

Louisiana Office of Public Health Laboratories	
Test Name	Acute, Generalized Vesicular or Pustular Rash Illness Testing
PHL Location	Office of Public Health Laboratory Baton Rouge
CPT Code	N/A
Synonyms	BT Test, Bioterrorism Test, Clinical Rule Out, Orthopox, Smallpox, VZV
Brief Description of Test	<p>If smallpox is suspected. Contact Infectious Disease Epidemiology at 800-256-2748 or BT Program Advisor at 225-219-5241.</p> <p>Testing consists of multiple Laboratory Response Network (LRN) procedures designed to rule in or rule out smallpox.</p>
Possible Results	<p>Possible results will depend on approved testing but may include:</p> <ul style="list-style-type: none"> • Cannot rule out Smallpox – Sent to CDC for further testing • Smallpox ruled out – Consider other diagnostic testing
Reference Range	Negative – smallpox ruled out
Specimen Type	<p>Direct Sample – clinical specimens require prior approval</p> <p>Vesicular Material Scab Specimens Biopsy Lesions Serum Autopsy Specimens</p>
Specimen Container(s):	Specimen must be transported in “triple” packaging (primary receptacle, water tight secondary packaging and durable outer packaging) required for a biological agent of human disease.
Minimum volume accepted:	See collection instructions below
Collection Instructions	<p>Safety Considerations</p> <p>Only recently, successfully vaccinated personnel (within 3 years) wearing appropriate barrier protection (gloves, gown, and shoe covers) should be involved in specimen collection for suspected cases of smallpox. Respiratory protection is not needed for personnel with recent, successful vaccination. Masks and eyewear or face shields should be used if splashing is anticipated.</p> <p>If unvaccinated personnel must be utilized to collect specimens, only those without contraindications to vaccination should be utilized as they would require immediate vaccination if the diagnosis of smallpox is confirmed. Fit-tested N95 masks should be worn by unvaccinated individuals caring for suspected patients.</p>

All manipulations of unfixed material must be carried out within a Class III Biological Safety Cabinet, or within a Class II Biological Safety Cabinet while using BSL-3 practices and safety equipment.

All procedures involving handling potentially infectious material should be performed in laboratories utilizing Biosafety Level 2 or 3 practices. Areas of the skin known to have come in contact with variola (or monkeypox) virus should be washed with soap and decontaminated with 0.5% sodium hypochlorite with at least a 1 min contact time. Administration of smallpox vaccination, and possibly Variola immune globulin (VIG) should be determined in coordination with CDC.

Specimen Collection

Vesicular Material

- a. Sanitize the patient's skin with an alcohol wipe and allow skin to dry.
- b. Open the top of a vesicle or pustule with a scalpel, sterile 26-gauge needle, or slide. Collect the skin of the vesicle top in a dry, sterile 1.5- to 2-mL screw-capped tube. Label the tube.
- c. Scrape the base of the vesicle or pustule with the wooden end of an applicator stick or swab and smear the scrapings onto a glass or plastic light microscope slide. Allow slide to dry for 10 minutes.
- d. Label the slide and place it in a slide holder. To prevent cross-contamination, do not place slides from more than one patient in the same slide holder.
- e. Take another slide, and touch it repetitively to the opened lesion using progressive movements of the slide in order to make a touch prep. Allow slide to dry for 10 minutes.
- f. Label the slides as touch preps and place in the same slide holder. To prevent cross contamination, do not place slides from more than one patient in the same slide holder. Parafilm may be used to wrap the slide holder to prevent accidental opening.
- g. If plastic-coated electron microscopic (EM) grids are available, lightly touch the shiny side of 3 EM grids to the base of the open lesion, allow EM grids to air-dry for 10 minutes, and place grids in an appropriately labeled grid box. Use varying degree of pressure (minimal, light, and moderately firm) in application of the 3 grids to the unroofed lesion. If a slide or EM grid is not available, swab the base of the lesion with a polyester or cotton swab, place in screw-capped plastic vial, break off applicator handle, and seal.
- h. Repeat this procedure for 2 or more lesions.

Scab Specimens

- a. Sanitize the patient's skin with an alcohol wipe and allow skin to dry.
- b. Use a 26-gauge needle to remove 2 to 4 scabs.
- c. Place 1 or 2 scabs in each of 2 dry, sterile screw-capped plastic tubes.
- d. Wrap parafilm around the juncture of the cap and vial.

e. Label the tube.

Biopsy Lesions (At least 2 specimens obtained by using a 3.5- or 4-mm punch biopsy kit.)

- a. Use sterile technique and appropriate anesthetic.
- b. Place 1 sample in formalin for immunohistochemical or histopathologic evaluation and store at room temperature.
- c. The second specimen should be placed dry (do not add transport medium) in a sterile 1.5- to 2-mL screw-capped container (do not add transport medium).
- d. Refrigerate if shipment occurs within 24 hours; otherwise, the specimen should be frozen.

Serum

- a. 10 ml of blood into a plastic marble-topped tube, or a plastic yellow-topped serum separator tube. If plastic tubes are not available, draw blood into a glass marble-topped or yellow-topped serum separator tube and place labeled tube into a Styrofoam protector for packaging and shipping.
- b. If possible, centrifuge to separate serum and decant before shipping. Store and ship at refrigeration temperature.

Autopsy specimens

- a. Autopsy specimens for virus isolation should be frozen (shipped with dry ice), including portions of skin containing lesions, liver, spleen, lung, lymph nodes, and/or kidney
- b. Formalin-fixed tissue is suitable for histopathology, immunohistochemistry and PCR but should not be frozen and must be packaged separately from autopsy specimens for virus isolation (which must be frozen). All major organs (liver, spleen, skin lung, lymph nodes, and/or kidney) should be adequately sampled and submitted for evaluation.

Packaging and Labeling

Package such that each sample is kept separate and is individually labeled, contained in its own bag/container and labeled with a unique identifier.

Decontamination of laboratory surfaces is easily accomplished using a fresh solution of 10% bleach.

Label specimen with Patient Name and a 2nd Unique Identifier such as a chart number or medical record number. DOB is not considered unique.

Complete a LAB Form 96 to accompany the sample. Lab submission form must be thoroughly completed with patient's first and last name, 2nd patient identifier, gender, date of birth, date and time of collection, specimen source, test requested, submitter's name,

	<p>address, fax and contact number. Additional information regarding patients' address is requested.</p> <p>The same two unique identifiers MUST be recorded on the tube AND the Lab 96 form.</p> <p>Transport specimen to laboratory as soon as possible after collection/incubation. Keep submission forms insulated from specimens.</p>
<p>Storage and Transport Instructions</p>	<p>For storage and transport of clinical samples, contact BT Program Advisor at 225-219-5241.</p> <p>Send sample to the Office of Public Health Laboratory Baton Rouge, 1209 Leesville Avenue, Baton Rouge, LA 70802</p> <p>LRN (Laboratory Response Network) guidance for the packaging and shipping of infectious substances and biological agents should be consulted for recent changes. IATA and DOT publications continue to be revised frequently. Submitters should frequently and regularly consult IATA publications, the Federal Register, and the publications of other governing agencies for more complete instructions. It is the shipper's responsibility to ensure adherence to the most current regulations.</p> <p>Useful web sites that address the shipping of infectious substances and biological agents: International Air Transport Association: https://www.iata.org Department of Transportation: http://phmsa.dot.gov American Society for Microbiology: http://www.asm.org American Biological Safety Association: http://www.absa.org/ Animal and Plant Health Inspection Service: http://www.aphis.usda.gov/ Centers for Disease Control and Prevention: http://www.cdc.gov/od/ohs/</p>
<p>Causes for Rejection</p>	<ul style="list-style-type: none"> • Incorrect source • Incorrect labeling • Expired collection tubes • Not approved for testing by Infectious Disease Epidemiology
<p>Limitations of the Procedure</p>	<p>N/A</p>
<p>Interfering Substances</p>	<p>N/A</p>
<p>References</p>	<p>Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases – <i>Smallpox</i>. https://www.asm.org/images/PSAB/Smallpox_July2013.pdf</p> <p>Laboratory Response Network</p>

Additional Information	If <i>smallpox</i> is suspected, contact Infectious Disease Epidemiology at 1-800-256-2748 or BT Program Advisor at 225-219-5241.
Release Date	05/18/2018
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