Key Code TSMX7850A

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IMAGEN Influenza virus A and B

REF K610511-2 Σ 50 **EN**

A direct immunofluorescence test for the detection of Influenza virus A and B.

1. INTENDED USE

The IMAGEN™ Influenza virus A and B test is a qualitative direct immunofluorescence test for the detection and differentiation of Influenza A virus and Influenza B virus in clinical specimens or for the confirmation and differentiation of Influenza virus A and B in cell cultures.

2. SUMMARY

Influenza A and B viruses are members of the genus *Influenza virus* classified within the family *Orthomyxoviridae*[1]. Influenza A virus strains infect a variety of animals including humans, horses, pigs, sea mammals and birds whereas Influenza B virus strains appear to infect humans only[2].

In humans Influenza A and B virus infections can cause acute and occasionally severe respiratory disease in immunocompetent and immunocompromised individuals. Influenza A and B viral infections occur in annual epidemics often with rapid onset and spread of infection. These epidemics may occur as small localised outbreaks or as worldwide epidemics depending on the type of strain prevalent[3]. Periodically, as a result of the ongoing evolution of Influenza A viruses, epidemics of disease occur which can have a significant impact on world health[2,4].

Transmission of Influenza virus infection occurs through inhalation of virus-laden droplets from respiratory secretions of symptomatic or asymptomatic carriers. Environmental conditions such as crowding enhance transmission of infection. Virus replication occurs in the ciliated columnar epithelial cells of the upper and lower respiratory tract resulting in necrosis and sloughing of cells. The period of peak viral shedding occurs from 1 day before, to 3-4 days after the onset of illness.

During the course of an influenza epidemic the prevalent virus strain may be associated with 15-50% of respiratory infections occurring in adults and children. The spectrum of respiratory disease may vary from mild upper respiratory disease to a severe pneumonia[3]. Acute pneumonia due to Influenza A or B viruses can be fatal particularly when associated with concomitant or secondary microbial infections in elderly or immunocompromised patients.

Influenza viruses have been associated with nosocomial outbreaks of respiratory tract infections in paediatric and geriatric wards resulting in prolonged hospitalisation and increased morbidity and mortality.

The rapid laboratory diagnosis of Influenza A or B virus infections plays an important role in patient management, influencing the use of antiviral therapy and enabling effective management and control of outbreaks[3,5]. Diagnostic methods include direct detection of virus or viral proteins in clinical specimens (eg nasopharyngeal aspirates), isolation of viable virus in cell culture monolayers inoculated with respiratory secretions and detection of Influenza virus specific immunoglobulins. Isolation of Influenza viruses from respiratory specimens can be accomplished in cell lines such as primary rhesus monkey kidney cells or Madin-Darby canine kidney cells (MDCK) using techniques such as haemadsorption or haemagglutination to identify the presence of the virus strain. A range of techniques have been used to confirm the identification of the Influenza virus isolates including haemadsorption inhibition, haemagglutination inhibition, neutralisation tests, electron microscopy or indirect immunofluorescence. These techniques are laborious, time consuming and require a degree of technical expertise which may not be available in all laboratories.

Direct immunofluorescence tests utilising specific monoclonal antibodies offer a rapid sensitive and specific method for the direct detection of Influenza viruses A and B in clinical specimens such as nasopharyngeal aspirates or for the confirmation of Influenza virus isolated in cell culture monolayers[6]. IMAGEN Influenza virus A and B is a direct immunofluorescence test for the detection and identification of Influenza virus strains A and B in clinical specimens or cell cultures. The test utilises species-specific monoclonal antibodies to detect epitopes of Influenza virus glycoproteins and fusion proteins specific to either Influenza virus type A or Influenza virus type B.

3. PRINCIPLE OF THE TEST

The IMAGEN Influenza virus A and B test contains monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) specific to either Influenza A virus or Influenza B virus. These are used in a one-step direct immunofluorescence technique. Specimens are incubated with the FITC conjugated antibody reagents for 15 minutes and excess reagent is washed off with phosphate buffered saline (PBS). The stained areas are mounted and viewed microscopically using epifluorescence illumination. If either Influenza A virus or Influenza B virus is present, characteristic bright apple green fluorescence is seen with the corresponding reagent, within the cytoplasm and nucleus of the cells, which contrasts with the red background staining of uninfected cells.

The monoclonal antibodies used in this test originated in the Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia, U.S.A.

The immunogen used to raise the influenza A antibodies included stains A / Puerto Rico / 8 / 34 (H1N1) and A / Bangkok / 1 / 79 (H3N2). The immunogen used to raise the Influenza B antibodies included strains B / Lee / 40 and B / Singapore / -222 / 79.

4. DEFINITIONS

The following symbols have been used throughout the product information.

REF	Catalogue number
	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 INFLUENZA VIRUS A AND B REAGENTS

Instructions For Use

POSITIVE CONTROL SLIDE 2 x 2 well positive control slides containing acetone fixed African green monkey kidney cells (Vero) infected with either Influenza A virus or Influenza B virus on separate well areas.

One bottle of each of the following:

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

REAGENT A 1.4mL of IMAGEN Influenza A virus test reagent. The reagent contains purified murine monoclonal antibodies specific to Influenza A virus, conjugated to FITC. The monoclonal antibodies are targeted against the matrix protein and nucleoprotein of Influenza A.

REAGENT B 1.4mL of IMAGEN Influenza B virus test reagent. The reagent contains purified murine monoclonal antibodies specific to Influenza B virus conjugated to FITC. The monoclonal antibodies are targeted against the nucleoprotein and haemagglutinin protein of Influenza B.

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

5.5. IMAGEN INFLUENZA A AND B REAGENTS - REAGENT

Ready to use. Store unused Reagent A and B at 2-8°C. Reagents should be stored in the dark at 2 8 °C and left at room temperature (15-30°C) for 5 minutes before use.

6. ADDITIONAL REAGENTS

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 IMAGEN Influenza Virus A and B. Contact your local distributor for further information.

General

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

IMAGEN Influenza Positive Control Slide (Code No. S611230-2).

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 (nasopharyngeal specimens only)

For Culture Confirmation

Sterile swabs, viral transport medium (VTM) and container suitable for collection, transportation and culture of Influenza viruses

Cell lines recommended for culture and isolation of Influenza viruses

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 Sodium azide, at a concentration less than 0.1%, has been added to certain components as an antimicrobial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, reagents should be discarded into sewerage only if diluted and flushed with large volumes of water.

8.1.2 Influenza viruses A and B on the Positive Control Slide have been processed by fixation methods such that they contain no detectable live micro organisms, however, the slide should be handled and disposed of as though potentially infectious.

8.1.3 If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to central reference laboratories for testing. Viral culture should not be attempted in these cases unless a biological safety level 3 facility or above is available to receive and culture specimens.

8.1.4 Evans Blue dye is present in the Reagent. Although present below the concentration for the product to be classified as carinogenic, contact with the skin should be avoided.

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

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

8.1.7 Do not pipette materials by mouth.

8.1.8 Wear disposable gloves while handling clinical specimens and infected cells, always wash hands after working with infectious materials.

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

8.1.10 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 reagent.

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

8.2.3 Prepare fresh Phosphate Buffered Saline (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[7,8]

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. 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 released. Avoid vigorous pipetting or 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 areas on slide.

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 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

Inoculation of Cell Cultures

Specimens collected for the diagnosis of Influenza 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 cytopathic effect (CPE) and haemadsorption tests performed at regular intervals. Any haemadsorption positive cultures, or cell cultures showing CPE, can be harvested and tested for the presence of Influenza A or Influenza B viruses

Preparation of Slides

Scrape the cell sheet into the 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) and repeat the centrifugation. Remove the supernatant and resuspend the cell deposit in a small volume of fresh PBS to maintain a high cell density.

Place 25µL aliquots of the cell suspension on to individual wells on the slides. 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 at –70°C until needed. 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.

10.1. ADDITION OF REAGENT

Add 25µL of Reagent A to one area of the fixed cell preparation on a 6mm well and 25µL of Reagent B to another area of fixed cell preparation on another 6mm well on the slide (see Section 6) or to appropriate wells on a Positive Control Slide. Ensure that the reagents cover the entire well areas. Reagent A must be used on A positive well and Reagent B must be used on B positive well.

10.2. FIRST INCUBATION

Incubate the slides with reagents 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

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).

10.4. 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.

10.5. 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 specimens should be examined immediately after staining, but may be stored at 2 8°C, in the dark, for up to 24 hours).

11. INTERPRETATION OF TEST RESULTS

11.1. CONTROLS

11.1.1 Positive Control Slides

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 counterstained material. These cells are slightly larger than respiratory epithelial cells but show similar nuclear and/or cytoplasmic fluorescence when infected with Influenza virus. Positive Control Slides should be used to check that the staining procedure has been satisfactorily performed.

These slides are prepared from Influenza 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 clinical material.

11.1.2 Negative Control

If a negative control slide is required, uninfected intact cells of the type used for the culture and isolation of Influenza virus are recommended. The cells should be prepared and fixed as described in Section 9.2 and stained as described in Section 10.

11.2. CLINICAL SPECIMENS

11.2.1 Appearance of Influenza virus infected cells

Intracellular, nuclear and/or cytoplasmic granular apple-green fluorescence is seen in respiratory epithelial cells with Influenza virus.

Uninfected cells stain red with the evans blue counterstain.

11.2.2 Interpretation

A positive diagnosis is made when one or more cells in the fixed stained specimen show specific fluorescence, described in Section 11.2.1 with either Influenza A or Influenza B virus reagent.

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

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.

For specimens collected from other sites (eg sputa) at least 50 uninfected respiratory epithelial cells are observed before a negative result is reported.

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 the cells 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

11.3.1 Appearance of Influenza virus infected cells

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

Uninfected cells will be counterstained red with evans blue counterstain and should be recorded as negative for Influenza.

11.3.2 Interpretation of Results

A positive diagnosis is made when one or more cells in the fixed, stained specimen show the typical fluorescence pattern described in Section 11.3.1 with either IMAGEN Influenza A or Influenza B reagents.

At least 50 uninfected cells of the cell culture being tested must be present in the slide well before a negative result is reported. See Section 11.2.3 if insufficient cells are present.

11.3.3 Insufficient Cells

If insufficient cells are present on the slide, the remainder of the clinical specimen should be centrifuged at 200g for 10 minutes at room temperature (15 30°C). Re suspend the cells in a smaller volume of PBS before re distribution (25µL) on the well area. Alternatively, a repeat clinical specimen should be requested.

12. PERFORMANCE LIMITATIONS

12.1. Use only the Mounting Fluid provided.

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

12.3. 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.

12.4. 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.

12.5. Failure to detect Influenza viruses 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 Influenza virus infection.

12.6. The IMAGEN Influenza virus A and B test detects type specific Influenza A and B antigens. It cannot be used for identification of subtypes of Influenza A and B.

12.7. The presence of Influenza virus in nasopharyngeal secretions does not necessarily exclude the possibility of concomitant infection with other pathogens. Test results should be interpreted in conjunction with information available from epidemiological studies, clinical diagnosis of the patient and other diagnostic procedures.

12.8. Non-specific staining is sometimes observed as an artifact in immuno-chemical test due to binding between antibody Fc regions and protein A antigen found in the cell wall of some strains of *Staphylococcus aureus*[9]. The IMAGEN Influenza virus A and B test reagent has been modified so that it does not bind to the protein A of Cowan 1 strain of *Staphylococcus aureus*.

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. Individuals who have received nasally administered influenza A vaccine may have positive test results for up to three days after vaccination.

13. EXPECTED VALUES

In temperate zones Influenza outbreaks caused by either type A or type B take place mainly in late Autumn to early Spring, but in tropical areas the season of prevalence is less well defined.

In general, the infection rates for Influenza A virus in non-immunised children and adults are similar, with the clinical manifestations of infection showing an inverse correlation with age[10,11,12]. During Influenza B virus epidemics the highest attack rates are usually reported amongst school age children[13,14]. During the course of a winter when the prevalent Influenza virus is one which has been in circulation for some years and therefore when a large proportion of the population are immune, Influenza viruses can be found to account for approximately 15% of all respiratory infections. When a new antigenic strain of Influenza virus has been introduced into the community, and a large proportion of those exposed have no immunity, that strain of Influenza virus may cause up to 50% of all respiratory infections. In a recognised defined outbreak the detection rate can approach 100% if both serology and antigen detection methods are used for diagnostic purposes[15].

14. SPECIFIC PERFORMANCE CHARACTERISTICS

14.1. REACTIVITY OF THE MONOCLONAL ANTIBODIES

The monoclonal antibodies utilised in this test have been shown to be type specific by immunoassay. The Influenza A virus antibodies will detect H_1N_1, H_2N_2, H_3N_2 Influenza A virus strains, and the Influenza B virus antibodies will detect various Influenza B viruses collected between 1940 and 1984[6,16,17].

14.2. CLINICAL STUDIES

The IMAGEN Influenza virus A and B test was evaluated for direct use at 2 clinical trial centres on nasopharyngeal secretions and sputa collected from children and adults hospitalised with symptoms of respiratory infection. The test was also evaluated at 5 trial centres on cell culture of stock strains of virus to confirm the presence of Influenza viruses. These studies were carried out in the USA, Europe and the Far East.

The trial centres performed direct tests on 213 clinical specimens and on 227 specimens for confirmation of cell culture. Strains detected by the monoclonal antibodies in the IMAGEN Influenza virus A and B test included 22 different strains of Influenza virus A and 20 different strains of Influenza virus B. The standard (reference) methods used were an indirect immunofluorescence test performed directly on specimens and virus culture in baboon kidney cells, MDCK cells or embryonated hens' eggs. Positive virus cultures were confirmed by indirect immunofluorescence using either monoclonal or polyclonal antibodies, or haemagglutination inhibition (HAI).

14.3. CLINICAL PERFORMANCE

14.3.1 Direct specimens

Clinical specimens were collected mainly during the winters of 1984-1987 and the trial centres compared the IMAGEN Influenza virus A and B test with standard methods. Both fresh clinical specimens and previously frozen specimens were used for these evaluations.

A result by the reference method was considered positive if either the cell culture or indirect immunofluorescence on direct specimen was positive. This allowed for the presence of non-viable virus to be detected by fluorescence or for cell-free virus to be detected by cell culture.

Table 14.3.1 shows the results obtained with the IMAGEN Influenza virus A reagent. The overall incidence of Influenza in these populations was 24.9%. The IMAGEN Influenza A results correlated with the standard tests in 211 cases (99.1%). Test sensitivity was 96.2% (51/53) and specificity 100% (160/160), assuming that the standard tests were 100% sensitive and specific. The predictive values for positive and negative results were 100% (51/51) and 98.8% (160/162) respectively.

Sensitivity, specificity and predictive values were calculated as previously described[18].

Table 14.3.2 shows the results with the IMAGEN Influenza virus B reagent. The overall incidence of Influenza B in these populations was 7.0%. This reflects the low prevalence of Influenza B in Europe during the clinical trials. The IMAGEN Influenza B results correlated with the standard tests in 210 cases (98.6%). Test sensitivity was 86.7% (13/15) and specificity 99.5% (197/198). The predictive values for positive and negative results were 92.9% (13/14) and 98.9% (197/199) respectively.

Table 14.3.1 Comparison of test results of the IMAGEN Influenza virus A reagent used directly on clinical specimens with the standard tests

TEST RESULTS				
Standard Method	**Neg**	**Pos**	**Pos**	**Neg**
IMAGEN Influenza virus A	**Neg**	**Pos**	**Neg**	**Pos**
Centre 1	59	35	1	0
Centre 2	101	16	1	0
TOTAL No. of Specimens (213)	160	51	2	0

Table 14.3.2 Comparison of test results of the IMAGEN Influenza virus B reagent used directly on clinical specimens with the standard tests

TEST RESULTS				
Standard Method	**Neg**	**Pos**	**Pos**	**Neg**
IMAGEN Influenza virus B	**Neg**	**Pos**	**Neg**	**Pos**
Centre 1	81	12	1	1
Centre 2	116	1	1	0
TOTAL No. of Specimens (213)	197	13	0	1

14.3.2 Culture confirmation

Five trial centres tested the IMAGEN Influenza virus A and B test on clinical isolates and stock strains isolated in cell culture. Virus isolation was performed using either primary or secondary baboon monkey kidney cells, or in Madin-Darby canine kidney cells (MDCK). Cell cultures were washed in PBS prior to being spotted on to slides (see Section 9.2). The slides were fixed in acetone and then tested by the IMAGEN Influenza virus A and B reagents. Both fresh clinical isolates and previously frozen specimens were used for this evaluation.

A total of 227 cultures were evaluated which included 54 cultures positive for Influenza virus A and 30 cultures positive for Influenza virus B. Cell culture isolates were confirmed by either immunofluorescence or haemagglutination inhibition (HAI).

The results (Tables 14.3.3 and 14.3.4) indicate that the Influenza virus A reagent detected all Influenza A viruses isolated (sensitivity 100%) and the Influenza virus B reagent detected all Influenza B viruses isolated (sensitivity 100%).

The specificity of both reagents was 100%.

Table 14.3.3 Comparison of test results of the IMAGEN Influenza virus A reagent for culture confirmation with the standard tests

TEST RESULTS				
Standard Method	**Neg**	**Pos**	**Pos**	**Neg**
IMAGEN Influenza virus A	**Neg**	**Pos**	**Neg**	**Pos**
Centre 1	59	13	0	0
Centre 2	27	1	0	0
Centre 3	43	13	0	0
Centre 4	23	22	0	0
Centre 5	21	5	0	0
TOTAL No. of Specimens (227)	173	54	0	0

Table 14.3.4 Comparison of test results of the IMAGEN Influenza virus B reagent for culture confirmation with the standard tests

TEST RESULTS				
Standard Method	**Neg**	**Pos**	**Pos**	**Neg**
IMAGEN Influenza virus B	**Neg**	**Pos**	**Neg**	**Pos**
Centre 1	69	3	0	0
Centre 2	25	3	0	0
Centre 3	54	2	0	0
Centre 4	27	18	0	0
Centre 5	22	4	0	0
TOTAL No. of Specimens (227)	197	30	0	0

14.4. CROSS REACTIVITY

The IMAGEN Influenza virus A and B test was performed against preparations of other viruses and organisms likely to be present in respiratory secretions or cell cultures. All organisms tested (Table 14.4) were negative with both IMAGEN Influenza virus A and B reagents.

Table 14.4 Organisms tested in the IMAGEN Influenza virus A and B Test and found to be non reactive

Acholeplasma laidlawii	*Mycoplasma pneumoniae*
Adenovirus types 1 5 & 7	*Mycoplasma salivarium*
Bordetella parapertussis	*Mycoplasma orale*
Bordetella pertussis	*Mycoplasma hominis*
Branhamella catarrhalis	*Mycoplasma arginini*
Candida albicans	*Mycoplasma hyorhinus*
Chlamydia psittaci	*Neisseria meningitidis A*
Chlamydia trachomatis	*Neisseria meningitidis B*
Coxsackie virus types A9 & B4	*Neisseria lactamica*
Cytomegalovirus	*Neisseria perflava*
Echovirus types 3, 6, 9, 11 & 22	*Neisseria cinerarea*
Epstein-Barr virus	*Parainfluenza virus types 1, 2 & 3*
Foamy virus	*Pneumocystis carinii*
Herpes simplex virus types 1 & 2	*Polio virus types 1 and 2*
Legionella pneumophila	*Respiratory syncytial virus*
Measles virus	*Rhinovirus*
Mumps virus	*Simian virus types 5 and 40*
Mycobacterium tuberculosis	*Staphylococcus aureus*
Mycobacterium avium	*Streptococcus gps A,B,C,D F G*
Mycobacterium intracellulare	*Varicella zoster virus*

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IFU X7850A Revised November 2013

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