



Alabama Department of Public Health
Bureau of Communicable Disease
Immunization Division
Vaccine-Preventable Disease (VPD) Surveillance Protocols

Purpose: To provide standard procedures for investigation of vaccine-preventable diseases (VPD) and potential VPD emerging diseases. These protocols include instructions on investigations, collection, handling, and shipping of specimens for presumptive Measles (rubeola), Mumps, Rubella, Diphtheria, Pertussis, Varicella, *Haemophilus influenzae* (invasive disease), *Streptococcus pneumoniae* (invasive disease), Meningococcal disease (*Neisseria meningitidis*) and Acute Flaccid Myelitis. In addition links to required forms, case definitions, and CDC's Vaccine Surveillance Manual are included.

Measles, Mumps, Rubella, Diphtheria, Pertussis, Varicella, Haemophilus Influenzae, Pneumococcal, Meningococcal and Acute Flaccid Myelitis Procedures:

1. When Area Immunization Manager (AIM) or designee receives a positive lab results for VPDs in Alabama NEDSS Based System (ALNBS) queue, presumptive VPD reports from an online REPORT Card, call from notifiable disease reporters (physicians, nurses, dentists, medical examiners, hospital administrators, nursing home administrators, laboratory directors, school principals, childcare/Head Start directors) or central office, open the investigation in ALNBS. The only exceptions to not opening an investigation are for varicella, rubella, and mumps IgG lab results, see 5 below. For calls received directly from reporter, notify central office staff, Data Quality and Surveillance Branch Director, VPD Surveillance Coordinator, or Immunization Director. If no central office staff is available, notify Dr. Karen Landers via e-mail, Karen.landiers@adph.state.al.us, or phone (256) 246-1714
2. At a minimum, vaccine-preventable disease (VPD) investigation must be opened in ALNBS on the same day for 4-hour diseases, within 24 hours for 24-hour diseases and outbreaks, and within 5 days for Standard Notification Diseases.
3. If the report is not from a physician, contact the provider about the presumptive case of VPD and obtain lab results performed to date and what specimens are available for further testing.
4. For VPD cases in child care center/homes and schools, please see the AAP Redbook regarding exclusions. Regardless of exclusions, contact the child care center/home director or school nurse to notify the parents of children who are not fully immunized for the specific VPD investigated.
5. For varicella, rubella, or mumps IgG positive lab results received, contact the provider and college/university who ordered the lab to educate, and document the education on the VPD Provider Education Spreadsheet. The education must include:
 - A. The Notifiable Disease Rule specifically required providers to report separately from the labs. Future positive IgGs will not be investigated unless the provider who ordered the labs reports the potential case. The preferred test methods for varicella are culture,

- DFA, serology, or PCR and for rubella and mumps are culture, PCR, or serology, please see TEST Webpage <http://www.adph.org/epi/Default.asp?id=5192>.
- B. How the provider can report a notifiable disease online, <https://www.adph.org/Extranet/Forms/Form.asp?ss=s&formID=4799>, or 1-800-469-4599.
 - C. Reasons to report timely include providing civil and criminal liability protection for the provider.
 - D. Ask if the provider looked for the patient’s vaccine history or previous titer results in ImmPRINT. If they don’t have access to ImmPRINT, educate the provider and college/university on ImmPRINT and encourage them to enroll.

After calling and educating the provider, document the education on the I:/drive, VPD folder, VPD Provider Education Spreadsheet, “Mark Lab as Reviewed” in ALNBS, and enter in the ALNBS General Comment section “Provider educated on date.” After calling and educating the college/university, document on the second tab of the provider spreadsheet and include the source for their recommendations.

6. Request lab at a private provider’s office for all VPDs reported. Please note ADPH registered nurses (RNs) should be trained to collect most VPD specimens, including NP swabs and nasopharyngeal aspirates. For specimens obtained by Immunization field staff, please see Lotus Notes, Document Library, Clinic Protocol Manual, Standing Orders 2016, 2016 Standing Order IMM TB Med Adm ER, 2. Collecting specimens needed to test for vaccine preventable diseases.
 - A. For **measles** and **rubella**, request a serology and throat swab, nasopharyngeal (NP) aspirate, or NP swab. AIM or designee is not allowed to collect a throat swab or nasopharyngeal aspirate. Physicians, nurse practitioner, physician’s assistant, and registered nurses (RNs) can collect NP swabs aspirates. Specimen collection must be within 3 days of rash onset for measles and 4 days of rash onset for rubella.
 - B. For **mumps**, request a buccal swab. AIM or designee is allowed to collect a buccal swab. Specimen collection must be as soon as mumps is suspected.
 - C. For **diphtheria**, request a nose, throat or wound swab. Physicians, nurse practitioner, physician’s assistant, and RNs can collect a swab.
 - D. For **pertussis**, whenever possible, a nasopharyngeal (NP) swab or aspirate should be obtained from all persons with suspected cases. The specimen should ideally be collected during the first three weeks of illness, but may provide accurate results for up to 4 weeks.
 - E. For **varicella**, to make a laboratory diagnosis of VZV infection using polymerase chain reaction (PCR) method, the presence of the virus DNA should be demonstrated in tissues, vesicular fluid, maculopapular lesions, or crusts from lesions.
 - F. For *Haemophilus influenzae* (HI) and **meningococcal** (mening), to make a laboratory diagnosis of HI or mening infections using isolation of the organism by culture or PCR method, the presence of the bacteria should come from a normally sterile body site.

- G. For **pneumococcal** (strep pneumo), to make a laboratory diagnosis of strep pneumo, the bacterial organism should be isolated from a normally sterile body site. Susceptibility testing should be performed in children <5 years of age.
- H. For **Acute Flaccid Myelitis** (AFM), to make a laboratory diagnosis of AFM, the onset of focal limb weakness should be present and a MRI should be performed and/or CSF should be collected. All specimens should be collected as early in onset as possible.
7. Review all investigations to ensure the case classification is selected, all fields are complete, and submit the investigation within 30 days of opening.
 8. Completed all fields on BCL Requisition Form, http://www.adph.org/bcl/assets/BCL_Requisition_Form.pdf, Wisconsin (WI) State Laboratories Hygiene Form, http://www.adph.org/bcl/assets/WSLH_Lab_Form.pdf or CDC Specimen Collection Form, http://www.adph.org/bcl/assets/CDC_Dash.pdf, see table below.

Table: VPD Labs Test and Location of Performing Lab

VPDs	BCL Division Responsible	Culture	Serology		PCR	Genotyping
			IgG	IgM		
Acute Flaccid Myelitis	Micro	CDC			CDC	
Diphtheria	Micro	CDC/BCL			CDC	
<i>Haemophilus influenzae</i>	Micro				BCL	
Measles	Micro		CDC	CDC	WI	WI
Meningococcal	Micro				BCL	
Mumps	Micro		CDC	CDC	WI	WI
Pertussis	EID	BCL	CDC	CDC	BCL	
Pneumococcal	Micro	CDC				
Polio	Micro	CDC				
Rubella	Micro		CDC	CDC	WI	WI
Tetanus	Micro					
Varicella	Micro		CDC	CDC	CDC	

Bureau of Clinical Laboratories (BCL)

Centers for Disease Control and Prevention (CDC)

Wisconsin State Laboratory (WI)

9. If the physician does not have the kit for collection of the PCR, please provide a kit for collection. Instruct the provider to properly package the specimens, with the completed lab forms, and drop off at the county health department. Alternatively, the provider can pay to have the specimen shipped to the BCL. If there is a problem with the physician sending the

specimen, please pick up the specimen and ship it to the BCL. For assistance with specimen collection, please request the BCL to contact the provider's lab.

10. For specimens obtained by ADPH staff for VPDs, complete the BCL Lab Requisition as follows:

Healthcare Provider Information		
Facility Name Immunization Division		
Physician/Requestor Name (Last and First)		NPI#
Karen Landers, MD		1609919380
Street Address 201 Monroe St		
City	State	Zip
Montgomery	AL	36104
Phone Number		Fax Number
334-206-5023		334-206-7901

Resources:

Case Definitions, <https://alnbs.adph.state.al.us/investigation-resources>

Surveillance Manual, <http://www.cdc.gov/vaccines/pubs/surv-manual/index.html>

Appendix A: Specimen Collection

- Measles**

Respiratory Swab - A throat, NP or nasal swab should be transferred to 1-3ml of viral transport medium (do not allow to dry out). The entire sample can be frozen at -70°C or if low temperature freezers are not available, keep the sample at 4°C and ship on cold packs.

Serology - Blood for serologic testing is collected by venipuncture or by finger/heel stick. Use tubes without additives—either a plain, red-top tube or a serum separator tube. The preferred volume for IgM and IgG testing at CDC is 0.5–1 ml of serum to allow for re-testing; however, testing can be done with as little as 0.1 ml (100 ul) if necessary. Generally, 5 ml of blood (yield about 1.5 ml of serum) can easily be collected from adults. Do not freeze the tube before serum has been removed. Centrifuge the tube to separate serum from clot. Gel separation tubes should be centrifuged no later than 2 hours after collection. Aseptically transfer serum to a sterile tube that has an externally threaded cap with an o-ring seal. Fresh, sterile serum can be shipped overnight on a wet ice pack. Hemolyzed and lipemic serum and plasma are noted and tested, usually without apparent interferences. Capillary tubes can be utilized for infants. Capillary tubes require the submitter to have access to the appropriate centrifuge for these capillary tubes. Clinical laboratories should have 50 or 100 ul capillary tubes that are typically used for a variety of tests such as hematocrits or total lipids on newborns. At least 3 of the 50 ul hematocrit capillary tubes should be collected and spun in a hematocrit centrifuge.

- Rubella**

Respiratory Swab - A throat swab or nasopharyngeal wash should be collected. Materials: sterile swabs, 3 ml aliquots of viral transport medium (VTM: sterile PBS or suitable isotonic solution such as Hanks BSS, containing antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin) and either 2 % fetal bovine serum or 0.5% gelatin in 15 ml polycarbonate or polystyrene centrifuge tubes, 5 ml plastic syringes, plastic aspirators or 30 ml syringe, Styrofoam shipping containers. Swab throat in the same manner as obtaining a bacterial culture. An alternative method for specimen collection is a nasal wash (nasopharyngeal aspirate) using a syringe attached to a small, plastic tube and 3–5 ml of VTM. After placing VTM in the nose, aspirate as much of the material as possible and rinse the tube with a small volume (2ml) of VTM. Alternatively, sterile swabs can be used to wipe the nose and throat. Place both swabs in a tube containing 2–3 ml of transport medium. The virus is extremely cell-associated, so attempt to swab the throat and nasal passages to collect epithelial cells.

Procedures for shipping of specimens Preferred method: Keep all specimens on wet ice or at 4° C and ship as soon as possible on wet ice (see address below). Other acceptable methods: If immediate, cold shipment (within 48 hrs) cannot be arranged or is not convenient please contact Bureau of Clinical Laboratories (BCL) at 334-260-3400 to ask if other acceptable methods are available. . Nose and throat swabs can be removed from the transport medium after allowing some time for elution of virus (at least 1 hr). Nasal wash specimens can be centrifuged at 2500 x g for 15 minutes at 4° C and the pellet resuspended in 1 ml of tissue culture medium. If possible, the supernatant can be saved in a separate tube. The samples should be frozen and shipped at -70° C (dry ice) if centrifugation is not available.

- **Rubella Serology – See Measles Serology**

- **Mumps**

Collect oral or buccal swab - Collect samples as soon as mumps disease is suspected. Samples collected when the patient first presents with symptoms have the best chance of having a positive result by RT-PCR. The buccal or oral swab specimens are obtained by massaging the parotid gland area for 30 seconds prior to swabbing the area around Stensen’s duct. A commercial product designed for the collection of throat specimens or a flocked polyester fiber swab can be used. Synthetic swabs are preferred over cotton swabs, which may contain substances that are inhibitory to enzymes used in RT-PCR. Flocked synthetic swabs appear to be more absorbent and elute samples more efficiently. Processing the swabs within 24 hours of collection will enhance the sensitivity of both the RT-PCR and virus isolation techniques. Swabs should be placed in 2 ml of standard viral transport medium (VTM)[1]. Allow the swab to remain in VTM for at least 1 hour (4°C). Ream the swab around the rim of the tube to retain cells and fluid in the tube. The swab can be broken off and left in the tube or discarded. Storage and shipment: Following collection, samples should be maintained at 4°C and shipped on cold packs (4°C) within 24 hours.

If there is a delay in shipment, the sample is best preserved by freezing at -70°C . Frozen samples should be shipped on dry ice.

- **Diphtheria**

Culture Swabs – Take a specimen swab from the nose, throat or wounds. Use polyester, rayon or nylon swabs (Copan flocced is what we have. Do not use cotton tipped applicators for this specimen.) The swabs should be placed in transport media such as Amies or Stuart and shipped overnight with ice packs. Dry swabs submitted in silica gel sachets are also acceptable. You can use the Reagan Lowe media for cultures to send to our state lab. Pieces of pseudo-membrane may also be submitted for culture (physicians only can collect this). Pseudo-membrane should be placed in sterile saline (not formalin) and shipped overnight with ice packs.

PCR Swabs - CDC does not perform PCR to rule out diphtheria unless diphtheria anti-toxin (DAT) has been requested to treat the patient. Swabs taken from the nose, throat or wounds can be tested by PCR for the presence of the A and B subunits of the diphtheria toxin gene (*tox*). The presence of *tox* does not necessarily indicate that toxin is being produced, and this PCR assay does not distinguish between *C. diphtheriae* and *C. ulcerans*. Swabs for PCR can be shipped in the same manner as swabs for culture, or they may be placed in a sterile tube or silica gel sachet. Swabs for PCR should be shipped overnight with ice packs. Pieces of pseudo-membrane may also be submitted for PCR testing. Pseudo-membrane should be placed in sterile saline (not formalin) and shipped overnight with ice packs.

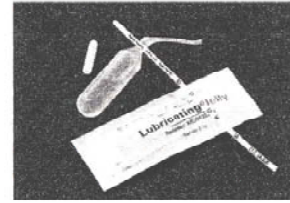
- **Pertussis**

Instructions for Collecting and Submitting Specimens for Pertussis Diagnosis

Collecting

Materials for Pertussis Nasal Wash/Aspirate

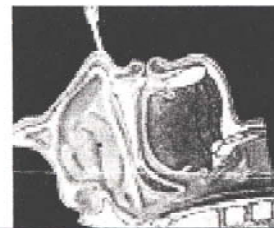
- Gloves
- Mask
- Biohazard Bag
- Eye protection
- Paper Towels (absorbent pad)
- Shipping kit: cardboard box enclosing a Styrofoam box, cold pack, absorbent pad, 3373 label, requisition form ADPH-F-CL-42/Rev.5/01, Regan-Lowe Transport Media Tube (expiration date is 6 weeks; call BCL to order more before it expires; Store media at 4 degrees until needed, and allow to come to room temperature before use), 1 Sterile Dry Tube, 1 Dacron or Rayon swab (*no calcium alginate*); N-Pak™ Nasopharyngeal Bulb Aspiration Kit.



N-Pak™ Nasopharyngeal Bulb Aspiration Kit

Nasopharyngeal (NP) Aspirate

1. Assemble all supplies.
2. Let the patient lie on their back (*supine*) with neck extended (*Neck extension is important as this allows pooling of the aspirate in the nasopharynx*).
3. Open N-Pak™ Nasopharyngeal Bulb Aspiration Kit.
4. Note calibration of catheter by age in years.
5. Remove the cap from bulb and attach the catheter to the bulb.
6. Generously lubricate the catheter.
7. If possible, ask patient to hold their breath during the procedure.
8. Expel the appropriate volume of saline from the bulb according to patient's age.
9. Insert catheter into right or left nares and follow the floor of the nose until resistance is met (*Insertion may induce coughing and tearing*).
10. Squeeze to dispense saline and release the bulb as catheter is withdrawn from the nose. The aspirate sample is adequate if at least 1cc ($\frac{1}{4}$ of the bulb) is collected.
11. Detach the catheter.
12. Dispense the aspirate as follows:
 - a) For Culture: Drop (*squeeze*) some of the aspirated material onto a swab; insert swab into the Regan-Lowe transport medium tube; and store at 4°C immediately (*Ship as soon as possible*).



<http://www.nasalaspirationkit.com/index.htm>

- b) For PCR: For PCR: Squeeze the remaining aspirated material into the sterile tube and store at 4°C immediately (*Ship as soon as possible*).
- 13. Make sure that the patient's name is written on the tubes.
- 14. Complete the requisition; however, make sure that the sections for the patient's name, collection date, birth date, and health care provider are filled-in.
- 15. Store specimens as indicated in #12 until ready for shipping to the laboratory from the county health department.
- 16. Place all waste materials in a disposable biohazard bag for discard.

Shipping

1. Ship specimens separately as UN 3373 Category B biological substance (see below).
 - a. Please adhere to the following packaging instructions to ship the Regan-Lowe transport tube and the tube containing the remaining aspirated material.
 - i. Wrap each tube in absorbent material. The tubes should not touch or bang together. If they rattle, use more cushioning. Use paper towels or other soft material for wrapping/cushioning.
 - ii. Place wrapped tubes in metal canister. Carefully screw cap onto metal canister.
 - iii. Place metal canister inside cardboard canister. Carefully screw cap onto cardboard canister.
 - iv. Place canister and cold pack (refrigerated) in the Styrofoam box. Secure canister in place with newspaper, paper, Styrofoam peanuts, and etc. if needed.
 - v. Close the Styrofoam box.
 - vi. Place the completed requisition on top of the Styrofoam box.
 - vii. Close the cardboard box.
 - viii. Place address label and UN 3373 Biological Substance Category B Label on the same side of the outside of the cardboard box.
2. Ship specimen by overnight courier Monday through Thursday. Specimens collected on Friday should be stored as indicated above and shipped on Monday.
3. Please ship specimen to: **Microbiology Division, Alabama Department of Public Health, Bureau of Clinical Labs, 8140 AUM Drive, Montgomery, AL 36117** using label that is provided.

• **Varicella**

PCR Swab - Polyester Swab Method is best suited to sampling vesicular lesions

1. A sterile needle should be used to unroof the top of the vesicle.
2. A sterile swab† is then used to vigorously swab the base of the lesion— applying enough pressure to collect epithelial cells without causing bleeding—and collect vesicular fluid. It is important to collect infected epithelial cells from the base of the lesion because they usually contain a significant amount of virus.

†We recommend swabs made from synthetic fibers, such as polyester, because it is difficult to elute virus from cotton swabs, and wooden swab supports usually absorb extraction buffer and inhibit PCR.

3. Swabs must be placed individually into separate, empty tubes to avoid contamination. Place swabs directly into tubes. Do not place transport medium into the tube; the specimen **MUST** be kept dry. Tubes must be individually labeled and must be resistant to breakage.

Collecting Crusts (Scabs)

1. Crusts are also excellent samples for PCR detection of VZV DNA. Crusts can be lifted off the skin (a glass slide is also useful for this purpose) and transferred directly into break-resistant, snap-cap or screw-top tubes. See Handling and Shipping PCR Specimens for shipping instructions.

Handling and Shipping PCR Specimens

1. Dried specimens for PCR can be stored at ambient temperature indefinitely, although we prefer to receive specimens as soon after collection as possible.
2. Do not refrigerate or freeze dry specimens intended for testing by PCR. Specimens can be mailed by regular post unless a result is urgently required. Do not suspend specimens in transport medium: they should be shipped dry.
3. In rare cases involving severe complications or death, other types of specimens (e.g., biopsied tissue, cerebrospinal fluid, peripheral blood, etc.) may be sent to the National VZV Laboratory for PCR testing. When possible, liquid specimens should be shipped frozen.

<http://www.cdc.gov/chickenpox/lab-testing/collecting-specimens.html>

• **Haemophilus influenza, Pneumococcal, and Meningococcal disease**

CSF Collection - The collection of CSF is an invasive procedure and should only be performed by experienced personnel under aseptic conditions. This specimen collection will only be performed by physicians in hospital or emergency based settings. NOTE: Information on CSF collection procedures is included in this document for informational purposes only. Physicians should follow their training and hospital procedures for collecting CSF specimens. . If bacterial meningitis is suspected, CSF is the best clinical specimen to use for isolation, identification, and characterization of the etiological agents.

A. CSF Collection Kit should contain:

1. Skin disinfectant: 70% alcohol swab and povidone-iodine
2. Sterile gloves (Be sure to check the expiration date)
3. Sterile gauze
4. Surgical mask
5. Adhesive bandage
6. Lumbar puncture needle (22 gauge/89 mm for adults and 23 gauge/64 mm for children)
7. Sterile screw-cap tubes
8. Syringe and needle
9. Transport container
10. T-I medium (if CSF cannot be analyzed in a microbiological laboratory immediately)
(should be refrigerated at 4°C and added to the kit immediately before use in the field.)
11. Venting needle (only if T-I is being used)

12. Instructions for lumbar puncture and use of T-I medium



B. Lumbar Puncture Procedure

1. Gather all materials from the CSF collection kit.
2. Label collection tubes.
3. Ensure patient is kept motionless during the lumbar puncture procedure
4. Disinfect the skin between the two posterior superior iliac crests with 70% alcohol or povidone-iodine to clean the surface from debris and oils. Allow to dry completely.
5. Position the spinal needle between the 2 vertebral spines at the L4-L5 level and introduce into the skin with the bevel of the needle facing up.
6. Remove CSF (1 ml minimum, 3-4 ml if possible) and collect into sterile screw-cap tubes. If 3-4 ml CSF is available, use 3 separate tubes and place approximately 1ml into each tube.
7. Withdraw the needle and cover the insertion site with an adhesive bandage. Discard the needle in a puncture resistant, autoclavable discard container.

C. Shipping – Transport the CSF to a microbiology laboratory within 1 hour for culture and analysis. If a delay of several hours in processing or getting specimen to the lab inoculate CSF into T-I medium or incubate CSF at 35-37°C with approximately 5% CO₂ and store in an approved location if the laboratory is closed, and warm to room temperature (25°C) before use. Specimens for culture should not be refrigerated or exposed to extreme cold, excessive heat, or sunlight. Transport specimens at temperatures between 20°C and 35°C.

Collecting Blood Specimens - Blood should be collected when a spinal tap is contraindicated, cannot be performed for technical reasons, or when bacteremia is suspected.

A. Venipuncture – 1-3 ml of blood should be collected from a child and 5-10 ml of blood should be collected from an adult. Collected blood should be diluted in blood culture broth in order to obtain blood cultures. Blood cannot be transported before being in a blood culture bottle

because the syringes do not contain any anticoagulant and the blood will coagulate within a few minutes. When using an alternative blood collection method, immediately inoculate specimens into the blood-culture bottle using a needle and syringe after disinfecting the top of the bottle with 70% alcohol.

Handling and Shipping Blood Specimens - Inoculated blood culture bottles should be transported to a microbiology laboratory immediately for overnight incubation at 35-37°C with ~5% CO₂ (or in a candle-jar) and subsequent culture onto a BAP and CAP. All inoculated blood culture media should be protected from temperature extremes (not less than 18°C or more than 37°C) with a transport carrier and thermal insulator (such as extruded polystyrene foam).

<http://www.cdc.gov/meningitis/lab-manual/chpt06-culture-id.html>

- **Acute Flaccid Myelitis** (see table below)

The CDC would like to receive CSF specimen, Upper respiratory tract specimen, serum samples, and two stool specimens

CSF – See Haemophilus influenza, Pneumococcal, and Meningococcal disease CSF collection.

NP/OP – See Pertussis

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	In order of preference below, highest to lowest			
	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.	1ml
	2. Nasal swab	As above	Store in viral transport	1ml

Specimen type		Optimal timing for collection	Collection specifications	Minimum amount
			medium	
	3. Nasopharyngeal wash or aspirate	As above	Collect in sterile container, no special medium required	1ml
	4.Oropharyngeal swab	As above	Store in viral transport medium	1ml
Serum		Paired acute and convalescent specimens are optimal. Single serum specimens are acceptable.		≥ 0.5 ml
Stool	In order of preference below, highest to lowest	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		
	1. Whole stool		Collect in sterile container, no special medium required	≥1 gram
	2.Rectal swab ^P		Store in viral transport medium	Place swab in viral transport medium
Specimen type	Handling	Storage	Shipping	Comments
Fresh-frozen	Place directly on	Freeze at -70°C	Ship on dry ice	Representative

Specimen type		Optimal timing for collection	Collection specifications	Minimum amount
tissue	dry ice or liquid nitrogen			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

Handling and Shipping

All samples should be frozen at -20°C (except for pathology specimens). All samples should be submitted with a hard copy of Page 1 of the Acute Flaccid Myelitis Patient Summary Form (completed), the specimen submission form (completed), and a print out of the specimen submission form. Contact your state epidemiologist prior to shipping and Allan Nix (wnix@cdc.gov) and Shannon Rogers (boo9@cdc.gov) regarding what is being shipped and include the name and phone number and email address of the shipper.

<http://www.cdc.gov/ncird/downloads/patient-summary-form.pdf>

<http://www.cdc.gov/laboratory/specimen-submission/pdf/form-50-34.pdf>