

National referral pathway for the confirmatory testing of poliovirus detected in wastewater

Background and poliovirus containment

Under the [Global Polio Eradication Initiative](#) (GPEI), the handling of poliovirus infectious materials (IM) or potentially infectious materials (PIMs), is restricted outside of poliovirus-essential facilities (PEFs). Australia has one designated PEF, the National Enterovirus Reference Laboratory (NERL) hosted by the Victorian Infectious Diseases Reference Laboratory (VIDRL).

Jurisdictions undertaking wastewater surveillance for poliovirus must comply with Australia's obligations for poliovirus containment under the GPEI. If poliovirus is detected in wastewater, the concentrated sample of that collection is subject to the containment requirements of the GPEI. Any detection of poliovirus in wastewater must be confirmed and characterised by the NERL using poliovirus detection methods approved by the World Health Organization (WHO).

Specimen collection and transfer to the NERL should be undertaken in accordance with the process specified in this document to ensure confirmatory testing can be undertaken rapidly to inform necessary public health action in a timely manner, while complying with Australia's poliovirus containment requirements.

Further information about containment requirements for poliovirus IM and PIMs can be found in the [Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses, 2nd edition](#) and the [WHO Global Action Plan for Poliovirus Containment, 4th Edition](#).

The 2015 working draft of the [WHO GPEI Guidelines on Environmental Surveillance for Detection of Polioviruses](#) provides guidance and considerations for facilities undertaking environmental surveillance for poliovirus.

Specimen collection and transport

When initial testing of wastewater detects poliovirus, the NERL should be notified, poliovirus@vidrl.org.au, alongside the relevant jurisdictional health contact and the Australian Government Department of Health and Aged Care (via the National Poliovirus Containment Coordinator, Gary.Lum@health.gov.au, and the National Authority for Containment at polio@health.gov.au).

Telephone contact with the NERL (03 9342 9607) is essential before any specimens are transferred to confirm the timing of the shipment and advice regarding safe packaging and transport. If the NERL is not contactable or when calling out of hours, the VIDRL on-call Medical Microbiologist can be contacted on 0438 599 437. In case of difficulty, back-up is provided by the VIDRL on-call laboratory manager (0438 599 439), and the Royal Melbourne Hospital Switchboard (03 9342 7000).

The NERL is located at The Peter Doherty Institute for Infection and Immunity, 792 Elizabeth Street Melbourne Vic 3000.

The essential specimen to be submitted for poliovirus confirmatory testing virus detection includes a sample of 500 mL of wastewater, which is required to be transported to the NERL for WHO-approved two-phase concentration, and poliovirus culture.¹

The following procedures should be followed:

- Appropriate personal protection equipment (PPE) should be worn during the wastewater collection and packaging process, including gloves, gown, and masks.
- 500 mL wastewater should be collected in a clean, watertight receptacle, which is wrapped in an absorbent layer and placed in a watertight secondary receptacle.¹
- The outside of each receptacle should be swabbed with an appropriate surface disinfectant and a label must be attached bearing the sample details including the date of collection. Samples should be classified as Category B biological hazard, assigned to UN 3373 with the shipping name “Biological substances, Category B”. They should be handled as required by International Air Transport Instruction (IATA) Hazard Class 6.2 and packaging instruction 650.
- Samples must be kept chilled throughout transport and received by the NERL within 48 hours of collection, or as soon as possible after detection, to maintain sample integrity.

In general, the specimens should be packaged as follows:

1. Place the specimens for transport in a tightly sealed, watertight collection container. The container should be clean but is not required to be sterile.
2. An information label should be fixed to the outside of the container, using waterproof markers and labels to avoid damage if the receptacle gets wet.
3. Wrap the primary collection container in sufficient absorbent material (e.g., absorbent paper) to absorb the entire contents in case the container leaks or breaks.
4. Place the primary collection container in a leakproof secondary receptacle. This secondary container should be sealed with tape and sturdy enough to avoid damage to the primary receptacle during transport.
5. On the outside of the secondary container, attach the specimen labels and other relevant information.
6. The specimen should be chilled using an appropriate number of ice bricks to last the duration of shipping.
7. Place the secondary container in a secure box addressed to:
National Enterovirus Reference Laboratory
Victorian Infectious Diseases Reference Laboratory (VIDRL)
The Doherty Institute
792 Elizabeth Street
Melbourne Vic 3000
8. A competent door-to-door courier should be used. Since individual commercial and non-commercial carriers or shipping services may apply different regulations for transporting biologic specimens, contact a representative of the chosen carrier beforehand to ensure all necessary formalities are fulfilled.
9. Notify the NERL of the dispatch of the specimen with flight time and number, courier or airway bill number.

¹ For jurisdictions with the capability to concentrate wastewater in accordance with the WHO-approved two-phase separation method (refer to [WHO GPEI Guidelines on Environmental Surveillance for Detection of Polioviruses](#)) a smaller volume of concentrate may be transferred to the NERL. This should be discussed with the NERL ahead of implementation.

10. If transport is by air, refer to the IATA Dangerous Goods regulations for appropriate packaging and documentation for Biological Substances, Category B, UN 3373.
11. Specimen delivery is to the foyer specimen receiving area at the Doherty Institute, accessed from Elizabeth Street where there are two short-term delivery parking spaces. For out of hours testing, the specimen should be handed to the VIDRL on-call scientist or on-call laboratory manager (0438 599 439) in foyer specimen receiving area at the Doherty Institute, who will have appropriate identification.
12. Full details of the contact person for reporting of results should be provided on the specimen form, including name and contact number.

Destruction of remaining specimen

Once the NERL has successfully completed confirmatory testing for poliovirus in wastewater samples, results should be notified to the primary referring laboratory, as well as the National Poliovirus Containment Coordinator, Gary.Lum@health.gov.au, and the National Authority for Containment at polio@health.gov.au. Poliovirus infectious and potentially infectious materials held at the primary referring laboratory must be inactivated and disposed of. This applies to samples which may have been transferred to other laboratories or used for other purposes, for example, monitoring for drugs or testing for other pathogens.

The appropriate destruction protocol is dependent on the type and quantity of samples. The following destruction protocols are appropriate for the different types of poliovirus IM and PIM.

Concentrated wastewater, derivatives, and small volumes of wastewater should be destroyed in an autoclave using a cycle of 121°C for 30 minutes.

Larger volumes of unconcentrated wastewater (2–3.8 L) can be destroyed in an autoclave using a cycle of 121°C for 60 minutes. In both instances, care should be taken not to overfill the autoclave and a biological test indicator should be used to demonstrate the autoclave cycle was successful.

Alternatively, unconcentrated wastewater can be inactivated using a chemical disinfection method effective against poliovirus, in accordance with the manufacturer instructions.

Once wastewater samples have been destroyed, please notify the Department of Health and Aged Care at polio@health.gov.au.