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Europe +800 135 79 135 US 1 855 2360 190

CA 1 855 805 8539 ROW +31 20 794 7071

IMAGEN Parainfluenza virus Types 1, 2 and 3

REF K610311-2, Σ 50

K610411-2 Σ 50

EN

A direct immunofluorescence test for the detection of **Parainfluenza 1, 2 and 3**.

1. INTENDED USE

IMAGEN™ Parainfluenza virus test kits are qualitative direct immunofluorescence tests for the detection and differentiation of Parainfluenza virus types 1, 2 and 3 directly in nasopharyngeal aspirates or in cell cultures. The kits are available in two formats:

IMAGEN Parainfluenza virus Group (Types 1, 2 and 3) (Code No. K6103) for the detection and confirmation of the presence of Parainfluenza virus antigens directly in nasopharyngeal aspirates and in cell culture preparations.

IMAGEN Parainfluenza virus Types 1, 2 and 3 (Code No. K6104) for the detection and differentiation of Parainfluenza virus types 1, 2 and 3 antigens respectively directly in nasopharyngeal aspirates and in cell culture preparations.

A negative result obtained following direct staining of nasopharyngeal aspirates should be considered presumptive until confirmed by culture.

2. SUMMARY

Parainfluenza viruses are members of the genus Paramyxovirus classified within the family Paramyxoviridae.[1] There are 6 species of Paramyxovirus known to infect man which include Parainfluenza viruses 1, 2, 3 and 4 (subtypes 4a and 4b), Mumps virus and Newcastle Disease virus. Of the 4 Parainfluenza viruses, types 1, 2 and 3 are now recognised as a major cause of acute respiratory disease in infants and children.[2,3]

Parainfluenza viruses are spread by direct contact or inhalation of virus-containing droplets shed from the respiratory tract of symptomatic individuals. Nasal secretions can contain high concentrations of virus. The virus multiplies in the ciliated columnar epithelial cells of the upper and lower respiratory tract causing cell necrosis and sloughing.

Viral shedding occurs from 1 day before, up to 7 days after the onset of symptoms. Shedding may persist in immunocompromised patients.[4,5]

Parainfluenza virus types 1, 2 and 3 can infect and cause disease throughout the upper and lower respiratory tract. Most Parainfluenza virus infections manifest clinically as acute laryngotracheobronchitis (croup) in infants and children.

However, Parainfluenza virus infections are also associated with tracheobronchitis, bronchitis, pneumonia and a range of symptoms associated with the upper respiratory tract.

Occasionally Parainfluenza virus infections can be severe in infants, causing obstruction of airways and respiratory distress which may lead to increased morbidity and mortality in patients with underlying disease such as cystic fibrosis or in immunocompromised patients.

Parainfluenza viruses have been associated with outbreaks of respiratory tract infections in paediatric wards and geriatric wards resulting in prolonged hospitalisation and increased morbidity and mortality.[6] Rapid diagnosis is important in the management of patients and the control of outbreaks.

At present the main diagnostic methods employed for the diagnosis of Parainfluenza virus infections are either isolation and identification in cell culture monolayers or direct detection of the virus in clinical samples.

Following virus isolation by cell culture, in order to identify the virus it is necessary to perform additional testing, neutralisation, haemagglutination inhibition or electron microscopy. These tests are laborious and time consuming and require a degree of technical expertise which may not be available in many laboratories.

More recently, indirect immunofluorescence tests using polyclonal or monoclonal antibodies for the identification and confirmation of Parainfluenza viruses in cell culture monolayers or directly in clinical specimens, have been described.[7,8,9]

Direct immunofluorescence tests utilising fluorescein labelled monoclonal antibodies offer a more rapid and specific method for the detection of viruses in clinical specimens or cell cultures.

The IMAGEN Parainfluenza virus kits are direct immunofluorescence tests for the detection and identification of Parainfluenza virus types 1, 2 and 3 directly in nasopharyngeal aspirates or in cell culture. The tests utilise type-specific monoclonal antibodies to detect and identify Parainfluenza virus types 1, 2 and 3.

3. PRINCIPLE OF TEST

The IMAGEN Parainfluenza virus test kits contain fluorescein isothiocyanate (FITC) conjugated monoclonal antibodies which bind specifically to either Parainfluenza virus types 1, 2 or 3. These are used in a one step direct immunofluorescence technique. Specimens are incubated with the FITC conjugated reagents for 15 minutes. Excess reagent is then removed by washing with phosphate buffered saline (PBS). The stained areas are mounted and viewed using epifluorescence illumination.

If Parainfluenza virus types 1, 2 or 3 are present then characteristic apple-green intracellular fluorescence is seen within infected cells, which contrasts with the red background staining of uninfected cells.

Acknowledgement
The monoclonal antibodies used in these tests originated in the Department of Respiratory and Enteric Viruses, Public Health Services, Centre for Disease Control, Atlanta, Georgia, USA.

4. DEFINITIONS

The following symbols have been used throughout the product information.

REF	Product code and catalogue number
i	Consult the instructions for use
Σ N	Contains sufficient for <N> tests
	Manufactured by
IVD	*In vitro* diagnostic medical device
	Use by
LOT	Batch Code
	Storage temperature limitations

5. REAGENTS PROVIDED

Σ 50 - Each kit contains sufficient materials for testing 50 cell culture preparations.

- The shelf-life of the kit is as indicated on the outer box label.

5.1. IMAGEN PARAINFLUENZA VIRUS REAGENTS PROVIDED

i Instructions For Use.

POSITIVE CONTROL SLIDE 2 x 3 well positive control slides containing acetone fixed African Green monkey kidney cells (Veros) infected with either Parainfluenza virus type 1, 2 or 3 (strain CDC V6-004, CDC V7-003 and CDC V5-003 respectively).

MOUNTING FLUID 3mL of mounting fluid. The mounting fluid contains a photobleaching inhibitor in a glycerol solution (pH 10.0).

One bottle of each of the following:

IMAGEN PARAINFLUENZA VIRUS GROUP REAGENTS – K6103

REAGENT G 1.4mL of IMAGEN Parainfluenza virus Types 1, 2 and 3 reagent. This reagent consists of a mixture of 3 purified murine monoclonal antibodies specific to Parainfluenza virus types 1, 2 and 3, conjugated to FITC, diluted in a protein stabilised phosphate buffered saline solution containing evans blue dye as a counterstain.

The monoclonal antibodies are targeted against Parainfluenza 1 F protein, Parainfluenza 2 haemagglutinin protein and Parainfluenza 3 haemagglutinin protein respectively.

OR

IMAGEN PARAINFLUENZA VIRUS TYPES 1, 2 AND 3 REAGENTS – K6104

REAGENT 1 1.4mL of IMAGEN Parainfluenza virus 1 reagent. This reagent consists of a purified murine monoclonal antibody specific to Parainfluenza virus type 1 (targeted against Parainfluenza 1 F protein), conjugated to FITC, diluted in a protein stabilised phosphate buffered saline solution containing evans blue dye as a counterstain.

REAGENT 2 1.4mL of IMAGEN Parainfluenza virus 2 reagent. This reagent consists of a purified murine monoclonal antibody specific to Parainfluenza virus type 2 (targeted against Parainfluenza 2 haemagglutinin protein), conjugated to FITC diluted in a protein stabilised phosphate buffered saline solution containing evans blue dye as a counterstain.

REAGENT 3 1.4mL of IMAGEN Parainfluenza virus 3 reagent. This reagent consists of a purified murine monoclonal antibody specific to Parainfluenza virus type 3 (targeted against Parainfluenza 3 haemagglutinin protein), conjugated to FITC, diluted in a protein stabilised phosphate buffered saline solution containing evans blue dye as a counterstain.

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

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

5.3. POSITIVE CONTROL SLIDES - POSITIVE CONTROL SLIDE

Positive control slides are provided individually sealed in foil pouches filled with nitrogen. Store unused slides at 2-8°C. The slide should be left in its pouch 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. REAGENTS, GROUP, 1, 2 AND 3 - REAGENT G/REAGENT 1/REAGENT 2/REAGENT 3

Ready to use. Store unused reagents in the dark at 2-8°C. The reagents 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.

6.2. ACCESSORIES

The following products are intended for use in conjunction with K6103 and K6104. 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. S6114).

Positive Control Slides (Code No. S6111).

7. EQUIPMENT

The following equipment is required:

Precision pipette and disposable tips to deliver 25µL.

Wash bath.

Coverslips suitable to cover 6mm diameter well.

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.

For Culture Confirmation
Sterile swabs, viral transport medium (VTM) and container suitable for collection, transportation and culture of Parainfluenza viruses. For information on use of suitable VTM please refer to reference 13 in Section 13 (available from your local Oxoid subsidiary).

Cell culture lines recommended for culture and isolation of Parainfluenza viruses.

A negative control slide prepared from uninfected intact cells of the type in use for the culture and isolation of Parainfluenza viruses. Cells should be prepared and fixed as described in Section 9.1 and stained as described in Section 10.

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 Parainfluenza virus test reagents contain <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 Parainfluenza viruses on the control slide has been shown to be non infectious in cell culture, however, slides 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 contains a known skin irritant. Although present below the concentration for the product to be classified as an irritant, 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 infected cells, 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 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 reagents are modified or stored under conditions other than those detailed in Section 5.

8.2.3 Typing reagents should not be pooled, or used together on one slide well, as the performance of the test may be adversely affected.

8.2.4 Prepare fresh Phosphate Buffered Saline (PBS) as required on the day of use.

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

8.2.6 Avoid microbial contamination of reagents.

8.2.7 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 Parainfluenza virus by cell culture. 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.

The recommended respiratory specimen is a nasopharyngeal aspirate (NPA) which should provide large numbers of respiratory epithelial cells.

9.1. CLINICAL SPECIMENS

9.1.1 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 maintained at 2-8°C and sent to the laboratory as soon as possible for processing.

Cell Separation
If necessary add 2mL phosphate buffered saline (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 is 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 stage.

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 onto 6mm diameter well areas on slide.

One well is required for the IMAGEN Parainfluenza virus Group test (Code No. K6103). Three wells are required for the IMAGEN Parainfluenza virus Types 1, 2 and 3 test (Code No. K6104).

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 then store at -70°C until needed. Stored slides should be tested within two weeks of preparation as deterioration may occur on long term storage.

9.2. CELL CULTURE CONFIRMATION AND TYPING

Inoculation of cell cultures
Specimens collected for the diagnosis of parainfluenza virus 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 a cytopathic effect (CPE) and haemadsorption tests carried out at regular intervals.

Any haemadsorption positive cultures, or cell cultures showing any CPE, can be harvested and tested for the presence of Parainfluenza viruses.

Preparation of slides
Scrape the cell sheet into the culture medium using a sterile pipette. Deposit the cells by centrifugation the cells 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 and repeat the centrifugation. Remove the supernatant and resuspend the cell deposit in a small volume (75-100µL per culture tube) of fresh PBS to maintain a high cell density.

Place 25µL aliquots of the cell suspension on to individual wells on the slides.

One well is required for the IMAGEN Parainfluenza virus Group test (Code No. K6103).

Three wells are required for the IMAGEN Parainfluenza virus test (Code No. K6104).

Allow to air dry thoroughly and fix in fresh acetone at room temperature (15 30°C) for 10 minutes. If the specimen is not stained immediately, store at 4°C overnight or freeze at –20°C for longer storage periods. Stored slides should be tested within 2 weeks of preparation as deterioration may occur on long term storage.

10. TEST PROCEDURE

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

For the IMAGEN Parainfluenza virus Group (Code No. K6103) test procedure refer to Section 10.1 and for the IMAGEN Parainfluenza virus Types 1, 2 and 3 (Code No. K6104) test procedure refer to Section 10.2

10.1. IMAGEN PARAINFLUENZA VIRUS GROUP (K6103)

10.1.1 Addition of Reagent

Add 25µL of Reagent G to the fixed cell preparation (see Section 9.1), positive control slide and negative control slide. Ensure that the reagent covers the entire well area.

10.1.2 First Incubation

Incubate 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.

10.1.3 Washing the Slide

Wash off excess reagent with phosphate buffered saline (PBS) then 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).

10.1.4 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.

10.1.5 Reading the Slide

Examine the entire well area 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 72 hours.)

10.2. IMAGEN PARAINFLUENZA VIRUS TYPES 1, 2 AND 3 - K6104

10.2.1 Addition of Reagent

Add 25µL of Reagent 1 to the first well, 25µL Reagent 2 to the second well and 25µL Reagent 3 to the third well of the fixed cell preparation (see Section 9.1), positive and negative control slides. Ensure that the reagent covers the entire well area.

10.2.2 First Incubation

Incubate 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.

10.2.3 Washing the Slide

Wash off excess reagent with phosphate buffered saline (PBS) and 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).

10.2.4 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.

10.2.5 Reading the Slide

Examine the entire well area 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 72 hours).

11. INTERPRETATION OF TEST RESULTS

11.1. CONTROLS

11.1.1 Positive Control Slide

When stained and viewed as described in Section 10, the positive control slide should show cells with intracellular nuclear and/or cytoplasmic apple-green 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.

These slides are prepared from Parainfluenza virus strains in cell culture monolayers and will only provide adequate control for the test procedure and not the specimen processing steps. Specimen processing procedures should be controlled using positive clinical material.

11.1.2 Negative Control

Negative control slides must be prepared from uninfected intact cells of the type in use for the culture and isolation of Parainfluenza viruses. These cells should be prepared and fixed as described in Section 9.2 and stained as described in Section 10. The cells on the negative control slide stain red with Evans blue counterstain.

Slides prepared in this way will only provide adequate control for the test procedure and not the sample processing steps. Sample processing steps should be controlled using negative clinical material.

11.2. CLINICAL SPECIMENS

11.2.1 Appearance of Parainfluenza virus infected cells

Intracellular, nuclear and/or cytoplasmic granular apple-green fluorescence is seen in respiratory epithelial cells infected with Parainfluenza virus. Uninfected cells stain red with the evans blue counterstain.

11.2.2 Interpretation of Results

A positive diagnosis is made when one or more cells in the fixed stained specimen shows the specific fluorescence described in Section 11.2.1 with the IMAGEN Parainfluenza virus Group reagent or one of the individual IMAGEN Parainfluenza virus type 1, 2 and 3 reagents. The result should be interpreted as positive for Parainfluenza virus antigen.

A negative diagnosis is made when fixed, stained specimens do not exhibit fluorescence with either the IMAGEN Parainfluenza virus Group reagent or any of the individual IMAGEN Parainfluenza virus types 1, 2 and 3 reagents. The result should be interpreted as negative for Parainfluenza virus antigen, culture result to follow.

For directly stained nasopharyngeal aspirate specimens, at least 20 uninfected respiratory epithelial cells must be observed 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 in the slide, 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 of PBS before re-distribution (25µL) on the well areas. Alternatively, a repeat clinical specimen should be requested.

11.3. CELL CULTURE CONFIRMATION AND TYPING

11.3.1 Appearance of Parainfluenza Virus Infected Cells

Infected cells will demonstrate intracellular, nuclear and/or cytoplasmic apple-green fluorescence and should be recorded as positive for Parainfluenza.

Uninfected cells stain red with evans blue counterstain and should be recorded as negative for Parainfluenza.

11.3.2 Interpretation of Results

A positive diagnosis is made when one or more of the cells in the fixed, stained specimen show the typical fluorescence pattern described in Section 11.3.1 with the IMAGEN Parainfluenza virus Group reagent or with one of the individual IMAGEN Parainfluenza virus Type 1, 2 and 3 reagents. At least 50 uninfected cells of the cell culture being tested must be observed on each slide well before a negative result is reported. See Section 11.2.3 if insufficient cells are present.

11.3.3 Quality Control

Positive and Negative controls should be stained and examined on each occasion that IMAGEN Parainfluenza reagents are used for determining the presence of Parainfluenza in infected cell cultures.

The positive control slides provided serve as a suitable control to

determine correct performance of staining technique and reactivity of the reagent with Parainfluenza virus infected cells. Additional positive control slides are available from Oxoid, (Code No S6111).

Reagents and technique should also be controlled by use of a negative control smear of cells made from uninoculated (non-infected) cell culture of the type in use for the isolation of Parainfluenza viruses.

If insufficient cells are observed (less than 50) on the test or negative control slide well preparation then the remainder of the cell culture sample should be centrifuged at 200g for 10 minutes at room temperature (15 30°C) and resuspended in a smaller volume (approximately 50µL) of PBS. Place 25µL of cell suspension onto individual wells and process as described in sections 9 and 10.

If both IMAGEN Parainfluenza virus Group and Typing reagents are used and negative results are obtained with the three individual reagents after a positive result is obtained with the group reagent, additional cell smears should be prepared and the test repeated. Alternatively, the culture should be passaged to amplify the amount of virus and the number of infected cells present before repeating immunofluorescence testing.

12. PERFORMANCE LIMITATIONS

12.1. Use only the mounting fluid provided.

12.2. Non specific staining is sometimes observed as an artefact in immuno chemical tests due to binding between antibody Fc regions and protein A antigen found in the cell wall of some strains of *Staphylococcus aureus*. The IMAGEN Parainfluenza virus test reagents have been modified to reduce binding to the protein A of *Staphylococcus aureus* Cowan 1 strain. However, the antibody used in the Parainfluenza virus type 1 reagent may show very weak cross-reaction with Protein A of *Staphylococcus aureus*. *Staphylococcus aureus* may be present in clinical specimens and therefore be inoculated into cell culture where its replication should be inhibited by antimicrobials.

Staphylococcus aureus invariably appears as tetrads or grape-like clusters of cocci (each approximately 1µM in diameter) and is likely to be extra cellular unlike the characteristic intra-cellular, fine granular staining seen in cell culture infected with Parainfluenza virus.

12.3. The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The end-point reaction may vary due to the type of microscope employed, the light source, age of the bulb, filter assembly and filter thickness, differences in sensitivity of the antigen substrate, or the assay procedure used. Each laboratory should establish its own criteria for reading of end-points using appropriate controls.

12.4. Failure to detect a Parainfluenza virus in cell culture may be a result of factors such as inappropriate collection, improper sampling and/or handling of specimen, failure of cell culture etc. A negative result does not exclude the possibility of a Parainfluenza virus infection.

12.5. Both the IMAGEN Parainfluenza Group and Typing reagents react with Parainfluenza virus antigens present in infected cells. A positive result, however, is not an indicator of the viability of the Parainfluenza virus present.

12.6. The monoclonal antibodies used in the IMAGEN Parainfluenza Group and Typing reagents have been raised against prototype Parainfluenza strains. The antibodies may not detect all antigenic variants or new strains of Parainfluenza virus.

12.7. Antigenic variation in the epitopes targeted by the monoclonal antibodies present in the IMAGEN Parainfluenza Group and Typing tests may give rise to new antigenic variants which may no longer be recognised by the antibodies.

12.8. The prevalence of the analyte will affect the predictive value of the assay.

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

12.10. 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.

12.11. All reagents are provided at fixed working concentrations. Test performance may be affected if the reagents are modified in any way or not stored under recommended conditions.

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

13. EXPECTED VALUES

The isolation rate for Parainfluenza viruses may vary depending on the type of test used, the method, the time and site of specimen collection, the handling, storage and transportation of samples. It is also dependent upon the population prevalence rates, as well as the age, geographic location and socio-economic status of the population tested.

Parainfluenza virus types 1, 2 and 3 are prevalent throughout the world and are associated with respiratory tract infections in temperate, tropical and arctic climates.[10] Most infections occur in infants under 5 years old in whom Parainfluenza viruses account for 20% of respiratory tract infections and 30 40% of cases of croup.[11,12]

Over 90% of infants will experience a primary infection with a Parainfluenza virus and up to 50% may experience symptomatic reinfections.[2]

Parainfluenza viruses show high prevalence rates during seasonal outbreaks of respiratory tract infections. In temperate zones Parainfluenza viruses are most commonly associated with outbreaks in the autumn and winter months.[13,14] In some countries outbreaks of Parainfluenza virus type 3 infections may also occur in the spring and summer months.[3]

Highest attack rates occur in children between the ages of 1 to 5 years. Infections or reinfections in older children and adults are usually associated with milder respiratory symptoms.

Parainfluenza viruses have been implicated in outbreaks of respiratory tract infections in hospitals, particularly paediatric wards and in geriatric institutions, where they have been associated with increased morbidity and mortality.[6]

During the winter of 1994-1995 the overall incidence of Parainfluenza virus at one trial centre based on viral isolation results was 12.5% (21/168) for Parainfluenza virus type 1, 1.8% (3/168) for Parainfluenza virus type 2 and 14.3% (24/168) for Parainfluenza virus type 3 virus. These figures do not accurately represent the prevalence of Parainfluenza virus in the overall population as specimens were taken from a selected population under investigation for respiratory tract infections.

14. SPECIFIC PERFORMANCE CHARACTERISTICS

14.1. CLINICAL STUDIES

14.1.1 Direct specimens

The IMAGEN Parainfluenza virus Group test and the IMAGEN Parainfluenza virus 1, 2 and 3 Typing test were evaluated for direct use on nasopharyngeal secretions at three centres, one in the US and two in the UK. Direct specimens were collected during the winter of 1994-1995.

The clinical trial was carried out using nasopharyngeal aspirate specimens from patients with suspected respiratory virus infections, which were collected and processed as outlined in section 9.1.1. Acetone fixed slides were tested either fresh (stored at 2-8°C and tested within 3 days) or frozen and tested at a later date. The standard (reference) methods used were a commercially available indirect immunofluorescence test and viral isolation and identification.

A specimen was scored positive with the IMAGEN Parainfluenza virus Group reagent or IMAGEN Parainfluenza virus types 1, 2 and 3 reagents if one or more respiratory epithelial cells showed the typical pattern of fluorescence described in Section 11.2.1.

14.1.2 Culture confirmation

The IMAGEN Parainfluenza virus Group test and the IMAGEN Parainfluenza virus 1, 2 and 3 Typing test were tested in clinical trials at 4 centres within the UK.

Clinical trials were undertaken during July-October 1991. During this time the overall incidence of Parainfluenza virus at one trial centre was 14.4% (15/104) for Parainfluenza 1 virus and 8.7% (9/104) for Parainfluenza 3 virus. These figures do not accurately represent the prevalence of Parainfluenza virus in the overall population as specimens were taken from a selected population under investigation for respiratory tract infections. Studies were performed on samples prepared from cell culture monolayers inoculated with clinical samples collected from patients with suspected Parainfluenza virus infection. The standard methods for identification of Parainfluenza virus in cell cultures were either neutralisation tests or indirect immunofluorescence. The IMAGEN Parainfluenza virus reagents were also tested for cross reactivity against a range of micro-organisms likely to be present in routine clinical samples (see Section 14.3).

14.2. CLINICAL PERFORMANCE

14.2.1 Direct specimens

A total of 222 samples from patients with suspected respiratory virus infections were tested in the IMAGEN Parainfluenza virus Group test and the IMAGEN Parainfluenza virus Types 1, 2 and 3 test. At a centre in the Northeast US during the winter of 1994-1995, a total of 184 samples were collected from a paediatric population and assessed in order to determine the sensitivity and specificity of the test.

The majority of these were tested in comparison with viral isolation, and a smaller number were tested in comparison with the reference indirect immunofluorescence test. Tables 14.1 and 14.2 show results from the US Centre. At the two UK centres, a selection of samples known to contain Parainfluenza virus were assessed in order to increase the number of positive samples obtained. These results can be seen in Tables 14.3 and 14.4. Results comparing the positivity rates for Parainfluenza viruses at all centres are shown in Tables 14.5 and 14.6.

IMAGEN Parainfluenza virus Group (Types 1, 2 and 3)
At the US centre the IMAGEN Parainfluenza virus Group (Types 1, 2 and 3) test showed a correlation of 97.6% against viral culture, and 98.4% against the reference indirect immunofluorescence test. The relative sensitivity and specificity of the IMAGEN Parainfluenza virus Group (Types 1, 2 and 3) test were 97.9% and 97.5% respectively when compared with viral isolation. In comparison with the reference indirect immunofluorescence test, the relative sensitivity and specificity were 96.2% and 99.0% respectively.

IMAGEN Parainfluenza virus Type 1
At the US Centre the IMAGEN Parainfluenza virus Type 1 reagent showed a correlation, sensitivity and specificity of 100% when compared with viral isolation and the reference immunofluorescence test.

IMAGEN Parainfluenza virus Type 2
At the US centre, the IMAGEN Parainfluenza virus Type 2 reagent showed a correlation, sensitivity and specificity of 100% when compared with viral isolation and the reference immunofluorescence test.

IMAGEN Parainfluenza virus Type 3
At the US Centre the IMAGEN Parainfluenza virus Type 3 reagent showed a correlation of 97.6%, and sensitivity and specificity of 91.6% and 98.6% respectively when compared with viral isolation. When compared with the reference immunofluorescence test, the correlation, sensitivity and specificity were 98.4%, 75% and 99.1% respectively.

Table 14.1 Comparison of performance of IMAGEN Parainfluenza virus reagents with viral isolation on nasopharyngeal aspirates at the U.S. Centre

			IMAGEN Parainfluenza virus reagents	
IMAGEN Parainfluenza virus reagent	No. of samples tested	No. of samples positive by viral isolation	Sensitivity	Specificity
Parainfluenza 1 (95% Confidence intervals)	168	21	100% (21/21) (84.0%-100%)	100% (147/147) (97.51%-100%)
Parainfluenza 2 (95% Confidence intervals)	168	3	100% (3/3) (29.3%-100%)	100% (165/165) (97.79%-100%)
Parainfluenza 3 (95% Confidence intervals)	168	24	92% (22/24) (73.0%-98.97%)	98.6% (142/144) (95.08%-99.83%)
Parainfluenza Group (95% Confidence intervals)	168	48	97.9 (47/48) (89.0% - 99.95%)	97.5% (117/120) (92.89% - 99.48%)

Table 14.2 Comparison of IMAGEN Parainfluenza virus reagents with reference immunofluorescence on nasopharyngeal aspirates at the U.S. Centre

			IMAGEN Parainfluenza virus reagents	
IMAGEN Parainfluenza virus reagent	No. of samples tested	No. of samples positive by reference immuno-fluorescence	Relative sensitivity	Relative specificity
Parainfluenza 1 (95% Confidence intervals)	127	21	100% (21/21) (84.0%-100%)	100% (127/127) (96.59%-100%)
Parainfluenza 2 (95% Confidence intervals)	127	1	100% (1/1) (2.5%-100%)	100% (127/127) (97.12%-100%)
Parainfluenza 3 (95% Confidence intervals)	127	4	75% (3/4) (19.4%-99.37%)	99.1% (122/123) (95.55%-99.98%)
Parainfluenza Group (95% Confidence intervals)	127	26	96.2% (25/26) (80.4% - 99.9%)	99.0% (100/101) (94.61% - 99.98%)

Note: Please be advised that 'relative' refers to the comparison of this assay's results with that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.

Table 14.3 Comparison of performance of IMAGEN Parainfluenza reagents with viral isolation on nasopharyngeal aspirates at 2 UK Centres

Viral isolation		**Pos**	**Neg**	**Pos**	**Neg**
IMAGEN reagent		**Pos**	**Neg**	**Neg**	**Pos**
Parainfluenza 1	n = 35	3	31	0	1**
Parainfluenza 2	n = 35	2	32	1*	0
Parainfluenza 3	n = 35	27	7	1*	0
Parainfluenza Group	n = 35	30	0	4***	1**

* Both specimens negative also by reference immunofluorescence
** This specimen was also positive by reference immunofluorescence
*** Two of these specimens negative also by reference immunofluorescence.

Table 14.4 Comparison of performance of IMAGEN Parainfluenza reagents with reference immunofluorescence on nasopharyngeal aspirates at 2 UK Centres

Reference immunofluorescence		**Pos**	**Neg**	**Pos**	**Neg**
IMAGEN reagent		**Pos**	**Neg**	**Neg**	**Pos**
Parainfluenza 1	n = 38	3	35	0	0
Parainfluenza 2	n = 38	3	35	0	0
Parainfluenza 3	n = 38	30	8	0	0
Parainfluenza Group	n = 38	34	2	2*	0

* One specimen was reported to contain few cells.

Table 14.5 Comparison of Parainfluenza positivity rates with IMAGEN Parainfluenza reagents and viral isolation on nasopharyngeal aspirates from all centres

IMAGEN reagent	Number detected by viral isolation	Number detected by IMAGEN	% Agreement
Parainfluenza 1	24	24	100%
Parainfluenza 2	6	5	83.3%
Parainfluenza 3	52	49	94.2%
Parainfluenza Group	82	77	93.9%

Table 14.6 Comparison of Parainfluenza positivity rates with IMAGEN Parainfluenza reagents and reference immunofluorescence on nasopharyngeal aspirates from all centres

IMAGEN reagent	Number detected by reference immuno-fluorescence	Number detected by IMAGEN	% Agreement
Parainfluenza 1	24	24	100%
Parainfluenza 2	4	4	100%
Parainfluenza 3	34	33	97.0%
Parainfluenza Group	62	59	95.2%

14.2.2 Culture confirmation

IMAGEN Parainfluenza virus Group
The IMAGEN Parainfluenza virus Group (Types 1, 2 and 3) test showed a correlation of 98.3% with the standard methods (Table 14.7). The relative sensitivity and relative specificity of the test were 98.3% and 98.5% respectively.

IMAGEN Parainfluenza virus Types 1, 2 and 3
The relative sensitivities for the individual Parainfluenza virus types 1, 2 and 3 reagents were 96.1%, 100% and 97.4% respectively. The relative specificities for the individual Parainfluenza virus types 1, 2 and 3 reagents were 99.3%, 99.7% and 98.4% respectively.

Table 14.7 Comparison of IMAGEN Parainfluenza virus reagents with standard methods for culture confirmation

IMAGEN Parainfluenza virus reagent	**No. of Samples tested**	**No. of Samples positive by Standard methods**	**IMAGEN Parainfluenza virus reagents[a]**	
			Relative Sensitivity	**Relative Specificity[b]**
Parainfluenza 1 (95% Confidence intervals)	322	51	96.1% (49/51) (86.5% - 99.5%)	99.3% (269/271) (97.4% - 99.9%)
Parainfluenza 2 (95% Confidence intervals)	315	24	100% (24/24) (85.8% - 100%)	99.7% (290/291) (98.1% - 100%)
Parainfluenza 3 (95% Confidence intervals)	345	155	97.4% (151/155) (93.5% - 99.3%)	98.4% (187/190) (95.5% - 99.7%)
Parainfluenza Group (95% Confidence intervals)	363	229	98.3% (225/229) (95.6% - 99.5%)	98.5% (132/134) (94.7% - 99.8%)

[a] Results from one of the four trial centres include a proportion of frozen samples.

[b] IMAGEN Parainfluenza virus reagents detected two type 1 strains and one type 3 strain which were not detected using the standard methods.

14.3. CROSS-REACTIVITY

The micro-organisms listed in Table 14.8 were tested in the IMAGEN Parainfluenza virus Group and the IMAGEN Parainfluenza virus Types 1, 2 and 3 tests and showed no cross reactivity. Cross reactivity studies were performed on slide preparations of stock cultures or recent microbial isolates.

Table 14.8 Organisms tested with all IMAGEN Parainfluenza virus reagents and found to be non reactive

Micro-organism	Source	No. of Samples tested
Acholeplasma laidlawii	*NCTC 10116 broth culture deposit*	*1*
Adenovirus type 1	*Cell Culture*	*9*
Adenovirus type 2	*Cell Culture*	*5*
Adenovirus type 3	*Cell Culture*	*8*
Adenovirus type 4	*Cell Culture*	*3*
Adenovirus type 5	*Cell Culture*	*4*
Adenovirus type 7	*Cell Culture*	*3*
Adenovirus type 10	*Cell Culture*	*1*
Adenovirus type 41	*Cell Culture*	*1*
Bordetella parapertussis	*Cell Culture*	*1*
Bordetella pertussis	*Culture Medium*	*1*
Branhamella catarrhalis	*Culture Medium*	*1*
Candida albicans	*Culture Medium*	*1*
Chlamydia pneumoniae	*Cell Culture & Culture Medium*	*1 of each*
Chlamydia psittaci	*Cell Culture*	*2*
Chlamydia trachomatis	*Cell Culture*	*2*
Cowpox virus	*Cell Culture*	*1*
Coxsackie virus type A7	*Cell Culture*	*1*
Coxsackie virus type A9	*Cell Culture*	*1*
Coxsackie virus type B3	*Cell Culture*	*3*
Coxsackie virus type B4	*Cell Culture*	*1*
Coxsackie virus type B5	*Cell Culture*	*2*
Cytomegalovirus	*Cell Culture*	*2*
Echovirus type 5	*Cell Culture*	*1*
Echovirus type 11	*Cell Culture*	*1*
Echovirus type 19	*Cell Culture*	*2*
Echovirus type 30	*Cell Culture*	*4*
Epstein Barr virus	*Continuous cell culture line*	*1*
Escherichia coli	*Culture Medium*	*1*
Foamy virus	*Cell Culture*	*1*
Haemophilus influenzae	*Culture Media & Sputum deposit*	*1 of each*
Herpes simplex virus type 1	*Cell Culture*	*3*
Herpes simplex virus type 2	*Cell Culture*	*2*
Influenza virus A	*Cell Culture*	*2*
Influenza virus B	*Cell Culture*	*2*
Klebsiella pneumoniae	*Culture Medium*	*1*
Legionella pneumophila	*Culture Medium*	*1*
Measles virus	*Cell Culture*	*3*
Mumps virus	*Cell Culture*	*8*
Mycobacterium avium	*Culture Medium*	*1*
Mycobacterium intracellulare	*Culture Medium*	*1*
Mycobacterium tuberculosis	*Culture Medium*	*1*
Mycoplasma arginini	*NCTC 10129 broth culture deposit*	*1*
Mycoplasma hominis	*NCTC 10111 broth culture deposit*	*1*
Mycoplasma hyorhinus	*NCTC 10130 broth culture deposit*	*1*
Mycoplasma orale	*NCTC 10112 broth culture deposit*	*1*
Mycoplasma pneumoniae	*NCTC 10119 broth culture deposit*	*1*
Mycoplasma salivarium	*NCTC 10113 broth culture deposit*	*1*
Neisseria cinerea	*Culture Medium*	*1*
Neisseria flavescens	*Culture Medium*	*1*
Neisseria gonorrhoeae	*Culture Medium*	*1*
Neisseria lactamica	*Culture Medium*	*1*
Neisseria meningitidis A,B,C&D	*Culture Medium*	*1 of each*
Neisseria mucosa	*Culture Medium*	*1*
Neisseria perflava	*Culture Medium*	*1*
Micro-organism	*Source*	*No. of Samples tested*
Neisseria pharyngis	*Culture Medium*	*1*
Parainfluenza virus 4a	*Cell Culture*	*2*
Pneumocystis carinii	*Clinical Specimen*	*1*
Polio virus type 1	*Cell Culture*	*1*
Polio virus type 2	*Cell Culture*	*2*
Polio virus type 3	*Cell Culture*	*3*
Respiratory syncytial virus	*Cell Culture*	*8*
Rhinovirus	*Cell Culture*	*6*
Sendai virus	*Cell Culture*	*1*
Streptococcus groups A, B, C, D, F and G	*Culture Medium*	*1 of each*
Streptococcus pneumoniae	*Sputum Deposit*	*1*
Varicella zoster virus	*Cell Culture*	*2*

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