

# ***Respiratory syncytial virus (RSV)***

## **Laboratory case definition**

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *respiratory syncytial virus*.

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## **1. Introduction**

Infection with *respiratory syncytial virus (RSV)* is typically an illness of children, most often occurring during winter. Spread to adults can occur, although illness in adults is usually less severe than that in children and neonates. RSV infection has a strong seasonality, present during winter and usually the peaks of RSV infection do not coincide with influenza or parainfluenza outbreaks. The infection is very common, often occurring during the first years of life and being somewhat ameliorated by passively transferred maternal antibody. Children infected as neonates have the most severe infections, and may present with non-specific symptoms such as lethargy and poor feeding, upper respiratory tract infection or lower respiratory tract infection with bronchiolitis. Children have RSV as a relatively common cause of pneumonia and bronchitis, and a very common cause of bronchiolitis. Older children typically present with a fever, and upper respiratory tract infection that may develop into bronchiolitis. The presence of worsening cough, tachypnoea, and dyspnoea with subsequent cyanosis is a typical course for progressive RSV infection. The illness is more severe in boys, in overcrowded areas and in industrialised countries. Infection is highly contagious between individuals. The most severe disease due to RSV occurs in premature infants, those with congenital heart disease, pulmonary hypertension, bronchopulmonary dysplasia and the immunosuppressed.

Adults with RSV infection have an upper respiratory tract infection, and less commonly develop lower respiratory tract infections. They may often have asymptomatic infection, typically acquired from children.

Testing for RSV infection involves clinical suspicion followed by chest x-ray, and in more severe cases bronchoscopy. Diagnosis in most laboratories is done using direct antigen detection on nasopharyngeal aspirates (NPA) with fluorescence labelled monoclonal antibodies. The virus can be isolated in tissue culture, and antigens can be detected also using enzymeimmunoassay. Serology is of little use in infants, as there is usually only a small rise in antibody, and seroconversion may take several weeks (up to 6 weeks).

Nosocomial spread is important, and can be prevented to some extent by isolation of the infected children, with limitation of the number of visitors, and avoiding fomite spread by careful handwashing, avoiding self-inoculation (touching the nose or mouth etc.).

RSV is of family *Paramyxoviridae*, genus *Pneumovirus*, with one species (RSV). It is a single-stranded RNA virus, with an envelope. The genome is encoded as three glycoproteins (G, F1 and F2).

## 2. Laboratory diagnosis

### 2.1 Clinical specimens

Nasopharyngeal aspirates (NPAs), swabs and washes are acceptable for culture, direct antigen detection and Nucleic acid testing (NAT) where this is available. Other acceptable specimens are endotracheal aspirates, bronchoalveolar lavage fluid and lung biopsy tissue. Swabs should be cotton, rayon or dacron-tipped, plastic-coated or aluminium shafted swabs. They should be placed into viral transport media and transported at 4°C or frozen at -70°C.

There is no centralised repository of RSV specimens in Australia.

### 2.2 Rapid diagnosis - direct immunofluorescence

A definitive laboratory diagnosis can be made by detection of RSV antigens or genetic material on the same clinical specimens used for culture, often using the same specimens used for influenza and parainfluenza diagnosis.

#### 2.2.1 Suitable specimens - children nasopharyngeal aspirates (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  
specimens are not acceptable for culture if they consist only of sputum. 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, which reduces test sensitivity.

2.2.2 Sensitivity - immunofluorescence has a sensitivity of 95-98% compared to culture [Grandien 1985]. False negative results usually result from inadequate specimens. False positive results may be due to improper handling and storage so that infectivity is lost whilst immunofluorescence or EIA assays remain positive.

2.2.3 Specificity of the test is 90% [Hendry, 1992] to 100% [Grandien, 1985].

2.2.4 Predictive Values - unknown.

#### 2.2.5 Suitable Test Acceptance Criteria

control slides containing infected cells with RSV positive (stored at -70°C).

2.2.6 Suitable Internal Controls - positive control slides containing infected cells with RSV (control slides are stored at -70°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 minimum background staining.

#### 2.2.7 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 line.

#### 2.2.8 Suitable External QC Program - participation in the National Association of Testing Authorities (NATA) quality control programs.

An Australia wide cell culture quality control program would be a reasonable addition to this, but is difficult to set up, would involve a small number of laboratories only, and may be unnecessary given the NATA laboratory.

#### 2.2.9 Special Considerations - RSV immunofluorescence may be positive in the presence of a negative culture due to:

improper handling of storage of specimens resulting in loss of infectivity but persistence of antigens  
presence of antibodies in nasal secretions that neutralize RSV infectivity but antigens remain present  
EIA and immunofluorescence tests have similar sensitivity and specificity, some authors find the sensitivity of EIA to be 85-95% (same as immunofluorescence) but the specificity lower at 70-95% (lower than immunofluorescence) [Grandien 1985], [Welliver 1998].

## 2.3 Isolation of RSV

Isolation of influenza *Respiratory Syncytial Virus* (RSV) provides a definitive diagnosis of infection. RSV can be readily isolated in epithelioid cells (eg. MDCK) or primary monkey kidney cells. Observation of typical cytopathic effect and/or using an RSV-specific monoclonal antibody (Mab) suggests infection.

Nasopharyngeal aspirates (NPAs) are obtained by inserting a number 5-8 French Paediatric feeding tube attached to a suction strap through both nostrils in a nasopharynx, applying gentle suction, and spending the aspirated contents in 1-2 ml volume of viral transport medium. The material should be stored at +40C until inoculation. Transport medium is standard viral transport medium, typically containing buffered salt solutions with some protein. Antibiotics are sometimes added and the VTM can be frozen, but should not be frozen once inoculated with the specimen.

#### 2.3.1 Suitable specimens - children nasopharyngeal aspirates (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

#### 2.3.2 Sensitivity -culture of RSV produces a distinctive CPE, particularly in Hep2 or HeLa cell line cpe is typically small syncytia, that extend over two days and eventually will attach the cell sheet.

#### 2.3.3 Specificity -cell culture is highly specific, as the CPE is typical, and the agent can be further identified using specific monoclonal antibodies.

#### 2.3.4 Predictive Values - unknown.

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#### 2.3.5 Suitable Test Acceptance criteria for RSV infection

direct immunofluorescence positive on fresh specimen  
typical CPE and/or immunofluorescence positive on cell culture material

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

#### 2.3.7 Suitable Test Validation Criteria for RSV detection by isolation

records of inoculation  
records of time specimen stored in the laboratory before inoculation  
evidence of regular mycoplasma testing of cell lines  
evidence of regular contamination testing of cell line

2.3.8 Suitable External QC Program - participation in the National Association of Testing Authorities (NATA) quality control programs. An Australia wide cell culture quality control program would be a reasonable addition to this, but is difficult to set up, would involve a small number of laboratories only, and may be unnecessary given the NATA inspections.

2.3.9 RSV Special Considerations - RSV is relatively labile to temperature and PH, and storage above 40C results in very rapid loss of infectivity.

- Freezing and thawing of specimens or the agent results in rapid loss of infectivity of RSV
- Definitive diagnosis of RSV requires laboratory confirmation.

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% (homologous type), single sera. 100% rising titre

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

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

## 2.5 Serology – CFT

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 -95% using CFT

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 - standard performance of positive and negative controls.

adequate performance of the laboratory on NATA accreditation

2.5.6 Suitable Internal Controls - standard performance of positive and negative controls. High positive, mid range positive and negative sera as controls.

2.5.7 Suitable Test Validation Criteria - Commercial assays. Control sera often from in-house stored samples.

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

2.5.9 Special Considerations -30-40% positive in reinfection. Some cross-reaction with mumps

most common method used in diagnostic laboratories  
may be falsely negative in young children.

## 2.6 Serology – EIA

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%. Sensitivity much reduced (50%) in children <4 months of age [Hendry, 1992].

2.6.3 Specificity -approximately 95% using EIA [Hendry, 1992].

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 - 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

2.6.6 Suitable Internal Controls -negative, mid-range positive, high positive sera for each run.

2.6.7 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%).

2.6.8 Suitable External QC Program - none

2.6.9 Special Considerations -used infrequently at this time.

No commercial test is currently available

EIA positives much lower in neonates. Response found in 50% of children <4 months age, and 90-95% of children >4 months age [Hendry, 1992].

## 2.7 Serology – IFA

2.7.1 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

2.7.2 Sensitivity -90 to 100%. Sensitivity reduced in young children - diagnostic rise in titre in children less than 6 months age in 75% [Hendry, 1992].

2.7.3 Specificity -95%

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

not available

2.7.5 Suitable Test Acceptance Criteria - standard performance of positive and negative controls

adequate performance of the laboratory on NATA accreditation

2.7.6 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.

2.7.7 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).

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

2.7.9 Special considerations --No commercial test is currently available

### 3. PHLN laboratory definition

In summary, RSV detection is particularly important in children, as it is a very common infection in the early years of life with occasional severe consequences. Clinical suspicion should be high during the winter months (usually slightly before or after the peak of influenza). Diagnosis is usually by direct immunofluorescence on nasopharyngeal aspirates. The NPA is reasonably sensitive and specific, although incorrect results may occur if specimens are not handled and stored adequately. Culture and nucleic acid testing are generally not used except in laboratories fulfilling reference functions. Serology is available, predominantly using complement fixation testing, although other test types are available. Serology is often difficult as significant infection occurs in children less than one year of age, where maternal antibodies may confuse interpretation of serology results.

### 4. References

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