Guideline for Obtaining Vaccinia Polymerase Chain Reaction (PCR) Assay

**Step #1**
- History of rash or serious illness <30 days following recent smallpox vaccination, contact with SPV* site, or exposed to vaccinia virus (HCP to adhere to appropriate infection control guidelines for patient isolation) * For Abbreviations, see Appendix C
  - No
  - Yes
    - See local HCP, diagnose and treat appropriately

**Points of Contact**
- Immunization Healthcare Support Center (24/7)
- Regional Vaccine Safety Hub
- CDC Emergency Operations Center
- State LRN Lab (Footnote 1)
- State/County Public Health Dept.
- Hospital (MTF) Lab
- Hospital (MTF) Lab (after hours)
- Phone Numbers 877-438-8222

**Step #2**
- Yes
  - Obtain Specimen(s)
    - Footnotes 4, 5, 6, 7

**IHB Provider**
- Guide test selection (serology, PCR, viral studies)
- Guide specimen collection
- Provide IHB/CDC/LRN POC info and CDC forms
- Obtain MTF Lab POC info
- Serve as MTF/CDC/LRN lab liaison
- Assign IHB case provider

**Step #3**
- Yes
- Package Specimen(s)
  - Footnote 8

**MTF Lab**
- Package sample(s) with parafilm wrap, double bag in zip-locked plastic bag with CDC and/or LRN lab virology request form(s) and seal
- Label outer bag with patient name, identifier, specimen type(s), location, collection date, name of person collecting specimen(s), and “R/O Vaccinia”
- If sent w/in 24hrs, refrigerate & ship w/ cold pack. Otherwise store at -70°C

**MTF Provider**
- Obtain specimen(s) [preferably by one prior immunized against Smallpox]
  - a. Vesicles/Pustule: de-roof 1-3 lesions from varying locations and place tissue in separate DRY 2 ml tube(s)
  - b. Vigorously swab deroofed site(s) with separate DRY DACRON swab(s) (nasopharyngeal swab) and place in DRY sterile container(s)
  - c. Scab: place 1-2 scabs in dry 2 ml tube (refrigerate)
  - d. Blood: 10ml red-, marble- or gold-topped tube, 5ml in purple top tube
- Label individual tubes/containers with name, date of collection, source and location of specimen (IE: L arm vesicle)
- Other labs: CBC, ESR, usCRP, infectious Disease evaluation (viral & bacterial studies) and Dermatology exam (w/ skin biopsy) may be considered.
- Send specimens to MTF lab with all required forms (Footnote 6)
- Continue to treat patient empirically pending laboratory results

**Step #4**
- Yes
- Ship Specimen(s)
  - Footnote 9

**MTF Lab**
- Mail to State lab or CDC
- Log specimen(s) for Urgent or Routine shipping
  - a. If urgent: contact MTF courier to arrange shipping (phone __________ )
  - b. If routine: ship in accordance with MTF guidance (FedEx, etc.)
- Inform IHB as to expected mail-out date/time
- Provide to IHB the tracking number when package shipped, if applicable

**IHB Provider**
- Establish contact with MTF lab
- Verify specimen receipt with MTF lab staff
- Provide IHB/CDC/LRN POC info
- Provide specimen packing guidance

**IHB Provider**
- Verify shipment sent/obtain tracking # or POC
- Notify receiving lab of shipment, provide POC info
- Contact Portsmouth RVSH, (757-953-9150) if suspect vaccinia contact transmission

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 deroof 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)

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.

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

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

Consultation with your state communicable disease unit / health laboratory is necessary BEFORE submission of specimens to CDC. Visit www.cdc.gov and www.aph.gov for listings of state epidemiologists and state laboratories.

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

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AGENT BEING TESTED FOR:
- ORTHOPOXVIRUS
- PARAPOXVIRUS
- TANAPOX
- HERPESVIRUS
- VARICELLA
- COWPOX
- MONKEYPOX
- ORF
- OTHER POXVIRUS
- HSV-1 or HSV-2
- VACCINA
- VARIOLA
- 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 cerebrospinal fluid when testing for post-vaccinal meningitis or encephalitis.

1. PATIENT NAME: ____________ 2. DATE OF BIRTH: ____________ 3. AGE (if DOB unit): ______
4. SEX: [ ] Female [ ] Male [ ] Unknown
5. DATE IDENTIFIED: ____________ 6. STATE ID NUMBER: ____________

11. HAS THIS PATIENT BEEN HOSPITALIZED BECAUSE OF ILLNESS? [ ] Yes [ ] No [ ] Unknown
12. WHAT WAS THE CLINICAL OUTCOME FOR THIS PATIENT? [ ] Recovering [ ] Recovered [ ] Died [ ] Unknown
13. DID THIS PATIENT EXPERIENCE A FEVER AS PART OF THEIR ILLNESS? [ ] Yes [ ] No [ ] Unknown

Page 1 of 3  Last modified: April 2011
14. **DID THE PATIENT EXPERIENCE A RASH AS PART OF THEIR ILLNESS?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown
   - **Rash onset date:**

**RASH TYPE:**
- [ ] MACULES (discolored and flat)
- [ ] PAPULES (raised and raised)
- [ ] VESICLES (raised and fluid filled)
- [ ] PUSTULES (raised and pus filled)
- [ ] OTHER (describe, nodules, etc.)

**APPROX #**

**LOCATION**

15. **HAS THIS PATIENT TRAVELED INTERNATIONALLY WITHIN ONE MONTH PRIOR TO ILLNESS ONSET?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

   **If yes, where:**

16. **HAS THIS PATIENT BEEN IN CONTACT WITH PERSON(S) RECENTLY VACCINATED AGAINST SMALLPOX?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

   **If yes, with who:**

17. **HAS THIS PATIENT BEEN IN CONTACT WITH ANIMAL(S) WITHIN ONE MONTH PRIOR TO ILLNESS ONSET?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

   **If yes, with what animals and where:**

18. **DOES THIS PATIENT WORK WITH POXVIRUSES IN A LABORATORY SETTING?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

   **If yes, what strain(s):**

19. **HAS THE PATIENT EVER BEEN VACCINATED WITH THE SMALLPOX VACCINE (VACOVIA VIRUS)?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

20. **IF YES, LIST DATES (if exact date unknown, indicate year):**
   - **Date:**
   - **Year:**

21. **DOES THIS PATIENT HAVE A SCAR FROM SMALLPOX VACCINATION?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

22. **HAS THIS PATIENT EVER BEEN INFECTED WITH SMALLPOX?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

23. **HAS THIS PATIENT BEEN RECENTLY EXPOSED TO SMALLPOX?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

24. **DOES THIS PATIENT HAVE A PREVIOUS HISTORY OF CHICKENPOX?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

25. **WAS THIS PATIENT EVER PREVIOUSLY VACCINATED AGAINST CHICKENPOX?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

26. **HAS THIS PATIENT BEEN RECENTLY EXPOSED TO CHICKENPOX?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

27. **HAS THIS PATIENT TAKEN ANY STEROIDS/IMMUNOSUPPRESSANT DRUGS ONE MONTH PRIOR TO RASH ONSET?**
   - [ ] Yes
   - [ ] No
   - [ ] Unknown

28. **PLEASE NOTE ANY ADDITIONAL CLINICAL OBSERVATIONS OR MEDICAL HISTORY NOT PREVIOUSLY COVERED.**

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ADDITIONAL SPECIMEN INFORMATION:
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:
     \[
     \text{UN 3373}
     \]
   - 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):
     \[
     \text{DIAGNOSTIC SPECIMENS}
     \]
   - 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

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).
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

Revised: 09 January 2017