Rotavirus

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 *rotavirus*.

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1 PHLN Laboratory Definition

1.1 Condition

Diarrhoea caused by *rotavirus*

1.1.1 Tests

1) Definitive Criteria

Detection of *rotavirus* by antigen assay in an unvaccinated patient*
Or
Detection of *rotavirus* by NADT
Or
Detection of *rotavirus* by EM
Or
Isolation of *rotavirus*

2) Suggestive Criteria
Nil

Where the patient is known to have been vaccinated, or comes from a routinely vaccinated population (i.e. young children) false positve results are likely, therefore positive sample in these patients should be retested by a confirmatory assay or by PCR.

2 Introduction

Rotavirus is one of nine genera amongst the family *Reoviridae*. It is, non-enveloped, 75 nm in diameter with a triple-layered icosahedral protein capsid with 11 segments of double stranded (ds) ribonucleic acid which code for six structural and six nonstructural proteins. The inner core contains VP1, VP2 and VP3, encoded by RNA segments 1-3; a middle capsid made up of VP6 encoded by segment 6, and an outer capsid of a VP7 shell encoded by segment 9 (or 7 or 8, depending on the strain) and a VP4 spike protein encoded by RNA segment 4 [1]. *Rotaviruses* are stable to heat, light and extremes of pH. The organism has a distinct ultrastructural appearance that resembles a wheel (rota, Latin). The *rotavirus* genus is divided into serological groups (A to E) based on the reactivity of the middle capsid protein VP6. All serogroups infect animals, but only group A commonly infects humans. While individual cases or clusters due to non-A serogroups have occurred, they are uncommon and the public health significance is uncertain. As nearly all *rotavirus* strains infecting humans belong to group A, the standard antigen detection and NADT in routine clinical use detect only group A. Groups B through G have been associated with human disease uncommonly and are variably called *pararotavirus*, atypical *rotavirus*, *rotavirus*-like *rotavirus* and adult diarrhoea *rotavirus* [2].

Rotavirus is a major cause of severe gastroenteritis in young children (with a peak incidence among children between 6 months and 2 years of age although younger children may be infected in developing countries) [2]. Although discovered as a human pathogen only 32 years ago, the role of the virus in the burden of diarrhoeal disease in developed and developing countries was quickly accepted [3] and in 1985 it was estimated to be the cause of about 600,00 deaths annually in developing countries [1]. It is rarely a cause of death in developed countries. It has been estimated that at least 30% of hospital admissions for acute gastroenteritis in young children are due to *rotavirus* infection and that it has a cyclical winter peak of disease in temperate climates. [2]

Rotavirus infection may produce a spectrum of illness ranging from subclinical infection to severe and, on occasion fatal, dehydrating illness. Typically the clinical presentation is 3 days vomiting and 5 days of watery diarrhoea with moderate fever, following a 1-3 day incubation period.

Routine *rotavirus* vaccination of infants was introduced in Australia in 2007. Two vaccines are in use: a live attenuated vaccine containing a single serotype of serogroup A (Rotarix) and a live reassortant vaccine containing antigens of five serotypes within serogroup A (RotaTeq). [4] As serotype-specific immunity is important ongoing surveillance of serotypes is important in monitoring vaccine effectiveness. [1]

Diagnosis was originally performed using electron microscopy, which is still occasionally used in centres where it is available. Routine diagnosis is now most commonly performed by antigen detection on faeces using commercially available, simple, rapid immunochromatographic dipstick style kits, latex particle agglutination and enzyme immunoassays. Reverse transcription polymerase chain reaction (RT-PCR) of faeces is available in a number of reference and research centres for diagnosis and strain characterisation, and is particularly useful for identification of outbreaks or cases occurring in recently vaccinated patients. Viral culture and serology are not routinely available and are used for research and for strain characterisation, and they do not have a role in diagnosis of acute disease.

Stool specimens collected from the first to fourth days of illness are optimal for *rotavirus* detection but virus may be shed for up to 3 weeks or longer depending on the severity of illness. Viral shedding usually coincides with the duration of diarrhoea but may stop prior to or after the cessation of diarrhoea.

Typing of *rotavirus* is based on the genetic and antigenic diversity of the 2 outer capsid proteins, VP4 and VP7. Surveillance of *rotavirus* G and P genotypes circulating in communities has become important with the recent introduction of vaccines for *rotavirus*. [1]

3 Tests

3.1 Culture

Rotavirus has been cultured in MA104 and primary African green monkey kidney cell cultures in roller bottles after trypsin pre-treatment. [5] This is not a practical method for routine diagnostic use as it is technically demanding, time consuming and expensive [6]. The sensitivity of this technique is only 50% compared to antigen detection from stool specimens. This method will not be discussed further

3.2 Electron Microscopy

This was the original method used to detect *rotavirus* and it is a sensitive and specific diagnostic tool due to the high viral load in acute disease and characteristic morphology of the organism. EM has the advantage of being able to detect non-*rotavirus* causes of diarrhoea or infection by strains of *rotavirus* not detected in the antigen assays. The disadvantages of EM are the limited availability, and the cost and impracticality of screening large numbers of specimens. Titres of approximately 106/mL are required for detection by EM: shedding at these levels is typical of the first 48 hours of illness. Sensitivity may be increased 10-100 times by immune electron microscopy, which also increases specificity, but availability of reagents is relatively limited.

3.2.1 Suitable specimens

Fluid stool

3.2.2 Test Sensitivity

80% to 90% compared to Immune Electron Microscopy. Fixation methods affect the results so proven methods must be used. Negative staining with 1% uranyl acetate at pH 4.3 or 2% phosphotungstic acid at pH 4.5 is recommended [6]. EM is less sensitive than both antigen detection tests and PCR [7].

3.2.3 Test Specificity

Immune EM can be used to group or serotype the virions. Specificity approaches 100% compared to EM, EIA, Latex agglutination 6,8.

3.3 Antigen-detection methods

All commercially available kits are based on detection of the VP6 antigen of group A *rotaviruses* so this is the only *rotavirus* serogroup that is detected. Strict adherence to the manufacturers' specifications are critical for optimal test performance [6] and, even then, it is recommended that

laboratories carry out their own evaluation of the test prior to introduction as performance characteristics may be different in their patient population.

Recent experience in Australia has found a high false positive rate for antigen detection tests compared with PCR, especially in vaccinated children. This may reflect the lower pre-test probability in vaccinated patients, so positve antigen tests in these patients should be interpreted cautiously and referred for PCR testing if a false positive result is suspected. Therefore confirmation of positive results should be done where there is a low pretest probability that the person has *rotavirus* infection, including vaccinated patients or cases occurring outside the *rotavirus* "season". This may be done by the use of an assay with confirmatory reagents [5,9] or referral of the sample to a reference laboratory for testing by PCR.

When the viral load is likely to be low e.g. late in the illness or in a rectal swab, retesting of negative samples by a more sensitive method such as PCR may be done if required.

3.3.1 Enzyme immunoassay (EIA)

3.3.1.1 Suitable specimens

Fluid stool

3.3.1.2 Test sensitivity and specificity

The performance of each kit depends on the "gold" standard method used for comparison and is often based on carefully controlled clinical trials. In these situations sensitivities and specificities of >95% are possible. In actual practice these characteristics may be less than 90% and different assays may yield different results when used to test the same specimen.

3.3.1.3 Suitable test acceptance/validation criteria

Follow the manufacturers guidelines if using a commercial kit and use the "kit" confirmatory test for validation [10].

3.3.1.4 Suitable internal controls

Known positive (external) controls should be processed with each new kit

3.3.1.5 Suitable external QC programs

Need to be developed.

3.3.2 Latex Agglutination

3.3.2.1 Suitable specimen

Fluid stool. (Rectal swabs are not suitable as they often have a low viral load)

3.3.2.2 Test sensitivity and specificity

This is a practical and cost effective way to test for group A *rotavirus*. The specificity is generally high so false positive results are unlikely unless the patient has a low pretest probability (see 3.3 above).

Sensitivity is less than PCR, equivalent to or lower than EIA, and greater than EM [7,11]. Non specific agglutination can be a problem.

3.3.2.3 Suitable test acceptance/validation criteria

Follow the manufacturers' guidelines if using a commercial kit

3.3.2.4 Suitable internal controls

Known positive (external) controls should be processed with each new kit (batch)

3.3.2.5 Suitable external QC programs

Need to be developed

3.3.3 Immunochromatographic tests

These are relatively cheap, simple to use, dip-stick style assays. Each dipstick usually contains a strip of immobilised monoclonal antibody to VP6 and a separate strip of monoclonal antibody to the hexon antigen of *adenovirus* for simultaneous detection of both viruses.

3.3.4 Special considerations

Wilhelmi et al (2001)[9] compared the performance characteristics of three different commercial rapid detection kits, resolving discordant results by RT-PCR. An EIA kit, latex agglutination kit and immunochromatographic kit were found to have, respectively, sensitivities of 96%, 68% and 99%; specificities of 99%, 99% and 96%; positive predictive values of 98%, 96% and 92%; and negative predictive values of 98%, 88% and 99%. However, these evaluations were performed in unvaccinated populations and may not be the same in contemporary Australia.

False positive results in rapid detection tests may be due to other viruses such as *reovirus*[1] or other undetermined causes.

3.4 Nucleic acid detection tests (NADT)

Reverse transcriptase polymerase chain reaction (RT-PCR) is the most sensitive method available to detect *rotavirus* and can be used. Most are designed to detect serogroup A *rotaviruses* only, though some assays have been designed for non-A *rotavirus* detection. One study found that real-time RT-PCR was 2-4 logs more sensitive than either conventional RT-PCR or nested PCR [12]. PCRs for *rotavirus* detection are often directed at the gene encoding the VP6 inner capsid protein while genotyping PCR assays usually target VP7 and VP4, using consensus primers for each gene that are designed to detect the common human genotypes. PCRs directed at genotype-specific sequences in the VP7 and VP4 genes can then be performed to determine genotype.

3.4.1 Suitable sample

Fluid stool

3.4.2 Test sensitivity

RT-PCR is the gold standard method for the detection of *rotavirus*. Sensitivity depends on the ability of the consensus primers to detect all possible genotypes circulating in the population and the RT- PCR method used. Most tests in use detect only serogroup A *rotaviruses*

3.4.3 Test specificity

Highly specific for *rotavirus*.

3.4.4 Predictive values and relevant populations

Positive and negative predictive values are high if primers are designed to detect currently circulating *rotavirus* subtypes.

3.4.5 Suitable test acceptance/validation criteria.

In house-assays should be validated according to the NPAAC *Requirements for the Validation of In-house In Vitro Diagnostic Devices (IVDs).*

3.4.6 Suitable internal controls

Internal and external controls should be included as described in the NPAAC *Laboratory Accreditation Standards and Guidelines for Nucleic Acid Detection Techniques.*

3.4.7 Suitable external QC programs

Need to be developed

3.4.8 Special issues

The NADT for *rotavirus* detection are highly sensitive and specific, but studies have shown that, compared with antigen detection tests, they are more likely to detect the virus in asymptomatic patients. [13] Therefore detection of *rotavirus* by PCR does not necessarily mean that it is causing disease, and a positive result must be interpreted taking into account the clinical and exposure history of the patient.

3.5 Typing Methods

Rotaviruses are classified according to the genetic and antigenic diversity of two outer capsid proteins, VP4 designating the P (protease sensitive) subtypes encoded on RNA segment 4, and VP7 encoded on segment 7, 8 or 9 designating G (glycoprotein) subtypes. The two genes segregate independently and various G and P combinations are seen in natural infections. [1] Both proteins are immunogenic and induce neutralizing antibodies. Epidemiological studies have shown that genotypically mixed infections can occur.

EIA using monoclonal antibodies to G serotypes (VP7) has been the most common method for serotyping *rotavirus* directly in faecal specimens. In 1990, a multiplexed, hemi-nested RT-PCR genotyping assay based on VP7 sequences was described and shown to correlate well with MAB-based G-serotyping [14]. While a similar genotyping assay was directed towards identifying the VP4 genotypes.

In Australia, surveillance for *rotavirus* genotypes is performed by Enteric Virus Research Group, Murdoch Childrens Research Institute, Royal Children's Hospital in Melbourne. They use 2 hemi-nested multiplex RT-PCR assays to characterize any specimens forwarded as *rotavirus* into the specific G and P genotypes. In instances where a sample is non-typeable for G and/or P type, the sample will be analysed by sequence analysis of the gene of interest. Annual reports of their typing results are published in Communicable Diseases Intelligence [15].

3.6 Serological tests

3.6.1 Suitable specimens

Clotted blood 1 week after illness. Stool at the commencement and one week after illness.

3.6.2 Sensitivity and specificity

These tests have been used for epidemiologic studies of *rotavirus* but high seropositivity for *rotavirus* group A in the majority of older children and adults has precluded the use of serology as a diagnostic tool. *Rotavirus* IgM may be detected in serum about a week after illness commences but this is not useful where stool is available to be tested on presentation. Serum IgA has been the most widely used marker for *rotavirus* infection in vaccine trials, and is the best serologic indicator of reinfection. There are no commercial kits available to measure *rotavirus* antibodies. This method will not be discussed further.

4. References

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