

Picornavirus Laboratory Instructions

Testing for enteroviruses, parechoviruses and cardioviruses

AUGUST 28, 2014: US Picornavirus season update

- 1) EV-D68 has been identified in a single cluster of severe respiratory disease, several other suspect locations are under investigation (ongoing)
- 2) CVA-6 continues to cause sporadic atypical severe rash in hand, foot and mouth disease cases (HFMD)
- 3) HPeV-3 has been identified in a single cluster of severe neonatal disease (June-July)
- 4) Sporadic neonatal sepsis-like illnesses; several EV types identified

INSTRUCTIONS

TIMING OF SPECIMEN COLLECTION: Collecting specimens during the first week of illness is ideal although the virus can be shed in stool for several weeks. A specimen set collected in the second week of illness should include a rectal swab or stool sample.

RECOMMENDED SWABS:

For nasopharyngeal (NP)/ oropharyngeal (OP) and/or rectal swabs please use only sterile dacron or rayon swabs with plastic shafts or if available, flocked swabs. DO NOT use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit some molecular assays. Place the NP/OP swabs immediately into a sterile vial containing 2 ml of viral transport media without antibiotics. If you do not have viral transport media or other media the swab(s) may be sent dry. Aseptically, cut or break applicator stick off near the tip to permit tightening of the cap. Sterile PBS or Hank's balanced salt solution (HBSS) (no antibiotics) can be used in lieu of viral transport medium.

HAND, FOOT AND MOUTH DISEASE (HFMD) SPECIMEN TYPES:

For each patient with HFMD, collecting specimens from more than one site is recommended. Preferred specimens are:

Vesicle fluid

Nasopharyngeal (NP)/oropharyngeal (OP) swabs

Stool / rectal swab

Serum

For each serum specimen, collect whole blood (adults and children >6kg: 5 ml, children <6kg: 2 ml) into a serum separator tube (marble or tiger top SST). A minimum of 1 ml of whole blood is needed for testing of pediatric patients. Allow to clot at room temperature for a minimum of 30 minute and centrifuge. Transfer the serum to a sterile vial and freeze.

SHIPMENT:

Please ship samples through your local or state public health department laboratory or obtain verbal/email permission to ship directly to us.

- Please ship specimens so that they arrive Monday-Friday
- Please fill out the specimen submission form online. Link to the form: <http://www.cdc.gov/laboratory/specimen-submission/form.html>

The pop-down menus may not offer exactly the testing you would like or you may be unsure about the menu choices; in either case make a choice and send an email/call to clarify what testing is requested.

- If ten or more patient specimens are submitted, please provide an electronic line listing by email. Ideally the line listing headers will be (in order): patient ID number; date of birth; sex; onset date; fatal y/n; specimen ID number; specimen collected date; specimen type; tissue culture—cell line and passage number.
- Print out the specimen submission form to send with the specimen; prior to shipping, please email Allan Nix (wnix@cdc.gov) and Shannon Rogers (boo9@cdc.gov) regarding what is being shipped and include the name, phone number and email address of the shipper.

Shipping Address

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Specimen collection and handling, by specimen type (other types of specimens not listed below may be submitted)

Specimen type	Test type	Minimum amount and collection	Storage	Shipping	Comments
CSF	Real-time PCR (generic) Nested PCR (typing)	≥150 ul Collect in sterile container, no special medium required.	Freeze at -20°C	Ship on dry ice	Collect within 0-4 days of symptom onset (earlier the better)
Serum	Real-time PCR (generic) Nested PCR (typing)	≥150 ul Ideally 300-500 ul	Freeze at -20°C	Ship on dry ice	Generally not recommended; exceptions: HFMD, neonates, infants
Respiratory NP/OP swab, or NP/OP wash or aspirate	Real-time PCR (generic) Nested PCR (typing)	2ml For washes and aspirates, collect in sterile container, no special medium required. For swabs, store in viral transport medium	Freeze at -20°C	Ship on dry ice	
Stool	Real-time PCR (generic) Nested PCR (typing)	≥1gram Collect in sterile container, no special medium required	Freeze at -20°C	Ship on dry ice	Virus present for longer than other specimen types
Rectal swab		Store in viral transport medium			
Fresh-frozen tissue (preferred over fixed)	Real-time PCR (generic) Nested PCR (typing)	Place directly on dry ice or liquid nitrogen.	Freeze at -70°C	Ship on dry ice	
Formalin-fixed or formalin-fixed, paraffin-embedded tissue (acceptable, but lower sensitivity)	Real-time PCR (generic) Nested PCR (typing)	Avoid prolonged fixation—can fix in formalin then transfer to 100% ethanol		Ship at room temperature with paraffin blocks in carriers to prevent breakage	