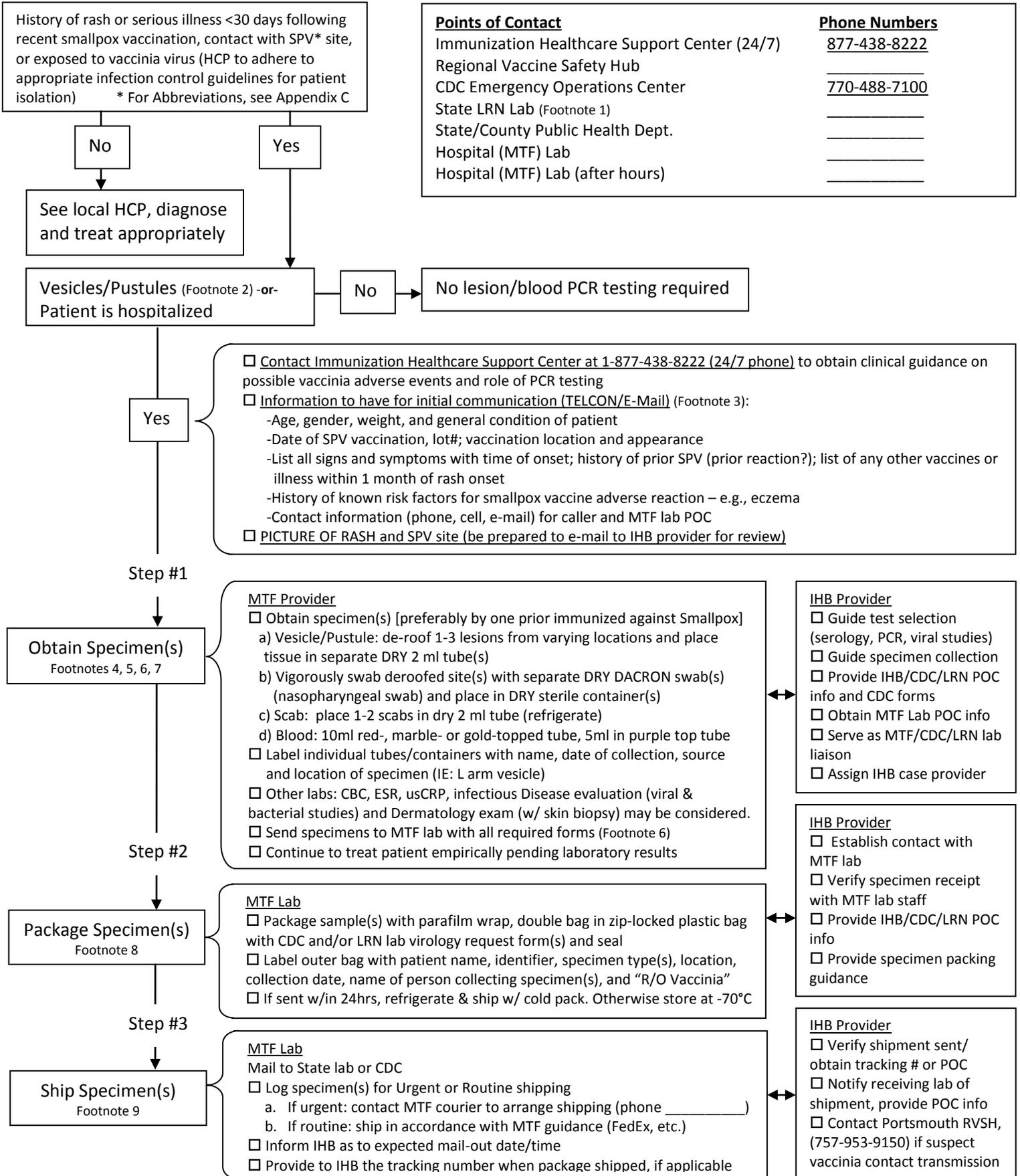


# Guideline for Obtaining Vaccinia Polymerase Chain Reaction (PCR) Assay



## **Footnotes**

1. State LRN lab contact info: <https://www.aphl.org/programs/preparedness/Crisis-Management/Pages/Emergency-Lab-Contacts.aspx>.
2. Rash description: (In addition to possible vaccine association, consider in the differential diagnoses other infectious, allergic, non-allergic, and immunologic/autoimmune causes.
  - A) Fine flat red itchy rash (differential dx includes, but not limited to: Non-viral pustulosis, folliculitis, erythema multiforme, heat rash, contact dermatitis):
    - (1) If confined to or around the vaccination site – send picture w/consult request to IHB. Do not apply topical medication.
    - (2) If diffuse body rash – send pictures w/consult request to IHB, describe additional systemic signs and/or symptoms.
  - B) Maculopapular, vesicular, or pustular rash (differential dx includes but not limited to: contact transmission/autoinoculation, non-viral pustulosis, contact dermatitis, erythema multiforme, eczema vaccinatum, eczema herpeticum, herpes simplex, chicken pox, progressive vaccinia, smallpox)
    - (1) Take multiple pictures and send to IHB for review
    - (2) Be prepared to activate this diagnostic testing algorithm
3. If Vaccinia Immune Globulin (VIGIV) is a possible therapeutic option, provide the patient's weight, the total dose required (@ 6,000 U/kg), a mailing address, and a physician or pharmacist POC at the hospital. (See IHB Vaccinia Immune Globulin Intravenous (Human) (VIGIV)).
4. Collection:
  - A) Materials
    - (1) Dacron or rayon swab placed in a tube (dry) after swabbing the lesion. Virus Transport Media tubes can be used, but dry swabs are preferred.
    - (2) Dacron or rayon swab placed in a tube (dry) after swabbing the oropharynx. Virus Transport Media tubes can be used, but dry swabs are preferred.
    - (3) 1 scalpel, sterile 26-gauge needle to derroof lesion and place collected skin specimen in separate dry container.
    - (4) 10cc glass red-, marble- or gold-topped serum separator tube for serum for IgG and IgM analysis.
    - (5) 5 cc purple topped tube (w/ EDTA, plastic tube preferable) for whole blood for PCR analysis.
  - B) Technique for macular, papular, vesicular, or pustular lesions
    - (1) Sanitize skin with an alcohol wipe, allow to dry.
    - (2) Use scalpel (or a sterile 26-gauge needle) to open, and remove, the top of the vesicle or pustule. Do not send the scalpel or sharp. Dispose of in appropriate biohazard container and dispenser.
    - (3) Place the skin of the vesicle top into a 1.5- to 2-mL sterile screw-capped plastic tube. Leave the material dry.
    - (4) Swab the base of the lesion with a polyester or Dacron swab and place in a screw-capped plastic vial, break off swab handle and screw on lid. **Do not add transport medium to the vial.**
  - C) Blood samples
    - (1) Obtain a serum sample. Collect 7 to 10 cc of patient blood into a marble-topped tube, or gold-topped serum separator tube. Spin samples to separate serum. Save the serum in at least two aliquots. Label tubes as acute serum, with other information listed in Specimen Labeling (e.g., case ID number, date of collection).
    - (2) Obtain whole blood sample for PCR. Collect 3 to 5 cc of blood into a purple-topped tube. Gently mix blood with anticoagulant in tube to prevent clotting. Label tube as whole blood, with other information listed in Specimen Labeling (e.g., case ID number, date of collection). Whole blood for PCR is appropriate for suspected acute systemic infection or progressive vaccinia.
5. Only vaccinated personnel wearing appropriate barrier protection (gloves, gown, and shoe covers) should be involved in specimen collection for suspected cases of generalized vaccinia, eczema vaccinatum, or progressive vaccinia. Respiratory protection is not needed. Masks and eyewear or face shields should be used if splashing is

anticipated. If unvaccinated personnel must be utilized to collect specimens, only those without contraindications to vaccination should be utilized. Fit-tested N95 masks should be worn by unvaccinated individuals caring for suspected patients.

#### 6. Specimen form(s) and collection

- A) MTF-specific mail-out (specimen processing) form(s) (if any).
- B) CDC specimen submission form: See [Appendix A](#)
- C) If using State LRN lab, contact lab for their specific submission form (if different from CDC form) (<https://www.aphl.org/programs/preparedness/Crisis-Management/Pages/Emergency-Lab-Contacts.aspx>)
- D) CDC specimen transport guidelines: See [Appendix B](#) (or [www.bt.cdc.gov/agent/smallpox/response-plan/files/guide-d.pdf](http://www.bt.cdc.gov/agent/smallpox/response-plan/files/guide-d.pdf) or <http://www.bt.cdc.gov/agent/smallpox/lab-testing>)

7. Pathogen (viral, bacterial, other) studies should be chosen based upon Infectious Disease and/or Dermatology consultation. IHB staff can assist as well. Pathogens may include, but are not limited to:

- a) Viral: herpes 1/2, EBV, coxsackievirus, parvovirus, rubella, rubeola, varicella (including zoster), HIV
- b) Bacterial: streptococcus, staphylococcus, pseudomonas, rickettsiae, meningococcus
- c) Other: candida, tinea, scabies

#### 8. Packaging guidance

See [Appendix B](#).

Detailed packaging guidance can be found at <http://www.asm.org/images/pdf/Clinical/pack-ship-7-15-2011.pdf>

#### 9. Shipping guidance

See [Appendix B](#).

- A) Separate any serum IAW GLP standards. Freezing of a serum specimen is preferred, but if sent immediately, can be shipped with a cold pack. Otherwise, transfer to an ultra-cold freezer for storage at -70°C until it can be shipped.
- B) Pack all items (swabs & blood vials) in the facility's standard mail out/specimen shipping container(s) IAW established standards.
- C) Ship container(s) via FEDEX account to:
  - Centers for Disease Control and Prevention
  - ATTN: STAT Lab / Poxvirus Program
  - 1600 Clifton Road NE, MS-G12
  - Atlanta, GA 30333
- D) IHB provider calls the CDC's general poxvirus line (404 639 4129) to alert them that a specimen shipment is on its way and provide them the container(s) tracking number(s).
- E) If require more rapid PCR turn around than shipping to the CDC, consider obtaining vaccinia PCR from your local DCLS State Lab (usually within 24 hrs.). See footnote #1 for State LRN Lab contact information.

**APPENDIX A**

Reset Form  
Print Form

**Poxvirus Human Specimen Submission Form**

Ship specimens to:  
Centers for Disease Control and Prevention  
ATTN: STAT Laboratory / Poxvirus Program  
1600 Clifton Road NE, MS G-12  
Atlanta, Georgia 30333  
Phone: 404.639.4129 Fax: 404.639.1060

CASE NUMBER (Poxvirus lab only):  
  
DATE RECEIVED:

Consultation with your state communicable disease unit / health laboratory is necessary **BEFORE** submission of specimens to CDC. Visit [www.cdc.org](http://www.cdc.org) and [www.aphis.org](http://www.aphis.org) for listings of state epidemiologists and state laboratories.

*Please remit one copy of the form with shipment of specimens.*

FORM COMPLETED BY: \_\_\_\_\_ DATE FORM COMPLETED: \_\_\_\_\_  
(mm/dd/yyyy)

PROVIDER (Submitted by):	INVESTIGATOR (State contact):
NAME: _____	NAME: _____
ORGANIZATION: _____	ORGANIZATION: _____
ADDRESS: _____	ADDRESS: _____
CITY, STATE: _____	CITY, STATE: _____
ZIP CODE: _____ COUNTRY: _____	ZIP CODE: _____ COUNTRY: _____
TELEPHONE 1: _____ FAX: _____	TELEPHONE 1: _____ FAX: _____
EMAIL: _____	EMAIL: _____

AGENT BEING TESTED FOR:

<input type="checkbox"/> ORTHOPOXVIRUS	<input type="checkbox"/> PARAPOXVIRUS	<input type="checkbox"/> TANAPOX	<input type="checkbox"/> HERPES VIRUS	<input type="checkbox"/> OTHER, SPECIFY: _____
<input type="checkbox"/> COWPOX	<input type="checkbox"/> BPSV	<input type="checkbox"/> OTHER POXVIRUS: _____	<input type="checkbox"/> VARICELLA	
<input type="checkbox"/> MONKEYPOX	<input type="checkbox"/> ORF		<input type="checkbox"/> HSV-1 or HSV-2	
<input type="checkbox"/> VACCINA	<input type="checkbox"/> PSEUDOCOWPOX			
<input type="checkbox"/> VARIOLA	<input type="checkbox"/> SEALPOX			

*Testing for herpes viruses is performed by the Measles, Mumps, Rubella, and Herpes Viruses Branch.  
Testing for poxviruses in formalin fixed or paraffin embedded specimens is performed by the Infectious Disease Pathology Branch.  
Serum must be submitted with cerebral spinal fluid when testing for post-vaccinal meningitis or encephalitis.*

1. PATIENT NAME: _____ <small>(last, first MI)</small>		2. DATE OF BIRTH: _____ <small>(mm/dd/yyyy)</small>		3. AGE (if DOB unk): _____	
4. SEX: <input type="checkbox"/> Female <input type="checkbox"/> Male <input type="checkbox"/> Unknown		5. DATE IDENTIFIED: _____ <small>(mm/dd/yyyy)</small>		6. STATE ID NUMBER: _____	
7. CITY, STATE: _____		8. ZIP CODE: _____		9. COUNTY: _____	
				10. COUNTRY (IF NOT USA): _____	
11. HAS THIS PATIENT BEEN HOSPITALIZED BECAUSE OF ILLNESS?				<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
				Hospital ID number: _____	
12. WHAT WAS THE CLINICAL OUTCOME FOR THIS PATIENT?				<input type="checkbox"/> Recovering <input type="checkbox"/> Recovered <input type="checkbox"/> Died <input type="checkbox"/> Unknown	
				Date of death: _____ <small>(mm/dd/yyyy)</small>	
13. DID THIS PATIENT EXPERIENCE A FEVER AS PART OF THEIR ILLNESS?				<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
				Fever onset date: _____ <small>(mm/dd/yyyy)</small>	



PLEASE COMPLETE ONE SPECIMEN SECTION PER SPECIMEN SUBMITTED  
(E.G., IF THREE SPECIMENS ARE SUBMITTED THEN THREE SPECIMEN SECTIONS SHOULD BE COMPLETED)

**SPECIMEN SECTION 1**      **DATE SPECIMEN COLLECTED:** \_\_\_\_\_  
*(month/year)*

<b>SPECIMEN MATERIAL:</b>		<b>COLLECTION METHOD:</b>		<b>DASH NUMBER:</b>
<input type="checkbox"/> BLOOD	<input type="checkbox"/> PUSTULE SKIN	<input type="checkbox"/> SWAB	<input type="checkbox"/> EM GRID	
<input type="checkbox"/> SERUM	<input type="checkbox"/> PUSTULE FLUID	<input type="checkbox"/> SLIDE ( <i>touch prep, smear, etc.</i> )	<input type="checkbox"/> SLIDE ( <i>formalin fixed</i> )	
<input type="checkbox"/> PLASMA	<input type="checkbox"/> VESICLE SKIN	<input type="checkbox"/> FRESH FROZEN TISSUE	<input type="checkbox"/> FORMALIN FIXED TISSUE	
<input type="checkbox"/> CSF	<input type="checkbox"/> VESICLE FLUID	<input type="checkbox"/> CONTAINER	<input type="checkbox"/> PARAFFIN BLOCK	
<input type="checkbox"/> CRUST	<input type="checkbox"/> BIOPSY	<input type="checkbox"/> BLOOD TUBE TYPE ( <i>blue red marble, pink, etc.</i> ) _____		
<input type="checkbox"/> OTHER _____		<input type="checkbox"/> OTHER COLLECTION TYPE _____		

ADDITIONAL SPECIMEN INFORMATION: \_\_\_\_\_

**SPECIMEN SECTION 2**      **DATE SPECIMEN COLLECTED:** \_\_\_\_\_  
*(month/year)*

<b>SPECIMEN MATERIAL:</b>		<b>COLLECTION METHOD:</b>		<b>DASH NUMBER:</b>
<input type="checkbox"/> BLOOD	<input type="checkbox"/> PUSTULE SKIN	<input type="checkbox"/> SWAB	<input type="checkbox"/> EM GRID	
<input type="checkbox"/> SERUM	<input type="checkbox"/> PUSTULE FLUID	<input type="checkbox"/> SLIDE ( <i>touch prep, smear, etc.</i> )	<input type="checkbox"/> SLIDE ( <i>formalin fixed</i> )	
<input type="checkbox"/> PLASMA	<input type="checkbox"/> VESICLE SKIN	<input type="checkbox"/> FRESH FROZEN TISSUE	<input type="checkbox"/> FORMALIN FIXED TISSUE	
<input type="checkbox"/> CSF	<input type="checkbox"/> VESICLE FLUID	<input type="checkbox"/> CONTAINER	<input type="checkbox"/> PARAFFIN BLOCK	
<input type="checkbox"/> CRUST	<input type="checkbox"/> BIOPSY	<input type="checkbox"/> BLOOD TUBE TYPE ( <i>blue red marble, pink, etc.</i> ) _____		
<input type="checkbox"/> OTHER _____		<input type="checkbox"/> OTHER COLLECTION TYPE _____		

ADDITIONAL SPECIMEN INFORMATION: \_\_\_\_\_

**SPECIMEN SECTION 3**      **DATE SPECIMEN COLLECTED:** \_\_\_\_\_  
*(month/year)*

<b>SPECIMEN MATERIAL:</b>		<b>COLLECTION METHOD:</b>		<b>DASH NUMBER:</b>
<input type="checkbox"/> BLOOD	<input type="checkbox"/> PUSTULE SKIN	<input type="checkbox"/> SWAB	<input type="checkbox"/> EM GRID	
<input type="checkbox"/> SERUM	<input type="checkbox"/> PUSTULE FLUID	<input type="checkbox"/> SLIDE ( <i>touch prep, smear, etc.</i> )	<input type="checkbox"/> SLIDE ( <i>formalin fixed</i> )	
<input type="checkbox"/> PLASMA	<input type="checkbox"/> VESICLE SKIN	<input type="checkbox"/> FRESH FROZEN TISSUE	<input type="checkbox"/> FORMALIN FIXED TISSUE	
<input type="checkbox"/> CSF	<input type="checkbox"/> VESICLE FLUID	<input type="checkbox"/> CONTAINER	<input type="checkbox"/> PARAFFIN BLOCK	
<input type="checkbox"/> CRUST	<input type="checkbox"/> BIOPSY	<input type="checkbox"/> BLOOD TUBE TYPE ( <i>blue red marble, pink, etc.</i> ) _____		
<input type="checkbox"/> OTHER _____		<input type="checkbox"/> OTHER COLLECTION TYPE _____		

ADDITIONAL SPECIMEN INFORMATION: \_\_\_\_\_

**SPECIMEN SECTION 4**      **DATE SPECIMEN COLLECTED:** \_\_\_\_\_  
*(month/year)*

<b>SPECIMEN MATERIAL:</b>		<b>COLLECTION METHOD:</b>		<b>DASH NUMBER:</b>
<input type="checkbox"/> BLOOD	<input type="checkbox"/> PUSTULE SKIN	<input type="checkbox"/> SWAB	<input type="checkbox"/> EM GRID	
<input type="checkbox"/> SERUM	<input type="checkbox"/> PUSTULE FLUID	<input type="checkbox"/> SLIDE ( <i>touch prep, smear, etc.</i> )	<input type="checkbox"/> SLIDE ( <i>formalin fixed</i> )	
<input type="checkbox"/> PLASMA	<input type="checkbox"/> VESICLE SKIN	<input type="checkbox"/> FRESH FROZEN TISSUE	<input type="checkbox"/> FORMALIN FIXED TISSUE	
<input type="checkbox"/> CSF	<input type="checkbox"/> VESICLE FLUID	<input type="checkbox"/> CONTAINER	<input type="checkbox"/> PARAFFIN BLOCK	
<input type="checkbox"/> CRUST	<input type="checkbox"/> BIOPSY	<input type="checkbox"/> BLOOD TUBE TYPE ( <i>blue red marble, pink, etc.</i> ) _____		
<input type="checkbox"/> OTHER _____		<input type="checkbox"/> OTHER COLLECTION TYPE _____		

ADDITIONAL SPECIMEN INFORMATION: \_\_\_\_\_

## **APPENDIX B**

### **Packaging and Shipping Diagnostic Specimens**

Diagnostic specimens are shipped under hazardous material guidelines of the US DOT 49 Code of Federal Regulations (CFR) Parts 171-180 and assigned UN 3373 under IATA regulations.

#### **1. What is considered a diagnostic specimen?**

- A diagnostic specimen is any human or animal material being transported for diagnostic or investigational purposes, but excluding live infected animals.

#### **2. How do you package diagnostic specimens?**

##### **PRIMARY PACKAGING**

- Primary receptacle(s) must be water tight, e.g., screw cap seal with parafilm or adhesive tape or similar positive means to prevent the cap from loosening.
- Multiple primary receptacles must be wrapped individually to prevent breakage.
- When determining the volume of diagnostic specimens being shipped, include the viral transport media.
- Primary receptacle(s) must not contain more than 500 ml or 500 g.
- The entire contents of the primary receptacle are the diagnostic specimen.

##### **SECONDARY PACKAGING**

- Use enough absorbent material in the secondary container to absorb the entire contents of all primary receptacles in case of leakage or damage.
- Secondary packaging must meet the IATA packaging requirements for diagnostic specimens including 3.9 foot drop test procedure. Since infectious substance packaging surpasses the requirements for diagnostic specimen packaging, in the IATA Packing Instruction 602, it can be used.
- Infectious substance packaging will have the required specification markings on packaging (“UN” will be in a circle), for example:



- Secondary packaging must be watertight. Follow the packaging manufacturer or other authorized party's packing instructions included with the secondary packaging.
- Secondary packaging must be at least 4 inches in the smallest overall external dimension.
- Must be large enough for shipping documents, e.g., air waybill.

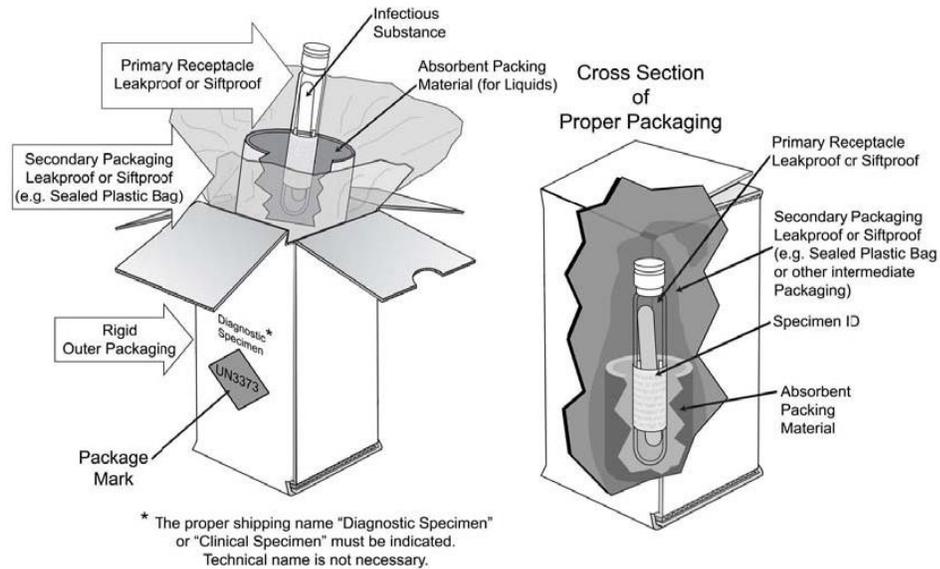
##### **OUTER PACKAGING**

- An overpack is used if the secondary packaging is not large enough for all the labels, markings, and documents OR if cold packs or dry ice is used.
- The outer packaging must not contain more than 4 L or 4 kg.
- Both dry ice and cold packs must be placed outside the secondary packaging.
- Dry ice packaging must permit the release of carbon dioxide gas and not allow a build-up of pressure that could rupture the packaging.
- Cold pack packaging must be leak-proof.
- Each package and the air waybill must be marked with the following text (exact wording):

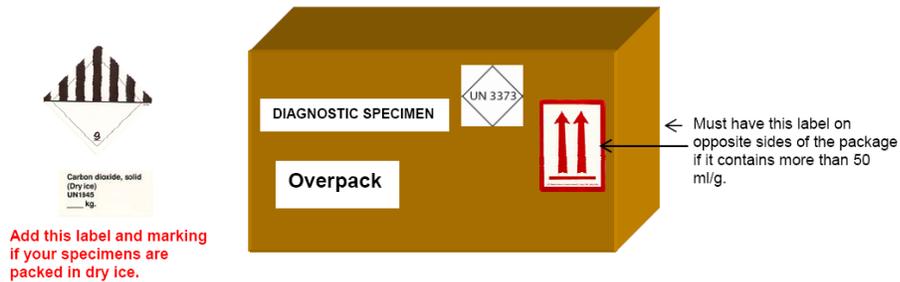


- An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. Place in a sealed plastic bag to protect from moisture.
- If overpack used, package must be marked “Overpack.” All secondary package markings must be on the overpack.
- Name, address, & telephone number of the responsible person must be on the package & the air waybill.
- You must put the words “DIAGNOSTIC SPECIMENS” or “CLINICAL SPECIMENS” and “UN 3373” in the “Nature and Quantity of Goods” box on the air waybill.
- A Shipper's Declaration for Dangerous Goods is **NOT** required.

## Packing and labeling of diagnostic specimens



### Example of outer packaging (overpack) for diagnostic specimens



### 3. What should be included with the specimens?

- The Poxvirus human or animal submission form which has been completed and is legible and any additional case history.

### 4. Where should specimens be shipped?

- Specimens for the CDC should be shipped to the following address:

Centers for Disease Control and Prevention  
ATTN: STAT Lab / Poxvirus Program  
1600 Clifton Road NE, MS-G12  
Atlanta, GA 30333

### 5. Do I need permits for shipping diagnostic specimens?

- The usage of permits for shipping should be evaluated on a case-by-case basis.
- The recipient of the shipment must hold the USDA permit.
- The shipper must hold the PHS permit.
- Permits may be necessary when shipping diagnostic specimens within the United States.
- Permits are required when shipping to the U.S. from foreign countries (contact the CDC Poxvirus lab).

## **APPENDIX C**

### **Glossary/Abbreviations**

CBC = Complete Blood Count  
CDC = Centers of Disease Control and Prevention  
CFR = Code of Federal Regulations  
usCRP = Ultra-Sensitive C-Reactive Protein  
DCLS = Division of Consolidated Laboratory Services  
EBV = Epstein Barr Virus  
EDTA = Ethylenediaminetetraacetic acid  
ESR = Erythrocyte Sedimentation Rate  
GLP = Good Laboratory Practice  
HCP = Healthcare Provider  
HIV = Human Immunodeficiency Virus  
IATA = International Air Transport Association  
IAW = In accordance with  
ID = Identification  
IHB = Defense Health Agency's Immunization Healthcare Branch  
LRN = Laboratory Response Network  
MTF = (Military) Medical Treatment Facility  
PCR = Polymerase Chain Reaction  
PHS = Public Health Service  
POC = Point of Contact  
R/O = Rule Out (Exclude)  
RVSH = Regional Vaccine Safety Hub (San Diego, San Antonio, Ft. Bragg, National Capital Region)  
SPV = Smallpox vaccine/vaccination  
TELCON = Telephonic Communication  
USDA = United States Department of Agriculture  
VIGIV = Vaccinia Immune Globulin