Influenza A and B viruses are members of the genus Influenza virus classified within the family Orthomyxoviridae. Influenza A viruses infect a variety of animals, including humans, horses, pigs, sea mammals and birds whereas Influenza B viruses appear to infect humans only. Influenza A and B virus infections can cause acute and occasionally severe respiratory disease in immunocompetent and immunocompromised individuals. Influenza A viruses cause epidemics annually in humans often with rapid onset and spread of infection. These epidemics may occur as small localised outbreaks or as large pandemics depending on the flu strain prevalent. Periodically, as a result of the ongoing evolution of Influenza A viruses, epidemics of disease which can have a significant impact on world health. Influenza virus infection occurs through inhalation of virus-laden aerosols from respiratory secretions of symptomatic or asymptomatic carriers. Environmental conditions that promote the formation of aerosols, such as fogs, mist and smoke, influence the transmission of influenza virus replication occurs in the ciliated columnar epithelial cells of the respiratory tract in respiratory secretions resulting in nasopharyngitis and coughing of the cells. The period of peak viral shedding occurs from 1 to 5 days after the onset of illness. The course of an influenza epidemic the prevalent virus strain may be associated with 15–50% of respiratory infections occurring in adults whereas in children it has been estimated to cause between 30% and 50% of mild upper respiratory infection, a similar rate reported in a severe pneumonia. Acute respiratory disease 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 mortality and morbidity. The clinical 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. Diagnostic methods include direct detection of virus or viral nucleic acid in clinical specimens (e.g. nasopharyngeal aspirates), isolation of viable virus in cell cultures inoculated with respiratory secretions and identification of influenza virus infections by exclusion of other respiratory infections. Detection of influenza viruses from respiratory specimens can be achieved by using rapid diagnostic tests (RLMCA, plaque reduction neutralisation test, haemadsorption or haemagglutination inhibition, nucleation tests, electrophoresis or indirect immunoperoxidase staining) and/or culture. Inoculation of cell cultures such as Madin-Darby canine kidney cells (MDCK) using respiratory specimens from patients may be used to identify the presence of the virus strain. A range of techniques have been used to confirm the identification of the influenza virus isolated in routine clinical specimens, including mouse inoculation, neutralisation tests, electron microscopy or indirect immunoperoxidase staining and/or culture. The isolation of virus from respiratory specimens can be used to confirm the diagnosis of Influenza A or B virus infection and may be used to identify the type of influenza virus, determine the type used for the culture and isolation of Influenza virus and to confirm the purity of the infected cell preparation. The remaining viable virus in the cell culture may be used for antigen detection, such as by enzyme immunoassay, for virus titre determination, in cell culture or in reverse transcription PCR, and for virus strain identification and characterisation. The determination of the type and subtype of the influenza virus can be used to identify the influenza virus test kit that is to be used for testing.

The collection and preparation of specimens is of fundamental importance in the detection 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. An additional specimen should be collected as soon as possible and prepared in such a way as to preserve either the viral antigen or RNA in a manner to allow detection of the virus by appropriate techniques. Two or three respiratory samples should be collected from each individual. At least one specimen should be collected 1 day before, to 3-4 days after the onset of illness. Virus replication occurs in the ciliated columnar epithelial cells of the nasal mucosa. Respiratory secretions are the predominant source of influenza virus shedding and can be demonstrated microscopically using epifluorescence illumination. If either nasal or nasopharyngeal secretions are used, the virus antigen will persist in the cell lines used for the culture and isolation of Influenza virus and may be used for antigen detection. The remaining viable virus in the cell culture may be used for antigen detection, such as by enzyme immunoassay, for virus titre determination, in cell culture or in reverse transcription PCR, and for virus strain identification and characterisation. The determination of the type and subtype of the influenza virus can be used to identify the influenza virus test kit that is to be used for testing.

For Culture Confirmation

- For Culture Confirmation

**5.3. POSITIVE CONTROL SLIDES - NA**

Collect specimens from the nasopharyngeal region into a mucus extractor through a site feeding tube. The mucus extrator and tubules should be inoculated with uninfected respiratory epithelial cells for the laboratory as soon as possible for processing.

**5.4. MOUNTING FLUID - NA**

Ready to use. Store at 2–8°C. The Mounting fluid should be stored in the dark at 2°C and at room temperature (15–30°C) for 5 minutes before use.

**5.5. INFLUENZA A AND B REAGENTS - NA**

Phosphate buffered saline (PBS) pH 7.5 for washing stained slides. The stained areas are mounted and viewed through a 10X objective lens.

**5.6. ADDITIONAL REAGENTS**

**6.1. REAGENTS**

Fresh acetone (for fixation).

**6.2. ADDITIONAL REAGENTS**

**6.3. POSITIVE CONTROL SLIDES - NA**

**6.4. MOUNTING FLUID - NA**

Ready to use. Store at 2–8°C. The Mounting fluid should be stored in the dark at 2°C and at room temperature (15–30°C) for 5 minutes before use.

**7. TEST PROCEDURE**

**7.1. SPECIMENS**

For clinical specimens or cell cultures. The test utilises specific monoclonal antibodies specific to Influenza A virus conjugated to FITC. The monoclonal antibodies are targeted against the matrix protein and nucleoprotein of influenza A virus. The reagent contains purified murine monoclonal antibodies specific to influenza B virus conjugated to FITC. The monoclonal antibodies are targeted against the haemagglutinin and haemagglutinin of influenza B virus.

One bottle of each of the following.

- **1.4mL of IMAGEN Influenza A virus test reagent**
- **1.4mL of IMAGEN Influenza B virus test reagent**
- **1.4mL of IMAGEN influenza A virus test reagent**

The reagents are provided for optimal working concentrations. Test performance will be adversely affected if the reagents are stored under conditions other than those described in Section 5.3.

**7.2. PREPARATION OF SPECIMENS**

**7.3. PREPARATION OF SPECIMENS**

**7.4. ADDITION OF MOUNTING FLUID**

1. Add 2 drops of Mounting fluid to the centre well of each slide. Use fresh Mounting fluid and specimen ensuring that no air bubbles are trapped.
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.

12.6. The IMAGEN Influenza virus A and B test results may not necessarily exclude the possibility of co-infecting pathogen. Test results should be interpreted in conjunction with information available from laboratory testing and other diagnostic procedures.

12.8. Non-specific staining is sometimes observed as an artifact in immunofluorescent test due to binding of antibody to antigenic regions of an unrelated agent. A negative result does not exclude the possibility of co-infecting pathogen. Test results should be interpreted in conjunction with information available from laboratory testing and other diagnostic procedures.

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

13. EXPECTED VALUES

In temperature extremes Influenza antibodies caused by either type A or type B take place mainly in late Autumn to Spring, but in tropical areas the season of prevalence is 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.

During Influenza B virus epidemics the highest attack rates average among school-age children. During the course of a winter when the prevalence of Influenza in one is in 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 35% of all respiratory infections. When a new antigenic strain of Influenza virus has been introduced into the community, and a large proportion of these individuals have no immunity, that strain of Influenza virus may cause up to 50% of all respiratory infections. In a recognized defined outbreak the detection rate can approach 100% if both serology and antigen detection methods are used for diagnosis purposes.

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 immunosassay. The Influenza A virus antibodies will detect H1N1, H1N2, H3N2, H4N1, Influenza A viruses, and the Influenza B antibodies will detect various Influenza B viruses collected between 1946 and 1987.

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 saliva collected from children and adults hospitalised with symptoms of respiratory infection. The test was also evaluated at 5 clinical centres on cell culture of virus strains to confirm the presence of Influenza viruses. These studies were carried out in the USA, Europe and the Far East.

The trial centres selected for direct testing on 213 clinical specimens and on 227 specimens for confirmation of cell culture. Strains detected were type A and B. The IMAGEN Influenza virus A and B test included 22 different strains of Influenza A virus and 20 different strains of Influenza B virus. The standard reference methods used were an indirect immunofluorescence test performed directly on virus and cell culture in balloon kidney cells (MDCK) or in 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 tests

Clinical specimens were collected mainly during the winters of 1984/85 and 1985/86 at 2 clinical centres compared the IMAGEN Influenza 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 cell culture or indirect immunofluorescence on direct cell culture was positive. This allowed for the presence of non-viable Influenza virus by immunofluorescence 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 and B test. The overall incidence of Influenza in these populations was 24.9%. The IMAGEN Influenza A results correlated with the standard tests in 211 cases (99.5%). Test sensitivity was 92.6% (51/53) and specificity 100% (160/160).

14.3.2. Culture confirmation

Five trial centres tested the IMAGEN Influenza A and B test on clinical isolates and stock strains isolated in cell culture. Virus isolation was performed using either primary or secondary baby hamster kidney cells, or 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 strains were used for this evaluation.

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

The results (Tables 14.3.1 and 14.3.2) 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 (specificity 100%).

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 culture. 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

15. REFERENCES


