3 and adenovirus in clinical specimens and in cell culture monolayers. The presence of a respiratory virus is indicated by a positive screening result. Any virus present can be identified by direct immunofluorescence using FITC conjugated monoclonal antibody reagents specific for individual respiratory viruses (see Sections 6.1 and 11.4).

3. PRINCIPLE OF THE TEST

The IMAGEN Respiratory Screen test contains a pool of monoclonal antibodies each of which has individual specificity for either RSV, influenza A or B virus, parainfluenza virus 1, 2 or 3 or adenovirus. The pooled antibody Screening Reagent is used in a two-step indirect immunofluorescence staining technique. The Negative Control Reagent provided is used to monitor the specificity of staining. Specimens are stained with the Screening Reagent and/or Negative Control Reagent for 15 minutes. Excess unbound reagent is then removed by washing with phosphate buffered saline (PBS). The specimens are then stained with a secondary FITC conjugate for 15 minutes. Excess unbound reagent is removed by washing with PBS. The stained areas are mounted and viewed microscopically using epifluorescence illumination. A positive result is indicated by the presence of characteristic apple-green intracellular fluorescence within infected cells which contrasts with the red background staining of uninfected cells. The respiratory virus present can be specifically identified using individual IMAGEN reagents (see Section 11.4).

Acknowledgements

The monoclonal antibodies used in this test were originated at:

The Department of Respiratory and Enteric Viruses, Public Health Services, Centers for Disease Control, Atlanta, Georgia, USA

The Institute for Research on Animal Diseases, Compton, Berkshire, UK

The Department for Health and Human Services, Public Health Services, Centers for Disease Control, Atlanta, Georgia, USA

Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale, London, UK

The Washington Research Foundation, Washington, USA

Although some of the monoclonal antibodies used in the test device originated at Centers for Disease Control (CDC), it should not be interpreted that the test is in anyway endorsed by CDC or the U.S. Department of Health and Human Services.

4. DEFINITIONS

The following symbols and definitions have been used in the product information.

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
Σ N	Contains sufficient for <N> tests
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

5. REAGENTS PROVIDED

2 °C – 8 °C

Σ 100 - Each kit contains sufficient materials for 100 direct specimens or cell culture preparations. The shelf life of the kit is as indicated on the outer box label.

5.1. IMAGEN RESPIRATORY SCREEN REAGENT

POSITIVE AND NEGATIVE CONTROL SLIDE — Instructions for Use

5 x 14 well, combined Positive and Negative Control Slides. Each slide consists of 7 wells containing acetone-fixed cells infected with either RSV (strain from clinical specimen), influenza A (strain CDC V7-002) or B virus (strain CDC V4-004), parainfluenza virus 1, 2 or 3 (strains CDC V6-004, CDC V7-003, and CDC V5- 003 respectively) and adenovirus (strain CDC V5-002), one well specific for each virus) and 7 wells containing acetone-fixed uninfected cells (negative control wells).

One bottle of each of the following unless indicated otherwise:

MOUNTING FLUID — 3 x 3mL of Mounting Fluid. The Mounting Fluid contains a photobleaching inhibitor in glycerol (pH 10.0).

SCREENING REAGENT — 4.4mL of Screening Reagent. The reagent consists of a pool of purified mouse monoclonal antibodies specific for RSV, influenza A and B virus, parainfluenza virus 1, 2 and 3 and adenovirus. The reagent is prepared in a protein stabilised buffer solution containing 15mmol/L sodium azide as a preservative.

The monoclonal antibodies used in the IMAGEN Respiratory Screen Screening Reagent have been shown to react with conserved epitopes of viral proteins present in the respective viruses, as detailed below:

Virus	Monoclonal antibody specificity
Respiratory Syncytial Virus	Nucleoprotein and fusion protein
Influenza A virus	Nucleoprotein and matrix protein
Influenza B virus	Nucleoprotein and haemagglutinin protein
Parainfluenza 1 virus	Fusion protein
Parainfluenza 2 virus	Haemagglutinin protein
Parainfluenza 3 virus	Haemagglutinin protein
Adenovirus	Hexon protein*

* This monoclonal antibody has been shown to detect all strains of adenovirus commonly associated with respiratory tract infection. Strains tested were adenovirus 1 - 41 inclusive.

CONTROL – — 2.6mL of Negative Control Reagent. The reagent consists of pooled mouse monoclonal antibodies with no anti-viral activity. The reagent is prepared in a protein stabilised buffer solution containing 15mmol/L sodium azide as a preservative.

FITC CONJUGATE — 2 x 3.5mL of anti-mouse FITC conjugated antibody. The reagent consists of an FITC conjugated F(ab')2 fragment of rabbit anti-mouse immunoglobulins, diluted in phosphate buffered saline solution containing stabilising proteins, Evans blue dye as a counterstain and 15mmol/L sodium azide as a preservative.

5.2. PREPARATION, STORAGE AND RE-USE OF KIT COMPONENTS

In order to ensure optimal kit performance, it is important that unused kit components are stored according to the following instructions:

5.3. POSITIVE AND NEGATIVE CONTROL SLIDES - POSITIVE AND NEGATIVE CONTROL SLIDE

The slides are provided individually in sealed foil pouches with nitrogen. Store unused slides at 2-8°C. The slide should be left for 5 minutes at room temperature (15-30°C) before opening.

Stain the slide immediately after opening.

5.4. MOUNTING FLUID - MOUNTING FLUID

Ready to use. Store unused Mounting Fluid at 2-8°C. The Mounting Fluid should be left at room temperature (15-30°C) for 5 minutes before use.

5.5. SCREENING REAGENT – SCREENING REAGENT

Ready to use. Store unused Screening Reagent at 2-8°C. The reagent should be left at room temperature (15-30°C) for 5 minutes before use.

5.6. NEGATIVE CONTROL REAGENT - CONTROL –

Ready to use. Store unused Negative Control Reagent at 2-8°C. The reagent should be left at room temperature (15-30°C) for 5 minutes before use.

5.7. ANTI-MOUSE FITC CONJUGATE REAGENT - FITC CONJUGATE

Ready to use. Store unused anti-mouse FITC conjugate reagent in the dark at 2-8°C. The reagent should be left at room temperature (15-30°C) for 5 minutes before use.

6. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

6.1. REAGENTS

Fresh acetone (for fixation).

Phosphate buffered saline (PBS) pH 7.5 for washing stained specimens and for specimen preparation.

IMAGEN reagents to confirm positive screening results, available from your local Oxoid subsidiary or distributor:

IMAGEN Adenovirus	Code No K610011-2
IMAGEN Respiratory Syncytial Virus (RSV)	Code No K610211.2
IMAGEN Parainfluenza Virus Types 1,2,3 (Typing)	Code No K610411-2
IMAGEN Influenza A and B Virus	Code No K610511-2

6.2. ACCESSORIES AND EQUIPMENT

The following products are intended for use in conjunction with IMAGEN Respiratory Screen. Contact your local distributor for further information.

Teflon coated glass microscope slides with single 6mm diameter wells (100 slides per box) available from your local distributor, (Code No. S611430-6).

IMAGEN Respiratory Screen Positive and Negative Control Slides (Code No. S612130-2).

7. EQUIPMENT

The following equipment is required:

Precision pipettes and disposable tips to deliver 20µL and 25µL

Wash bath

Coverslips suitable to cover either single 6mm diameter wells or 14 well slides

Non-fluorescing immersion oil

Epifluorescence microscope with filter system for FITC (maximum excitation wavelength 490nm, mean emission wavelength 520nm) and x200-x500 magnification

Incubator at 37°C

Low speed centrifuge

For direct specimens

Mucus extractor (nasopharyngeal specimens only)

For culture confirmation

Sterile swabs, viral transport medium (VTM) and container suitable for collection, transportation and culture of RSV, influenza A and B virus, parainfluenza virus 1, 2 and 3 and adenovirus.

Cell lines recommended for culture and isolation of RSV, influenza A and B virus, parainfluenza virus 1, 2, 3 and adenovirus.

8. PRECAUTIONS

IVD - For *in vitro* diagnostic use. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.

8.1. SAFETY PRECAUTIONS

8.1.1 The IMAGEN Respiratory Screen reagents contains <0.1% sodium azide, which is a poison. Sodium azide may react with copper and lead plumbing systems to form explosive metal azides. Always dispose of materials containing azide by flushing with large quantities of water.

8.1.2 The virus antigens on the control slides have been shown to be non-infectious in cell culture, however, the slide should be handled and disposed of as though potentially infectious.

8.1.3 Evans blue dye is present in the Reagent. Although present below the concentration for the product to be classified as carcinogenic, contact with the skin should be avoided.

8.1.4 Care should be taken when using the Mounting Fluid as it may cause skin irritation. Skin should be flushed with water if contact occurs.

8.1.5 Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.

8.1.6 Do not pipette materials by mouth.

8.1.7 Wear disposable gloves while handling clinical specimens and virus cultures, always wash hands after working with infectious materials.

8.1.8 Dispose of all clinical specimens in accordance with local legislation.

8.1.9 Safety data sheet available for professional user on request.

8.2. TECHNICAL PRECAUTIONS

8.2.1 Components must not be used after the expiry date printed on the labels. Do not mix or interchange different batches/lots of reagents.

8.2.2 The reagents are provided at fixed working concentrations. Test performance will be adversely affected if the reagents are modified or stored under conditions other than those detailed in Section 5.

8.2.3 Prepare fresh PBS as required on the day of use.

8.2.4 Washing in PBS is necessary. Use of other wash solutions such as tap water or distilled water will compromise test results.

8.2.5 Avoid microbial contamination of reagents.

8.2.6 The reagents must not be frozen.

9. COLLECTION AND PREPARATION OF SPECIMENS

The collection and preparation of specimens is of fundamental importance in the diagnosis of respiratory virus infection by direct immunofluorescence and cell culture methods.

Specimens must be collected from the site of infection during the time of peak viral shedding so that they contain as much infected material as possible and prepared in such a way as to preserve either intact cells which are free from adherent mucus etc for direct microscopy of specimens or the viability of viruses in specimens to be cultured.

9.1. NASOPHARYNGEAL ASPIRATES/SECRETIONS

Collection

Collect specimens from the nasopharyngeal region into a mucus extractor through a size 8 feeding tube. The mucus extractor and tubing should be transported as soon as possible, maintained at 2-8°C and sent to the laboratory for processing. Cell separation techniques are necessary for direct immunofluorescence staining. Specimen or supernatant material from cell separation techniques may be used for virus culture inoculation.

Cell Separation

If necessary add 2mL PBS to the specimen prior to centrifugation to reduce the viscosity and dilute the mucus. Centrifuge the mucus extractor at room temperature (15-30°C) for 10 minutes at 380g. Remove the supernatant which can be used for cell culture. Resuspend the cell deposit in 2mL PBS and gently pipette the cells up and down with a wide bore pipette, or vortex gently, until the mucus is broken up and cellular material released. Avoid vigorous pipetting/vortexing to prevent damage to the cells. When a smooth suspension has been obtained add further PBS as required, pipetting or vortexing after addition of the extra PBS to wash the cells further. Remove and discard any visible flecks of mucus remaining at this point. Excess mucus must be removed as it will prevent adequate penetration of the reagent and may result in non specific fluorescence.

If all secretions remain in the feeding tube and none reach the mucus extractor, wash all secretions out of the tube into PBS. This is best achieved by inserting a pasteur pipette into the end of the tube which was attached to the mucus extractor. Suck up the appropriate fluid into the tube and expel it repeatedly until the secretions adhering to the wall of the tube have been dislodged. Pipette the suspension up and down until the mucus has been adequately broken up.

Preparation of Slides

After completing the cell separation process, centrifuge the resultant cell suspension at room temperature (15-30°C) for 10 minutes at 380g and discard the supernatant.

Resuspend the cell deposit in sufficient PBS to dilute any remaining mucus, while at the same time maintaining a high cell density. Place 25µL of the resuspended cell deposit into the well area on the slide.

NOTE: It is important that duplicate wells are prepared for each specimen to be tested using the IMAGEN Respiratory Screen, one well for Screening Reagent and one well for Negative Control Reagent (see Section 10.8).

Allow the specimen to air dry thoroughly at room temperature (15-30°C) and fix in fresh acetone at room temperature (15-30°C) for 10 minutes. If the specimen is not stained immediately store slides at 4°C overnight or freeze at –20°C for longer storage periods.

NOTE: For follow-up testing for specific virus identification, an additional slide containing at least 8 wells per specimen is required (see Section 11.4).

9.2. INOCULATION OF CELL CULTURES

Inoculation of Cell Cultures

Specimens collected for the diagnosis of RSV, influenza A and B virus, parainfluenza virus 1, 2 or 3 and adenovirus infections should be inoculated into the cell lines routinely used in the laboratory according to established laboratory methods. Cell cultures should be examined regularly for the appearance of cytopathic effect (CPE) and haemadsorption tests carried out at regular intervals. Any haemadsorption positive cultures or cell cultures showing CPE, can be harvested and tested for the presence of RSV, influenza A and B virus, parainfluenza virus 1, 2 or 3 and adenovirus.

Preparation of Slides

Scrape the cell sheet into the liquid culture medium using a sterile pipette. Deposit the cells by centrifugation at 200g for 10 minutes at room temperature (15-30°C) and remove the supernatant.

Wash the cells by resuspending the cell deposit in PBS (see Section 6.1) and repeat the centrifugation. Remove the supernatant and resuspend the cell deposit in a small volume (250µL per culture tube) of fresh PBS to maintain a high cell density.

Place 25µL aliquots of the cell suspension on to the appropriate number of individual wells on the slides. Allow to air dry thoroughly and fix in fresh acetone for 10 minutes room temperature (15-30°C). If the specimen is not stained immediately, store at 4°C overnight or freeze at –20°C for longer periods.

NOTE: It is important that duplicate wells are prepared for each specimen to be tested using the IMAGEN Respiratory Screen, one well for Screening Reagent and one well for Negative Control Reagent (see Section 10.8).

NOTE: For follow-up testing for specific virus identification, an additional slide containing at least 8 wells per specimen is required (see Section 11.4).

10. TEST PROCEDURE

PLEASE REFER TO SECTION 8.2 TECHNICAL PRECAUTIONS, BEFORE PERFORMING THE TEST PROCEDURE.

10.1. ADDITION OF SCREENING REAGENT AND NEGATIVE CONTROL REAGENT

For each specimen two wells of fixed cell preparation are required. To one well of the test slide add 25µL of Screening Reagent, and to the other well add 25µL Negative Control Reagent. To the control slide add 20µL of Screening Reagent to each well. Ensure that the reagents cover the entire area of each well.

10.2. FIRST INCUBATION

Incubate the slides with reagent in a **moist chamber** for **15 minutes** at **37°C**. **Do not** allow the reagent to dry on the specimen, as this will cause the appearance of non-specific staining.

10.3. WASHING THE SLIDE

Rinse gently with PBS using a squeeze wash bottle . Do not focus the PBS stream directly onto the wells. Tilt the slide down and run the PBS stream across the slide above the wells, allowing the PBS to run off the bottom edge of the slide. Gently wash the slide in an agitating bath containing PBS for 5 minutes. Drain off PBS and allow the slide to air dry at room temperature (15-30°C).

10.4. ADDITION OF ANTI-MOUSE FITC CONJUGATE

Add 25µL of FITC reagent to each specimen well and add 20µL FITC reagent to each well of the control slide. Ensure that the reagent covers the entire area of each well. When using control slides which have wells with a diameter of less than 6mm it is recommended that 20µL of reagent is used.

10.5. SECOND INCUBATION

Incubate the slides with FITC reagent in a **moist chamber** for **15 minutes** at **37°C**. **Do not** allow the reagent to dry on the specimen, as this will cause the appearance of non-specific staining.

10.6. WASHING THE SLIDE

Rinse gently with PBS using a squeeze wash bottle . Do not focus the PBS stream directly onto the wells. Tilt the slide down and run the PBS stream across the slide above the wells, allowing the PBS to run off the bottom edge of the slide. Gently wash the slide in an agitating bath containing PBS for 5 minutes. Drain off PBS and allow the slide to air dry at room temperature (15-30°C).

10.7. ADDITION OF MOUNTING FLUID

Add one drop of Mounting Fluid to the centre of each well and place a coverslip over the Mounting Fluid and specimen ensuring that no air bubbles are trapped.

10.8. READING THE SLIDE

Examine the entire well areas containing the stained specimen using an epifluorescence microscope. Fluorescence, as described in Section 11, should be visible at x200-x500 magnification. (For best results slides should be examined immediately after staining, but may be stored at 2-8°C, in the dark, for up to 24 hours).

If a positive screening result is obtained (see Section 11.2) specific identification of the virus present can be performed on an additional 8 well slide preparation (see Section 11) using individual IMAGEN direct immunofluorescence reagents listed in Section 6.1.

11. INTERPRETATION OF TEST RESULTS

11.1. CONTROLS

11.1.1 Control Slide Positive Wells

When stained and viewed as described in Section 10, positive wells on the control slide should show cells with intracellular apple-green cytoplasmic and/or nuclear fluorescence contrasting against a background of red counterstained material. Positive control slides should be used to check that the staining procedure has been satisfactorily performed.

11.1.2 Control Slide Negative Wells

When stained and viewed as described in Section 10, negative wells on the control slide should show cells with no intracellular apple-green fluorescence. Only background red counterstaining should be visible.

11.2. CLINICAL SPECIMENS

11.2.1 Appearance of Virus Infected Cells

Stained infected cells will demonstrate apple-green cytoplasmic and/or nuclear fluorescence. Uninfected cells stain red with Evans blue counterstain.

11.2.2 Interpretation

A positive diagnosis is made when one or more cells in the fixed, stained specimen show the typical fluorescence pattern described in Section 11.2.1.

A negative diagnosis is made when fixed, stained specimens do not exhibit fluorescence with the screening reagent.

For directly stained nasopharyngeal aspirate specimens, at least 20 uninfected respiratory (columnar) epithelial cells must be observed within each slide well area before a negative result is reported. See Section 11.2.3 if insufficient cells are present.

11.2.3 Insufficient Cells

If insufficient cells are present on the slide, then the remainder of the clinical specimen should be centrifuged at 380g for 10 minutes at room temperature (15-30°C). Resuspend in a smaller volume (approximately 50µL) of PBS before re-distribution (25µL) on each well area. Alternatively a repeat clinical specimen should be requested.

11.2.4 Quality Control

A Positive and Negative Control Slide should be stained and examined on each occasion that IMAGEN Respiratory Screen reagents are used.

The Control Slides provided with the kit serve as a suitable

control to determine correct performance of staining technique and reactivity of the reagent with RSV, influenza A and B, parainfluenza virus 1, 2 or 3 and adenovirus in infected cells.

11.2.5 Reporting of Results

The following guidelines are recommended for reporting of results:

Positive result as defined in 11.2.2:

Presumptively positive for one or more of RSV, influenza A and B virus, parainfluenza virus 1, 2 or 3 and adenovirus. Culture isolation and specific identification to follow. For specific identification refer to Section 11.4

Negative result as defined in 11.2.2:

Presumptively negative for RSV, influenza A and B virus, parainfluenza virus 1, 2 or 3 and adenovirus. Culture confirmation to follow

11.3. CELL CULTURE CONFIRMATION

11.3.1 Appearance of Virus Infected Cells

Infected cells will demonstrate intracellular, nuclear and/or cytoplasmic apple-green fluorescence and should be recorded as positive. Uninfected cells will be counterstained red, with Evans blue counterstain.

11.3.2 Interpretation of Results

A positive diagnois is made when at least one fixed, stained cell shows the fluorescence pattern described in Section 11.3.1 after staining.

A negative result is indicated when fixed, stained specimens do not exhibit fluorescence after staining with the reagents.

At least 50 uninfected cells of the cell culture being tested must be visible within each slide well area, before a negative result is reported. See Section 11.3.4 if insufficient cells are present.

11.3.3 Reporting of Results

The following guidelines are recommended for reporting of results:

Positive result as defined in 11.3.2:

Positive for one or more of RSV, influenza A and B virus, parainfluenza virus 1, 2 or 3 and adenovirus. Specific identification to follow. For specific identification refer to Section 11.4.

Negative as defined in 11.3.2:

Negative for RSV, influenza A and B virus, parainfluenza virus 1, 2, or 3 and adenovirus.

11.3.4 Insufficient Cells

If insufficient cells are present in the slide preparation, the remainder of the cell culture specimen should be centrifuged at 200g for 10 minutes at room temperature (15-30°C). Resuspended in a smaller volume (approximately 50µL) of PBS before re-distribution (25µL) on each well area.

Alternatively, a repeat specimen should be re-inoculated onto fresh cell monolayers and the virus culture repeated.

11.4. SPECIFIC IDENTIFICATION OF RESPIRATORY VIRUS

Specific identification of the respiratory virus present can be made by confirming positive results using IMAGEN direct immunofluorescence (see Section 6.1, for preparation of slides see Section 9).

12. PERFORMANCE LIMITATIONS

12.1. Use only the Mounting Fluid provided.

12.2. Although the monoclonal antibodies used in this test have been raised against prototype strains and selected on their reactivity to a conserved epitope they may not detect virus strains which have undergone antigenic variation in the structure of the target epitope or new strains of the virus.

12.3. The Screening Reagent and Negative Control Reagent may non-specifically stain *Staphylococcus aureus* strains containing protein A. This is due to the non-immune interaction of protein A with the Fc region of the monoclonal antibodies, an observation reported for other monoclonal and polyclonal based fluorescence assays[20].

12.4. The visual appearance of the fluorescence image obtained may vary due to the type of microscope and light source used.

12.5. It is recommended that 25µL of reagent is used to cover a 6mm diameter well area. A reduction in this volume may lead to difficulties in covering the specimen area and may reduce sensitivity.

12.6. All reagents are provided at fixed working concentrations. Test performance will be affected if the reagents are modified in any way or not stored under the recommended conditions as outlined in Section 5.

12.7. IMAGEN Respiratory Screen cannot be used to specifically identify respiratory viruses. For specific identification positive specimens should be further tested using individual direct immunofluorescence IMAGEN reagents (see Section 6).

12.8. Failure to detect a virus by direct tests or by cell culture confirmation may be a result of factors such as collection of specimen at an inappropriate time of the disease, improper sampling and/or handling of specimen, failure of cell culture etc. A negative result does not exclude the possibility of a viral infection.

12.9. Test results should be interpreted in conjunction with information available from epidemiological studies, clinical assessment of the patient and other diagnostic procedures.

12.10. Inherent in the manufacture of the control slide, an occasional mix of material may occur (i.e. a few positive cells in the negative well.). The slide will still serve as a suitable control to determine correct performance of staining technique and reactivity of the reagent with RSV, influenza A and B, parainfluenza virus 1, 2 or 3 and adenovirus in infected cells.

13. EXPECTED VALUES

The detection rate for respiratory viruses is influenced by geographical, climatic and seasonal factors as well as demographic factors such as overcrowding, socio-economic and nutritional status. In addition, specimen quality factors including the type of specimen tested, the time of specimen collection during the course of infection, the handling and storage of specimens, including procedures used for preparation of smears, may all influence the success of diagnostic testing by direct detection techniques.

Generally, respiratory viruses occur on a world-wide basis and may cause sporadic infection, small outbreaks, epidemics or occasional pandemics with the emergence of new antigenic variants of virus. Despite seasonal occurrences of respiratory virus infection it is possible that during the course of a season viruses such as parainfluenza virus 1 and 2 and influenza B may not be isolated or detected due to low prevalence rates.

RSV is seasonally associated with significant outbreaks of upper and lower respiratory tract infection. Infants in the first six months of life and all immuno-compromised groups are most at risk. Approximately 50% of all infants experience RSV infection in the first year of life[21]. RSV may account for 20% of all respiratory tract infections during a respiratory season[21].

Influenza A or B virus may cause outbreaks of infection. Influenza A infection rates are influenced most by the immune status of a population in association to circulating strains. When a high proportion of immunity is achieved influenza may account for 15% of respiratory tract infections but with the emergence of new strains, to which there is little or no indigenous immunity, influenza may account for more than 50% of all respiratory tract infections[22].

With influenza B, highest attack rates are usually reported amongst school age children[23].

Adenoviruses may be responsible for 5% of acute respiratory infection in children under 4 years and 10% of all respiratory infection in hospitalised children[12]. Adenoviruses are sometimes responsible for small localised epidemics of acute respiratory disease in young adults especially those living in confined conditions.

Parainfluenza virus infections account for up to 20% of respiratory tract infection, in infants 2-4 years of age who may suffer severe symptoms including laryngotracheobronchitis (croup)[24]. Outbreaks of infection, especially with parainfluenza virus 3, may occur in older children and adults. Greater than 90% of infants become infected with parainfluenza virus early in life and symptomatic re-infections are common[8]. Outbreaks of infection due to parainfluenza virus 3 may occur during spring and summer months.

Direct detection of respiratory viruses in respiratory epithelial cells provides rapid, clinically relevant diagnostic information when either virus viability or the absence of virus culture facilities are limitations to successful diagnosis.

During the winter of 1994-95, the following prevalence rates were recorded by the three hospital laboratories performing the clinical evaluation.

At a Children's Hospital in Western New York State USA, RSV was identified by direct specimen immunofluorescence in 43.6% (478/1096) of respiratory specimens, influenza A was identified by direct specimen immunofluorescence and cell culture isolation in 3.1% (34/1096) of respiratory specimens and influenza B was identified by direct immunofluorescence and cell culture isolation in 1.1% (12/1096) of respiratory specimens. Adenovirus was identified by cell culture isolation in 4.4% (39/883) of respiratory specimens and a parainfluenza virus was identified by cell culture isolation in 2.9% (26/883) of respiratory specimens (including 2 specimens containing parainfluenza virus 1, 3 containing parainfluenza virus 2 and 21 containing parainfluenza virus 3).

At a hospital in the North West of the UK with a varied patient population, RSV was identified by direct specimen immunofluorescence in 49.68% (467/940) of respiratory specimens. Influenza B was identified by direct specimen immunofluorescence or cell culture isolation in 1.7% (16/940) of respiratory specimens. Adenovirus was identified by direct specimen immunofluorescence or cell culture isolation in 1.17% (11/940) of respiratory specimens. Parainfluenza virus 1 was identified by direct specimen immunofluorescence or cell culture isolation in 0.1% (1/940) of respiratory specimens.

At a hospital in the South West of the UK with a varied patient population RSV was identified by direct specimen immunofluorescence in 10.6% (195/1840) of respiratory specimens. Influenza B was identified by direct specimen immunofluorescence or cell culture isolation in 3.04% (56/1840) of respiratory specimens.

Adenovirus was identified by cell culture isolation in 1.2% (22/1840) of respiratory specimens and a parainfluenza virus was identified by direct specimen immunofluorescence or cell culture isolation in 1.14% (21/1840) of respiratory specimens. In addition to the above viruses, the following viruses were isolated from respiratory specimens: picornavirus 1% (18/1840), cytomegalovirus 0.38% (7/1840), rhinovirus 1.7% (32/1840) Herpes simplex virus type 1 1.03% (19/1840) and Herpes simplex virus type 2 0.5% (1/1840).

14. SPECIFIC PERFORMANCE CHARACTERISTICS

14.1. CLINICAL STUDIES

IMAGEN Respiratory Screen was evaluated for direct use at two clinical trial centres in the UK; one located in the north-east of the country and the other in the south-west, and one trial centre in the State of New York, USA. Testing was done on respiratory specimens collected from children and adults hospitalised with symptoms of respiratory infection. The test was also evaluated for detection of virus antigen in cell cultures inoculated with respiratory specimens.

Clinical specimens were collected during winter 1994 - spring 1996, and the trial centres compared the IMAGEN Respiratory Screen test with the standard methods used, and a commercial indirect immunofluorescence test comprising a Screening Reagent and individual typing reagents. Fresh clinical specimens and frozen specimens were used for these evaluations, in addition stored frozen specimens from 1993-94 were tested.

The trial centres performed direct tests on 343 clinical specimens and on 87 cell culture monolayers for confirmation of the presence of virus. For direct specimens the IMAGEN Respiratory Screen was evaluated against a commercially available indirect immunofluorescence screening test and virus culture. For cell cultures the IMAGEN Respiratory Screen was evaluated against a commercially available indirect immunofluorescence screening test and either haemadsorption inhibition, viral neutralisation or direct immunofluorescence testing.

14.2. CLINICAL PERFORMANCE

14.2.1 Direct Specimens

A total of 343 nasopharyngeal aspirates (NPAs) were evaluated at the three trial centres. Specimens included:

144	specimens positive for RSV
11	specimens positive for influenza A
5	specimens positive for influenza B
19	specimens positive for parainfluenza virus 1
4	specimens positive for parainfluenza virus 2
26	specimens positive for parainfluenza virus 3
8	specimens positive for adenovirus
141	negative specimens
5	Specimens with coinfections:
2	coinfections of RSV and parainfluenza 1
1	coinfection of RSV and adenovirus
1	coinfection of RSV and adenovirus
1	coinfection of adenovirus and parainfluenza 3

Table 14.2.1 shows results of IMAGEN Respiratory Screen and a commercial indirect immunofluorescence screening test compared to viral isolation on direct respiratory specimens. A correlation of 94.1% (317/337) was obtained for the IMAGEN Respiratory Screen compared to the standard method. Test sensitivity was 96.7% (205/212) compared to viral isolation.

Table 14.2.1 Comparison of IMAGEN Respiratory Screen and a commercial indirect immunofluorescence screening test with viral isolation for direct respiratory specimens

		IMAGEN Respiratory Screen		Commercial IIF Test	
		+	-	+	-
Viral Isolation[a]	+	205	7[c]	207	5[e]
	-	13[b]	112	15[d]	110
% Relative Sensitivity 95% Confidence Intervals		96.7 (205/212) (94-99%)		97.6 (207/212) (95-100%)	
% Relative Specificity 95% Confidence Intervals		89.6 (112/125) (83-94%)		88.0 (110/125) (81-93%)	
% Correlation 95% Confidence Intervals		94.1 (317/337) (91-97%)		94.1 (317/337) (91-97%)	

a Viral isolation was performed in Hep-2, A549, primary rhesus monkey kidney, MK, HEL, RK13, or G293 cell lines and was confirmed by specific indirect immunofluorescence and either haemadsorption inhibition, viral neutralisation or direct immunofluorescence testing.

b Thirteen specimens also positive by direct specimen tests using both IMAGEN and commercially available typing tests (See Table 14.2.4) including:

RSV	7
Influenza A	3
Parainfluenza 1	1
Adenovirus	2

c Two specimens positive for adenovirus; two specimens positive for parainfluenza 2; three specimens positive for parainfluenza 3 (See Table 14.2.4).

d Fifteen specimens, including thirteen detailed in footnote[b] and two other specimens, positive only by commercial IIF (See Table 14.2.4).

e Five specimens negative by commercial IIF including one influenza A, two parainfluenza virus 2, one parainfluenza virus 3 and one adenovirus (See Table 14.2.4).

Table 14.2.2 shows a comparison of IMAGEN Respiratory Screen with a commercial indirect immunofluorescence screening test on nasopharyngeal aspirate specimens at each of the trial centres. At Centre 1 and 2 all specimens tested (with the exception of 7 specimens at Centre 2) had been stored frozen. At Centre 3, 121 specimens were tested fresh within 24 hours of collection and 68 specimens were tested after frozen storage.

Table 14.2.2 Comparison of IMAGEN Respiratory Screen with a commercial indirect immunofluorescence screening test (Bartels VRK) on respiratory specimens

			Commercial IIF test	
			+	-
Trial Centre 1 (South-west UK) n = 74	IMAGEN Respiratory Screen	+	49	0
		-	0	25
Trial Centre 2 (North-east UK) n = 80	IMAGEN Respiratory Screen	+	46	0
		-	4[g]	30
Trial Centre 3 (State of New York, USA) n = 189	IMAGEN Respiratory Screen	+	126	1[f]
		-	1[g]	61

f One specimen culture positive for influenza A which was also typed in both the direct specimen test and culture by IMAGEN Influenza A reagent

g Includes one specimen from Centre 2 and one specimen from Centre 3 recorded as positive only in the commercial IIF screening test

Table 14.2.3 shows a summary of data from all three trial centres comparing IMAGEN Respiratory Screen with the commercial indirect immunofluorescence screening test on nasopharyngeal aspirate specimens.

Table 14.2.3 Summary of comparison of IMAGEN Respiratory Screen with a commercial indirect immunofluorescence screening test (Bartels VRK) on respiratory specimens

		Commercial IIF test	
		+	-
IMAGEN Respiratory Screen	+	221	1[f]
	-	5[g]	116
Relative Sensitivity 95% Confidence Intervals		97.8% (221/226) (95-99%)	
Relative Specificity 95% Confidence Intervals		99.1% (116/117) (95-100%)	
% Correlation 95% Confidence Intervals		98.3% (337/343) (96-99%)	

f One specimen culture positive for influenza A which was also typed in both the direct specimen test and culture by IMAGEN Influenza A reagent

g Includes one specimen from Centre 2 and one specimen from Centre 3 recorded as positive only in the commercial IIF screening test

Table 14.2.4 Detailed results of specimens referenced in footnotes to Tables 14.2.1 to 14.2.3

Footnote Reference	Specimen No.	Culture Result	IMAGEN Screen Result	IMAGEN Typing Result	Commercial IIF screen test	Commercial IIF typing test
B	1	Negative	Positive	Adenovirus	Positive	Adenovirus
B	2	Negative	Positive	Adenovirus	Positive	Negative
B	3	Negative	Positive	Influenza A	Positive	Influenza A
B	4	Negative	Positive	Influenza A	Positive	Influenza A
B	5	Negative	?Positive	Influenza A	Positive	Influenza A
B	6	Negative	Positive	RSV	Positive	RSV
B	7	Negative	Positive	RSV	Positive	RSV
B	8	Negative	Positive	RSV	Positive	RSV
B	9	Negative	Positive	RSV	Positive	RSV
B	10	Negative	Positive	RSV	Positive	RSV
B	11	Negative	Positive	RSV	Positive	RSV
B	12	Negative	Positive	RSV	Equivocal	RSV
B	13	Negative	Positive	Parainfluenza 1	Positive	Parainfluenza 1
C and E	14	Parainfluenza 3	Negative	Negative	Negative	Negative
C and E	15	Adenovirus 2	Negative	Not tested	Negative	Not tested
C and E	16	Parainfluenza 2	Negative	Undiagnosable	Negative	?Parainfluenza 2
C and E	17	Parainfluenza 2	Negative	Negative	Negative	Undiagnosable
C	18	Parainfluenza 3	Negative	?Parainfluenza 3	Positive	?Parainfluenza 3
C	19	Parainfluenza 3	Negative	Parainfluenza 3	Positive	Parainfluenza 3
C	20	Adenovirus 4	Negative	Negative	Positive	Adenovirus
D and G	21	Negative	Negative	?Influenza A	Positive	? Influenza A
D and G	22	Negative	Negative	Negative	Positive	Negative
E and F	23	Influenza A	Positive	Influenza A	Negative	Influenza A

14.2.2 Culture Confirmation

The trial centres tested the IMAGEN Respiratory Screen on viral isolates from clinical specimens and stored virus strains. Clinical specimens were collected mainly during the winter of 1994-95 and the trial centres compared the IMAGEN Respiratory Screen test with the standard methods. Virus isolation was performed using HEp-2, MRC-5 or RMK cells and isolates identified and confirmed using haemadsorption inhibition, viral neutralisation or direct immunofluorescence.

A total of 87 cultures were tested which included 7 cultures positive for RSV, 10 positive for influenza A, 15 positive for influenza B, 6 positive for parainfluenza 1, 8 positive for parainfluenza 2, 5 positive for parainfluenza 3, 9 positive for adenovirus and 27 negative cultures.

Table 14.2.5 Comparison of IMAGEN Respiratory Screen with viral isolation and commercial IIF test (Bartels VRK) for cell culture confirmation

			Commercial IIF test		Viral Neutralisation	
			+	-	+	-
Trial Centre 1 (South-west UK) n = 32	IMAGEN Respiratory Screen	+	17	1[a]	18	0
		-	0	14	0	14
Trial Centre 3 (State of New York, USA) n = 52	IMAGEN Respiratory Screen	+	39	0	39	0
		-	0	13	0	13
Oxoid (Ely) Ltd n = 3	IMAGEN Respiratory Screen	+	3	0	3	0
		-	0	0	0	0

a Specimen from which RSV was isolated and typed by culture

Table 14.2.6 Summary of comparison of IMAGEN Respiratory Screen with viral isolation and commercial IIF test (Bartels VRK) for cell culture confirmation

		Commercial IIF test		Viral Neutralisation	
		+	-	+	-
IMAGEN Respiratory Screen	+	59	1[a]	60	0
	-	0	27	0	27
% Relative Sensitivity 95% Confidence Intervals		100(59/59) (94-100%)		100 (60/60) (94-100%)	
% Relative Specificity 95% Confidence Intervals		96.4(27/28) (82-100%)		100 (27/27) (87-100%)	
% Correlation 95% Confidence Intervals		98.9 (86/87) (94-100%)		100 (87/87) (96-100%)	

a Specimen from which RSV was isolated and typed by culture

14.3. CROSS REACTIVITY

The micro-organisms listed in Table 14.3.1 were tested in the IMAGEN Respiratory Screen and showed no cross-reactivity. Cross reactivity studies were performed on slide preparations of stock cultures or recent microbial isolates.

Table 14.3.1 Micro-organisms tested with IMAGEN Respiratory Screen and found to be non-reactive

Micro-organism	Source
Acholeplasma laidlawii	NCTC 10116 Broth culture deposit.[2]
Bordetella pertussis[1]	Chocolate agar[3]
Branhamella catarrhalis[1]	Chocolate agar[3]
Candida albicans[1]	Fungal medium[3]
Chlamydia pneumoniae[1]	Commercial preparation[4]
Chlamydia psittaci[1]	Commercial preparation[4]
Chlamydia trachomatis A-K, LGV1-LGV3[1]	Commercial preparation[4]
Coxsackie virus A9	Commercial preparation[5]
Coxsackie virus B1, B2, B3, B4, B5	Stock strains[5]
Cytomegalovirus	Human clinical isolate[5]
Echovirus 9, 11, 19, 22	Human clinical isolates[5]
Epstein-Barr virus	Commercial preparation used in routine serological tests[5]
Herpes simplex virus types 1 and 2	Commercial preparation[5]
Legionella pneumophila	Serogroup 1. Formalised yolk sac. Commercial preparation used in routine serological tests[4]
Mycoplasma arginini	NCTC 10129 Broth culture deposit[2]
Mycoplasma hominis	NCTC 10111 Broth culture deposit[2]
Mycoplasma hyorhinus	NCTC 10130 Broth culture deposit[2]
Mycoplasma orale	NCTC 10112 Broth culture deposit[2]
Mycoplasma pneumoniae	NCTC 10119 Broth culture deposit[2]
Mycoplasma salivarium	NCTC 10113 Broth culture deposit[2]
Neisseria cinerea[1]	Blood agar[3]
Neisseria lactamica1	Blood agar[3]
Neisseria meningitidis A, B, C[1]	Blood agar[3]
Neisseria perflava[1]	Blood agar[3]
Poliovirus types 1,2 and 3	Human clinical isolates[5]
Pneumocystis carinii	Commercial preparation used in routine serological tests[4]
Rhinovirus	Human clinical isolate[5]
Streptococcus groups A, B, C, D, F and G[1]	Columbia agar[3]

1 Data acquired at Oxoid (Ely) Ltd.

2 Smears prepared from three day broth cultures, centrifuged at 3000g for 30 minutes, washed in PBS, resuspended in 100µL of PBS and 15µL of suspension added to microscope slide wells.

3 Milky suspensions prepared in PBS from culture medium, centrifuged, washed, resuspended in 1mL PBS and 20µL of suspension added to microscope slide wells.

4 Commercial slide preparations.

5 Smears prepared from viral tube cultures showing 50% CPE, washed, resuspended in 0.2mL PBS and 15µL of suspension added microscope slide wells.

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X7852A Revised March 2017

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