

Poliovirus infection case definition summary

Public Health Laboratory Network case definitions

PHLN0003

Version: 1

Consensus Date: 21 February 2000

Authorisation: PHLN

Introduction

Poliovirus is a single stranded RNA virus, a member of the family Picornaviridae, genus poliovirus. There are three strains - poliovirus types 1-3. Poliovirus is an important cause of acute flaccid paralysis (AFP), which when attributable to poliovirus is known as paralytic poliomyelitis. Paralytic poliomyelitis follows approximately 0.1-2% of poliovirus infections and typically presents as an acute onset over 48hrs of progressive weakness and flaccidity of the extremities progressing to maximum severity within 1-10 days (1,2). Muscles of respiration and swallowing may be involved. There may be a febrile prodrome. Poliovirus infection is clinically inapparent in 90-95% of cases. Some 4-8% of infections present as abortive illness characterised by upper respiratory infection, gastroenteritis or influenza like illness, and a further 1-2% present as aseptic meningitis (1).

Poliomyelitis typically occurs in outbreaks during the tropical rainy season, or the temperate summer and autumn, mainly affecting young children. The risk of infection is directly correlated with poor hygiene and poor sanitation and overcrowding, typically among inadequately vaccinated populations (2). This is a major health problem in the developing world causing mortality and morbidity among thousands of children annually.

Diagnosis is usually by viral culture performed on faeces, or less commonly blood or CNS material, with subsequent viral characterisation by neutralisation using specific antisera (2). Polymerase chain reaction (PCR) nucleic acid amplification has begun to be employed more recently. Specific antibody may be detected by neutralisation for determination of immunity. Serology has a limited role in diagnosis of poliomyelitis.

Surveillance for poliomyelitis is executed via surveillance for AFP with the expectation that 1 case of non-poliomyelitis AFP will be identified per 100,000 children under 15 years of age. Laboratory testing of appropriate specimens from AFP cases is undertaken in Australia by the WHO Western Pacific Regional Poliovirus Reference Laboratory (RRL), and Australian National Poliovirus Reference Laboratory, VIDRL, North Melbourne. All polioviruses grown or detected at another laboratory must also be referred to VIDRL for confirmation of identity, and differentiation of wild from vaccine strains. Poliomyelitis is a diagnosis with grave public health implications that must not be made without reference laboratory confirmation.

Tests

Diagnosis

2.1.1 Poliovirus (enteroviral) culture

Specimens:

Faeces - faeces is the preferred specimen as polioviruses multiply in the gastrointestinal tract for several weeks after infection (2). Faecal samples of approximately 4-8g (2x adult thumbnails) should be collected within 14 days (preferably 7 days) of illness onset and placed in a dry, clean, leakproof container with a screw cap. Isolation rates are increased by obtaining 2 samples 24-48 hrs apart. If it is impossible to obtain faeces a rectal swab may be substituted, but the small quantity of faecal material thus obtained is inadequate for routine use. After collection faeces should be immediately placed at 4°C and shipped to the laboratory at this temperature within 72hrs of collection. If this is not possible the specimen must be frozen, preferably at -70°C (although -20°C is adequate), and transported to the laboratory frozen, preferably on dry ice.

Throat swab - throat swabs are inferior to faeces as the titre of virus is 10 fold lower and the duration of shedding is short (7-10 days). They are not recommended for poliovirus surveillance by WHO (2). If swabs are employed they should be cotton, rayon or dacron-tipped, plastic coated or aluminium shafted. Once the swab is taken it should be placed into viral transport medium and transported to the laboratory at 4°C.

CSF - polioviruses are rarely detected in CSF by culture which is therefore not recommended for poliovirus surveillance by WHO (2). PCR amplification of poliovirus sequences from CSF may be possible (see below), but should not replace culture of faeces. If CSF is collected a minimum of 0.5 ml should be placed into a sterile, leak proof tube, chilled at 4°C and transported immediately to the laboratory.

Biopsies and post mortem specimens - In fatal cases neurological tissue of approximately 1cm³, preferably from cervical and lumbar cord, medulla and pons, should be obtained aseptically and placed in separate sterile containers with sufficient viral transport medium to keep the specimens moist (2). Also a segment of descending colon 3-5cm long, containing faecal material should be taken.

Sensitivity:

Analytical sensitivity: 1 TCID₅₀ of poliovirus is about 1000 particles

Clinical sensitivity: 100% by definition ie poliovirus case definition is in terms of poliovirus culture, but poorly defined in practice. Culture appears to be approximately 50-75% sensitive compared to PCR (see below).

Specificity: little systematic data available - specificity of culture approaches 100%

Suitable external QA programs: WHO annual proficiency panel (participation by RRL)

2.1.2 Enteroviral (potentially including poliovirus) PCR

Specimens:

PCR is increasingly used in of detection enteroviruses, especially in the CNS, and may detect an enterovirus (poliovirus) when poliovirus infection is not clinically suspected eg aseptic meningitis, or may be used to complement culture of faeces by providing rapid detection of an enterovirus. PCR amplification product may be further characterised, for example by nucleic acid sequencing, and a suspected poliovirus may potentially be identified in this manner (see below). Specimens (faeces) should immediately be obtained for viral culture and referred to the RRL for confirmation should this situation arise. Viral culture and subsequent characterisation remains the standard method of laboratory surveillance for poliovirus. For PCR a minimum of 0.5 ml CSF is collected into a sterile, leak proof tube, chilled at 4°C and transported immediately to the laboratory. Swabs, biopsies, and faeces are collected as above.

Sensitivity:

PCR for enteroviruses is not currently standardised and assay performance may vary significantly between laboratories.

Analytical sensitivity: typically 0.01-1.0 TCID₅₀ of enterovirus (about 10-1000 particles) (3)

Clinical sensitivity: culture appears to be about 50-75% as sensitive as PCR (4, 5, 6).

Specificity: specificity of well executed PCR approaches 100% but assay performance may vary site to site.

Suitable external QA programs: not available

2.1.3 Poliovirus serology (neutralization assay)

The serological diagnosis of poliovirus infection in patients with poliomyelitis is not recommended due to difficulties in interpreting results in the context of high coverage with polio vaccine (2). Also serology cannot distinguish antibody to wild-type poliovirus from antibody to a vaccine strain (Sabin). Serology is used for determination of immunity to poliovirus. A standard microneutralization procedure is employed using 100 TCD₅₀ of vaccine (Sabin) strains of poliovirus 1, 2 and 3 as challenge viruses grown in HEp-2 cells, and doubling dilutions of patient sera from a starting dilution of 1/8 (2). The neutralization end point is calculated by the Karber formula and is expressed as international units by comparison with the International Standard Poliovirus Antiserum, National Institute for Biological Standards and Control, UK (NIBSC).

Specimens:

Serum: for determination of immunity a single blood sample is required of minimum 0.5ml volume, preferably 2ml. Serum should be transported to the laboratory at 4°C within 72hrs of collection, or frozen and transported frozen.

Sensitivity: ill defined - highly sensitive

Specificity: approaches 100%

Suitable external QA programs: none available

2.2 Characterisation

2.2.1 Enteroviral typing (virus neutralization, PCR)

Enteroviruses may be classified by antigenic type, based on serum neutralizing assay. The most commonly employed approach to serotyping employs type-specific polyclonal reference antisera mixed into “intersecting” pools (ie contains antisera to individual enteroviruses in some pools but not in others) (7). Attempted neutralization of an enterovirus isolate with each antiserum pool allows inference regarding the serotype of the isolate. The most commonly used scheme is that of Lim, Benyesh-Melnick (LBM) which employs eight pools A-H containing antisera to 42 enteroviruses, and supplementary pools and antisera for the remainder.

In some circumstances, for example when a particular enterovirus is known to be the dominant serotype circulating in the community, laboratories may elect to dispense with intersecting pools and attempt neutralization with only antiserum to the expected (homologous) virus, with antiserum to an unrelated (heterologous) enterovirus used as a negative control.

More recently nucleic acid sequence of the VP1 gene has been demonstrated to correlate with enteroviral serotype (8). Generic RT-PCR primers have been developed which amplify all enterovirus serotypes and allow amplified partial sequence of the VP1 region to be compared with available databases of prototype enterovirus strains.

Specimens: enteroviral isolate from cell culture

Sensitivity: not applicable.

Specificity: ill defined but very high, approaches 100%

Suitable external QA programs: not available

2.2.2 Confirmation of Poliovirus Identity and Typing (RRL only)

Polioviruses are identified in the first instance using selective growth in L20B cells, a transgenic mouse L cell line expressing the poliovirus receptor, and RD cells (2). Polioviruses are conventionally typed as poliovirus types 1, 2 or 3 by standard neutralization assay using WHO standard type-specific antisera supplied by the National Institute for Public Health and the Environment (RIVM), Netherlands. More recently poliovirus serotype specific PCR primers targetting the VP1 gene developed by CDC have been implemented as the first line technique for poliovirus typing in regional poliovirus reference laboratories including Australia, and selected national reference laboratories. Sequence of the VP1 gene has been shown to correlate with serotype (9).

Specimens: enteroviral isolate (presumed poliovirus) from cell culture

Sensitivity: not applicable.

Specificity:

Specificity of selective cell culture growth is high, but on occasion reoviruses and some

coxsackie A viruses may also grow in this cell line. These would not give interpretable results in serotyping assays and would be subsequently identified using standard methods for these viruses. If necessary they could be referred to a WHO global specialist poliovirus laboratory for identification. Specificity of poliovirus typing by either neutralization or PCR is very high approaching 100%.

Suitable external QA programs: Annual WHO proficiency panels (participation by RRL)

2.2.3

Intratypic differentiation of Polioviruses (EIA, nucleic acid hybridization, PCR, RRL only) Wild type and vaccine strain (Sabin) polioviruses are conventionally differentiated by enzyme immunoassay (EIA) and nucleic acid hybridisation (NAPH) (2, 10). The EIA was developed by RIVM Netherlands and employs cell culture grown test virus as antigen, with cell culture grown wild-type and Sabin viruses as control antigens, and cross absorbed polyclonal antisera to wild-type, Sabin viruses and both (total). The NAPH assay was developed by CDC and employs digoxigenin labelled synthetic oligonucleotide probes that recognise the poliovirus VP1/2a region (either of Sabin poliovirus 3 or of all polioviruses from all three types) (11). More recently vaccine strain (Sabin) and wild-type specific poliovirus PCR primers developed by CDC have been shown to correlate with serologic reactivity (12). Use of vaccine strain (Sabin) specific poliovirus PCR has been implemented as the first line technique for intratypic differentiation of poliovirus in regional reference laboratories including Australia, and selected national reference laboratories. Were an Australian poliovirus isolate determined to be a wild type its identity would immediately be confirmed by PCR and nucleic acid sequencing, and referred to a WHO global specialist poliovirus laboratory such as the Centers for Disease Control & Prevention, USA (CDC), for confirmation of these results.

Specimens: typed poliovirus isolate from cell culture

Sensitivity: not applicable

Specificity: ill defined - approaches 100% for vaccine strain (Sabin) strains

Suitable external QA programs: Annual WHO proficiency panels (participation by RRL)

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3. PHLN laboratory definition

3.1 Definitive criteria

Culture of wild-type poliovirus with RRL confirmation, or

PCR detection of wild-type poliovirus with RRL confirmation

(NB. WHO does not yet endorse use of PCR for primary detection of poliovirus as distinct from use in poliovirus typing. This criterion should only be invoked where a poliovirus isolate cannot be obtained)

3.2 Suggestive criteria

A four fold rise in poliovirus type-specific antibody

(NB. serology cannot distinguish antibody to wild-type poliovirus from antibody to a vaccine strain (Sabin). This criterion should only be invoked where a poliovirus isolate cannot be obtained.)

4. Surveillance case definitions

4.1 Poliovirus Infection:

Culture of wild-type poliovirus from the throat, blood, faeces or CNS material confirmed by the RRL.

4.2 Paralytic Poliomyelitis Potential Case = Acute Flaccid Paralysis:

Any case of acute flaccid paralysis (AFP), a syndrome characterised by acute onset of progressive weakness and flaccidity of the extremities without sensory loss, plus/minus weakness of muscles of respiration & swallowing, progressing to maximum severity within 1-10 days.

4.3 Paralytic Poliomyelitis Confirmed Case:

A case of acute flaccid paralysis with culture of wild-type poliovirus from the throat, blood, faeces or CNS material confirmed by the RRL.

4.4 Vaccine Associated Poliomyelitis:

A case of acute flaccid paralysis occurring 7-30 days after receipt of oral polio vaccine (OPV), with no sensory or cognitive loss and with paralysis still present 60 days after the onset of symptoms, with culture of sabin-like poliovirus from the throat, blood, faeces or CNS material confirmed by the RRL.