Parainfluenza infection case definition summary

Public Health Laboratory Network case definitions

PHLN0010
Version: 1
Consensus Date: 7 August 2000
Authorisation: PHLN

Introduction

The parainfluenza virus is a single-stranded RNA virus, a member of the family Paramyxoviridae, genus Paramyxovirus. There are three species identified in diagnostic laboratories- named Parainfluenza 1, 2, 3. A fourth type (consisting of two subtypes) is not identified routinely.

Parainfluenza viruses, especially type 1, are important causes of childhood laryngotracheobronchitis (croup). Children typically present with a cough, with or without inspiratory stridor, and indrawing of the chest wall, usually without fever. Spread to the lower respiratory tract with bronchiolitis and bronchopneumonia can occur, and in some cases is fatal. Infection in adults is uncommon, and presents with influenza-like illness or laryngotracheobronchitis. Infection occurs typically during winter, and the virus may co-circulate with influenza, RSV and other respiratory viral agents. Unlike influenza (which has the WHO Collaborating Centre for Reference and Research), there is no centralised service for parainfluenza diagnosis and study within Australia.

Testing is most often by detection of antigen using direct immunofluorescence on nasopharyngeal aspirates (NPAs) in children, and on nasal and throat swabs in adults. Culture, and subsequent detection of cytopathic effect (CPE) in cell culture with monoclonal antibody based immunofluorescent assays is also performed. Nucleic acid testing (NAT) techniques are currently not used routinely, although tests are research tools in a small number of laboratories. Serology is useful for retrospective diagnosis and surveillance.

2. Laboratory diagnosis

2.1 Clinical Specimens

As for other respiratory viruses, nasopharyngeal aspirates, swabs and washes, endotracheal aspirates, bronchoalveolar lavage fluid and lung biopsy tissue are acceptable for culture, direct antigen detection and NAT (where available). Swabs should be cotton, rayon or dacron-tipped, plastic-coated or aluminium shafted. They should be placed into viral

transport media and transported at 4^{o}C or frozen at -70^{o}C. Specimens for culture or IF are best taken as early as possible in the illness.

2.2 Rapid Diagnosis – direct immunofluorescence (IF)

2.2.1 Suitable specimens - in children NPA

- adults NPA usually not available, usually nasal (best) and/or throat swab
- specimens collected as early as possible during illness, preferably within the first 4 days
- Paramyxoviruses (including parainfluenza virus) are relatively labile, especially during freezing and thawing, so specimen should be processed immediately or stored (short term) at +4^{0}C or (longer term) at –70^{0}C. The maximum acceptable duration of storage at room temperature is 5 days for IF and 2 days for culture. Prolonged storage will result in lower sensitivities, predominantly because of bacterial overgrowth and cell lysis.

2.2.2 Sensitivity

- direct detection is less sensitive than cell culture but gives a result the same day
- Sensitivity of IF is 95% compared to cell culture

2.2.3 Specificity - rapid diagnosis is highly specific, 99-100% in most laboratories [Grandien, 1985]

- Specificity 100% [Grandien, 1985]
- cell culture appearance typical but not specific. Definitive detection requires haemadsorption or, more commonly, staining with monoclonal antibody (Mab) [Monto, 1992].

2.2.4 Predictive Values - variable, and dependent upon the season. That is, the predictive values are low out of season and very high at the peak of the season. Absolute values for predictive values are not published.

2.2.5 Suitable Test Acceptance Criteria

- control slides containing parainfluenza-infected cells show positive immunofluorescence in each run. These control slides are stored at -70^{0}C
- Positive control should exhibit apple green fluorescence in the cytoplasm of infected cells. The fluorescence should be brightly stained inclusion-like bodies or fine granular structures. There should be minimal background staining.
- sputum specimens are not acceptable for culture. Results from specimens that have been processed later than 1 day after collection should carry an indication that the specimen was processed at this time, as delayed processing reduces test sensitivity.

2.2.6 Suitable internal controls - positive control slides containing infected cells with parainfluenza positive. These control slides are stored at -70^{0}C

2.2.7 Suitable Test Validation Criteria -records of time specimen stored in the laboratory before testing.

2.2.8 Suitable External QC Program - participation in the National Association of Testing Authorities (NATA)-RCPA quality control programs for the laboratory. No specific program exists for parainfluenza immunofluorescence.

2.2.9 Special Considerations - there are four types of parainfluenza virus, although only three are diagnosed in the routine laboratory - 1, 2 and 3. Type 1 and 2 infections most often occur during autumn, type 1 may occur as second yearly epidemics. Parainfluenza virus type 3 occurs endemically, with occasional perioids of increased infection for 2-3 months.

- Recurrent infection with parainfluenza virus can occur.
- Most laboratories use direct immunofluorescence tests as the major diagnostic test in a clinical setting.
- A direct antigen test based on the use of ELISA has been available for some time, and has been commercialised recently [Grandien, 1985].

2.3 Isolation of Parainfluenza Virus

2.3.1 Suitable specimens

- Children NPA
- Adults as above
- Cell lines generally used are Madin - Darby canine kidney (MDCK) lines. Primary monkey or human kidney cell lines can also be used, and new cell lines are compared with these cell lines as reference.

2.3.2 Sensitivity – culture is more sensitive than direct IF, although culture os slower and more labour-intensive to perform. Maximum cpe occurs 4-7 days after inoculation in LLCK, HEK, Vero and human diploid fibroblasts. The parainfluenza virus can be detected using haemadsorption (positive 2-5 days prior to CPE), or more commonly Mab staining.

Trypsinisation should be performed in order to clear the F protein, otherwise viral culture in MDCK or other cell lines is not possible. This is done usually once to twice per week.

2.3.3 Specificity -is determined by monoclonal antibody staining of the cell monolayer. The cpe is typical, but not diagnostic. CPE is more commonly observed with type 2 virus, than type 3 or type 1 virus.

approaches 100%

2.3.4 Predictive Values – undefined.

2.3.5 Suitable Test Acceptance Criteria

- typical cpe and/or immunofluorescence positive on cell culture material.

2.3.6 Suitable Internal Controls - cell cultures maintained at the same time without inoculation with NPA material.

2.3.7 Suitable Test Validation Criteria - auditors should have available:

- evidence of records of inocula;
- records of time specimen stored in the laboratory before inoculation;
- evidence of regular mycoplasma testing of cell lines; and
- evidence of regular contamination testing of cell lines.

2.3.8 Suitable External QC Program - as above

2.3.9 Special Considerations - trypsinisation of the cell line must be performed in order to allow viral replication by cleavage of the F protein, otherwise viral propagation cannot occur.

parainfluenza outbreaks can co-circulate with other respiratory outbreaks

2.4 Serology – CFT

2.4.1 Suitable specimens - Acute phase serum (within 10 days of the acute illness, preferably as close to the acute illness as possible)

- Convalescent serum more than 14 days after infection

2.4.2 Sensitivity -Single titre 95%, rising titre 100%

2.4.3 Specificity -95% using CFT

2.4.4 Predictive Values -not available

2.4.5 Suitable Test Acceptance Criteria - EIA standard performance of positive and negative controls. Adequate performance of the laboratory on NATA accreditation

2.4.6 Suitable Internal Controls -negative, mid-range positive, high positive

2.4.7 Suitable Test Validation Criteria - Control sera

2.4.8 Suitable External QC Program - none; suggest RCPA QAP add to program

2.4.9 Special Considerations -special problem with paramyxoviruses is the presence of parainfluenza antibody in the guinea pig complement [Monto, 1992]. This is usually not a practical problem and should be detected by the controls used in each test. That is the complement control should react non-specifically with the antigen, indicating the presence of contaminating antibody in the guinea pig complement.

2.5 Serology – EIA

2.5.1 Suitable specimens - Acute phase serum (within 10 days of the acute illness, preferably as close to the acute illness as possible)

- Convalescent serum more than 14 days after infection

2.5.2 Sensitivity - Single titre 95%, rising titre 100%

2.5.3 Specificity -97% using EIA

2.5.4 Predictive Values - reflects how definitive a positive or negative result is in the relevant population

- not available

2.5.5 Suitable Test Acceptance Criteria - EIA standard performance of positive and negative controls. These consist of a high positive, mid range positive and negative control sera for each run. Adequate performance of the laboratory on NATA accreditation

2.5.6 Suitable Internal Controls -negative, mid-range positive, high positive sera for each run. The control sera usually are available in the kit.

2.5.7 Suitable Test Validation Criteria - Control sera run with each test in parallel. Adequate performance of controls (within recommended levels of variation - usually plus or minus 10%).

2.5.8 Suitable External QC Program - none; suggest RCPA QAP add to programme.

2.5.9 Special Considerations

2.6 Serology – IFA

2.6.1 Suitable specimens - Acute phase serum (within 10 days of the acute illness, preferably as close to the acute illness as possible)

- Convalescent serum more than 14 days after infection

2.6.2 Sensitivity -Single titre 95%, rising titre 100%

2.6.3 Specificity -95%

2.6.4 Predictive Values - reflects how definitive a positive or negative result is in the relevant population

- not available

2.6.5 Suitable Test Acceptance Criteria - standard performance of positive and negative controls. Adequate performance of the laboratory on NATA accreditation

2.6.6 Suitable Internal Controls - negative, minimal reactive positive, and high positive control sera. Usually the minimal reactive is obtained by diluting the high positive serum to

be minimally reactive on the test.

2.6.7 Suitable Test Validation Criteria - control sera run with each test in parallel. Adequate performance of controls (within recommended levels of variation - minimal reactive serum shows reproducibility from run to run).

2.6.8 Suitable External QC Program - none; suggest RCPA QAP add to program

2.6.9 Special Considerations

3. PHLN laboratory definition

In summary, most testing for parainfluenza virus is by direct immunofluorescence on nasopharyngeal aspirates in children, and on nasal and throat swabs in adults. Rapid diagnosis using direct immunofluorescence is sensitive, specific and widely available in Australia. Culture and nucleic acid testing are not routinely used in most laboratories. Serology using complement fixation, EIA or immunofluorescence are all available and generally sensitive and specific.

References

1. Gardner P.S., McQuillin J (1980). Rapid Virus Diagnosis Application of Immunofluorescence (2nd edition). Butterworth & Company Limited, London.

2. Grandien M., Pettersson C.A., Gardner P.S., Linde A., Stanton A. (1985) Rapid viral diagnosis of acute respiratory infections: Comparison of enzyme linked immunosorbent assay and the immunofluorescence technique for detection of viral antigens in nasopharyngeal secretions. Journal of Clinical Microbiology 22(5): 757-760.

3. Monto A.S., Shaw M.W. (1992). Parainfluenza Viruses 573-590 in Lennette E.H. (Ed) Laboratory Diagnosis of Viral Infections (2nd edition) (1992), Marcel Dekker Incorporated New York.