



Specimen Collection Instructions

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Follow these instructions for collecting specimens from patients that meet the [clinical case definition](#) for AFM.

For patients that are **currently hospitalized**:

- CDC would like to receive **EACH** of the following:
 - CSF specimen
 - Upper respiratory tract specimen (ranked by first to last preference)
 - Nasopharyngeal swab >>nasal swab>>nasal wash/aspirate>>oropharyngeal swab
 - Serum samples (acute and convalescent, if possible*) and whole blood
 - Two stool specimens, in accordance with the recommendations for poliovirus testing in all patients with a compatible clinical picture (ranked by preference)
 - Whole stool>>rectal swab
- See [Table](#) for further information, optimal timing of specimen collection, minimum amount of samples, collection and shipping instructions. Although minimum sample volumes are listed, please send any remaining samples, irrespective of available volume.
- We request that all specimens be sent, even if testing for EV-D68 has been performed and has tested negative. This is because we are also concerned about other potential etiologies of the neurologic illness and we would like to test for those etiologies as well.
- If you have not collected the samples detailed above, or if all of the available specimens have been utilized and no specimen is remaining, we request that repeat specimen(s) be collected and sent to CDC. We recognize that this may clearly be challenging for certain specimens (i.e., CSF and NP swabs); however, if it is possible to collect these specimens, it would be extremely useful.

For patients that have already been **discharged from the hospital**:

- If it has been **less than 30 days** since the hospital admission date, please send any stored specimens from the list above. In addition, if any specimen on the list above was never collected or is no longer available, please strongly consider obtaining the sample now from the patient, for testing at CDC.
- If it has been **30 days or more** since the hospital admission date, please send any stored specimens from the list above.

Comments:

- Please recognize that this testing will be done for investigational purposes, and it is unlikely that results would be available in a timely fashion to guide clinical management. Results will be provided from the CDC laboratory to the sender of the specimens.
- Your assistance and participation in this important endeavor is greatly appreciated. We realize that collection of these specimens may be an added burden on already busy schedules, but through your assistance and cooperation, we hope to gain further insights into the nature of this unusual illness.

*If any of the serum samples that you are sending to CDC were collected after the patient had received intravenous immune globulin (IVIG) or plasmapheresis/plasma exchange, please indicate the date of that therapy on the [Patient Summary Form](#).

Specimens to collect from **hospitalized** and from **discharged** patients:

Specimen type	Optimal timing for collection	Collection specifications	Minimum amount
Cerebrospinal fluid (CSF)	As early in illness as possible, preferably at time of first evaluation/admission	Collect in sterile container, no special medium required.	Please send as much sample as available, since multiple tests may be performed at CDC.
Upper respiratory tract specimen	Ranked below by first to last preference		
	1. Nasopharyngeal swab (see figure below)^p	As early in illness as possible, preferably at time of first evaluation/admission	Store in viral transport medium

Specimen type		Optimal timing for collection	Collection specifications	Minimum amount
	2. Nasal swab	As early in illness as possible, preferably at time of first evaluation/admission	Store in viral transport medium	1ml
	3. Nasopharyngeal wash or aspirate	As early in illness as possible, preferably at time of first evaluation/admission	Collect in sterile container, no special medium required	1ml
	4. Oropharyngeal swab	As early in illness as possible, preferably at time of first evaluation/admission	Store in viral transport medium	1ml
Blood	1. Serum	Paired acute and convalescent* specimens are optimal. Single serum specimens are acceptable.		Please send as much sample as available (Ideally 0.8 – 2 ml), since multiple tests will be performed at CDC.
	2. Whole blood	Acute specimens are optimal.	Prefer specimens with no anticoagulants (EDTA, heparin, or citrate)	≥1 ml
Stool	Ranked below by first to last preference			
	1. Whole stool	Two samples total, collected at least 24 hours apart, both collected as early in illness as possible and ideally within 14 days of illness onset	Collect in sterile container, no special medium required	≥1gram

Specimen type		Optimal timing for collection	Collection specifications	Minimum amount
	2.Rectal swab²	Two samples total, collected at least 24 hours apart, both collected as early in illness as possible and ideally within 14 days of illness onset	Store in viral transport medium	≥1gram

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In the event of a **death**, please send both types of specimens below, if possible:

Specimen type	Handling	Storage	Shipping	Comments
Fresh-frozen tissue	Place directly on dry ice or liquid nitrogen	Freeze at -70°C	Ship on dry ice	Representative sections from various organs are requested, but particularly from brain/spinal cord (including gray and white matter), heart, lung, liver, kidney, and other organs as available.
Formalin-fixed or formalin-fixed, paraffin-embedded tissue	Avoid prolonged fixation—tissues should have been fixed in formalin for 3 days, then transferred to 100% ethanol	Room temperature	Ship at room temperature with paraffin blocks in carriers to prevent breakage	See comment above regarding frozen tissue

*Convalescent sera should be collected 10-14 days after the first serum specimen, or at time of patient discharge, whichever comes first.

Handling and Shipping

- **All above samples should be frozen at -20°C** (except for pathology specimens—see Table above). Hospitals should make arrangements with Health Department (HD) to transport the samples frozen to the HD, and shipped from HD to CDC on dry ice.

- Samples from each patient should be submitted with a hard copy of
 - Page 1 of the completed Acute Flaccid Myelitis: Patient Summary Form (Page 1 contains the patient demographic and ID information) and
 - A completed specimen submission form 50.34 (<http://www.cdc.gov/laboratory/specimen-submission/form.html>). Please note the pop-down menus may not offer the exact testing you would like or you may be unsure about the menu choices; in either case, make a choice and send an email or call to clarify what testing is requested.
- Please ship specimens so they arrive at CDC Monday through Friday.
- If 10 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.
- Prior to shipping, please email Allan Nix (wnix@cdc.gov (<mailto:wnix@cdc.gov>)) and Shannon Rogers (boo9@cdc.gov (<mailto:boo9@cdc.gov>)) regarding what is being shipped and include the name, phone number and email address of the shipper.

Shipping Address

W. Allan Nix

Centers for Disease Control and Prevention

SMB/STAT

ATTN: Polio and Picornavirus Laboratory Branch; Building 17, Room 6066

1600 Clifton Road, NE

Atlanta, GA 30333

Office: +1-404-639-1689 Mobile: +1-404-398-8310 Email: wnix@cdc.gov (<mailto:wnix@cdc.gov>)

[¶]For nasopharyngeal (NP)/ oropharyngeal (OP) 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 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.

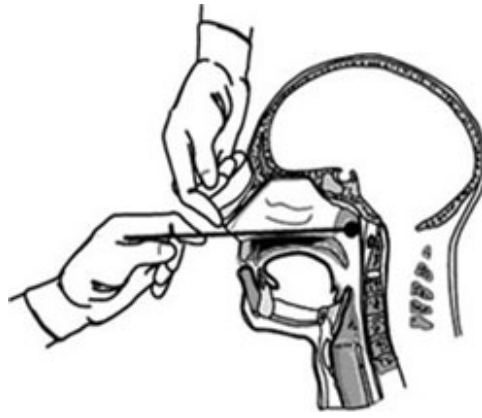


Figure. Technique for collection of a nasopharyngeal swab. For more information on the proper technique, see the videos at [Pertussis \(Whooping Cough\) Specimen Collection](http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.html) (<http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.html>).

Image: Manual for the Surveillance of Vaccine-Preventable Diseases, 4th ed, 2008

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Page last reviewed: January 22, 2016

Page last updated: January 22, 2016

Content source: National Center for Immunization and Respiratory Diseases (<http://www.cdc.gov/ncird/>),

Division of Viral Diseases (<http://www.cdc.gov/ncird/DVD.html>)