

Smallpox Response Plan for State of Utah Public Health Laboratory

Revision 4/30/2004 Prepared by Dr. June Inez Pounder

i. SPECIMEN HANDLING

A. Specimen Collection Safety Procedures

1. Only personnel who have been successfully vaccinated against smallpox in the last 3 years, or non-vaccinated personnel with no contraindication to vaccination, should participate in specimen collection. Vaccination of non-vaccinated personnel should occur as soon as possible after specimen acquisition.

2. All procedures for obtaining, processing, packing, and shipping potentially infectious material should be performed by using BSL-2, or if available, BSL-3 practices. Appropriate respiratory and barrier protective equipment such as N-95 or HEPA-filtered mask, gloves, gown, shoe covers, and protective eyewear should be used in specimen collection for suspected cases of smallpox.

3. While working with specimens, laboratory personnel should avoid any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, or applying makeup.

4. After removing gloves, personnel should thoroughly wash their hands with antimicrobial soaps before leaving the laboratory. Areas of skin known or suspected to have come in contact with variola virus should be washed with the recommended soap, followed by 60-95% ethanol containing gel (available from Fisher Scientific (800)766-7000). If possible, skin should be decontaminated with 0.5% sodium hypochlorite for at least one minute.

B. Specimen Collection

The laboratory's test request form (Bioterrorism Test Request Form) is available at the end of this document, or at www.health.utah.gov/els/microbiology under Microbiology Client Services Manual.

Collection Kit Guidelines:

Most of the following items are available from Fisher Scientific (800)766-7000, VWR (800)932-5000, or ISC BioExpress (800)999-2901. Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the Utah Department of Health.

Personal Protective Equipment: gloves, gown, shoes cover, N95 mask, and eye protection

Alcohol wipes

Scalpel with a number 10 blade

26 gauge needle

Wooden applicator

1.5 to 2 ml screw capped plastic sample tubes

Snap capped plastic sample tubes
Formalin for fixed tissues (Sigma (800) 325-3010)
Clean glass microscope slides
50 ml plastic conical centrifuge tubes for holding glass slides or commercial slide holders
Polyester swabs
Permanent marking pens for labeling tubes
Marble-top vacutainer tube for collection of serum collection 10cc
Purple-top vacutainer tube for whole blood collection 5cc
Vacutainer collection supplies
Sharps containers
M4 transport medium (REMEL (800)255-6730)
Gauze

1. Label all tubes, vials, and microscope slide holders with patient's name, unique identifier, date of collection, source of specimen (vesicle, pustule, scab, or fluid), and name of person collecting sample.

2. Avoid cross-contamination of samples. Use one sample per primary container. Collect sufficient amount of lesion material to permit multiple diagnostic tests and confirmations. Suitable specimens for virologic tests of suspected smallpox cases are biopsy tissues, 2 to 4 scabs, and vesicular tissues and fluid. Serologic testing requires at least 1 ml of serum. Other potentially useful specimens for viral sampling are throat swabs and whole blood, however, quality-controlled diagnostic tests have not been completed for these specimens.

3. Biopsy specimens of individual lesions should be made with a 3.5 or 4.5 mm punch biopsy device. This should sample the entire lesion. The biopsied material should be bisected with sterile scissors or scalpel and placed into 2 labeled containers: one half of the sample should be placed in formalin for immunohistochemical or histopathological evaluation, and the container should be kept at room temperature; the other half of the lesion material should be placed in a dry, sterile 1.5 to 2 ml screw capped sample tube (do NOT add transport medium), and refrigerate the container if shipment occurs within 24 hours (if shipment will be longer than 1 day, freeze this sample).

4. Scabs should be removed by using a sterile 26 gauge needle and collected in a dry, sterile 1.5 to 2 ml screw capped sample tube.

5. Vesicular material should be sampled after the skin area has been sanitized with an alcohol wipe and allowed to dry. Unroof the lesion with a sterile 26 gauge needle or with a scalpel. Place the skin "roof" in a dry, sterile 1.5 to 2 ml screw capped sample tube. Scrape the base of the blister with a wooden applicator and smear the scrapings onto a clean glass light-microscope slide. Touch a clean glass light-microscope slide to the open lesion multiple times. If additional tests are required electron microscope slides will be provided. Lightly touch the shiny side of 1 or 2 plastic coated grids to the base of the open lesion. Allow slides and grids to air dry for approximately 5 minutes, then place in appropriate containers. Repeat this procedure for 2 or more lesions.

6. Autopsy specimens from major organs collected for virus isolation and immunohistochemical and histopathological evaluation include skin, spleen, lymph node, liver, lung, kidney, and heart. Specimens for virus isolation should be frozen. Specimens for immunohistochemical and histopathological evaluation should be fixed in formalin.

7. Blood should be drawn into a purple-topped tube for possible viral identification and into a marble-topped serum separator tube for serological testing.

8. A cotton or polyester swab should be used for sampling tonsillar tissue in the posterior pharynx. Collect the specimen as a throat swab. Break off the end of applicator into a 1.5 to 2 ml screw capped sample tube. Do not add transport medium.

9. Package each patient's lesion specimens separately to avoid cross-contamination. See the next section for sample transport information.

10. When specimen collection has been completed, all protective materials and sample collection materials must be double bagged in biohazard bags and autoclaved or incinerated.

11. Specimens for Variola virus testing are dried vesicular fluid on a microscope slide, vesicular tissue (skin from unroofed vesicle), swab of vesicular fluid. Specimens for Variola virus PCR should be dried vesicular fluid on slide, scab or swab material. To obtain a microscope slide preparation, unroof the scab with a sterile scalpel or a 26 gauge needle. Place the scab in a labeled plastic sample tube, do not add transport medium. Touch a clean glass microscope slide to the open lesion several times. Allow the slide to air dry and place in an appropriate labeled container. Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid. Specimens for PCR testing may be stored indefinitely and shipped at room temperature.

12. Specimens from cases that require testing to rule-in varicella zoster virus (VZV) may be analyzed by the direct fluorescent antibody (DFA) method. To collect specimens for the DFA test, unroof the vesicle using a 26 gauge needle. Scrub the base of the lesion vigorously enough with a sterile swab to collect lesion cells. Avoid contaminating the sample with blood. Place the swab in a sterile tube containing 1 to 2 ml of suitable transport medium (M4 viral transport medium) Keep swab moistened. Transport with a cold pack, do not freeze. Specimens for VZV PCR should be scab or swab material. Collect by lifting the scab using the beveled point of a sterile 26 gauge needle and place in a snap cap tube or other suitable container. Do not add transport medium. Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid. Specimens for PCR testing, may be stored indefinitely and shipped at room temperature.

13. Specimens that require testing to rule-out Vaccinia or monkeypox virus will be analyzed by PCR. To obtain a microscope slide preparation, unroof the scab with a sterile scalpel or a 26 gauge needle. Place the scab in a labeled plastic sample tube. Touch a clean glass microscope slide to the open lesion several times. Allow the slide to air dry and place in an appropriate labeled container.

C. Shipping

1. Procedures have been established to contact the CDC Emergency Duty Officer, and the Rapid Response and Advanced Technology Laboratory staff, and the FBI Hazardous Materials Response Unit to coordinate transport of suspected smallpox specimens to the appropriate testing facility. The State of Utah Public Health Laboratory staff have been trained in safe packaging and shipping protocols for smallpox. Chain of custody procedures and secure containment to maintain specimens as evidence are in place at the state lab. Specimens will be packed using the “triple” packaging scheme of primary receptacle, water-tight secondary packaging and durable outer packaging. Adequate absorbent material will be packed with the specimens to contain all fluids. A rigid, crush-proof overpack will be used. No transport medium or glycerol will be added to the specimens. Formalin-fixed, electron microscopy grids, and PCR specimens will be stored and shipped at room temperature, not frozen. These specimens should not be packed with dry ice because dry ice vapors may cause a change in the pH of the specimens. Additional specimens will be stored at 2-8°C or frozen according to specific directions for the type of specimen. All IATA regulations will be adhered to for preparing packages, labeling packages, and paperwork to accompany the specimens.

ii. TESTING PROCEDURES AND CAPACITY

1. Currently the State of Utah Public Health Laboratory is capable of testing all samples for rule-out of smallpox for human acute, generalized vesicular or pustular rash illness, suspect vaccine related adverse reactions, and environmental samples.

2. Varicella, or chicken pox, is the disease most frequently misdiagnosed as smallpox by physicians. The state lab can perform the rule-in DFA for VZV. This test takes about 2 hours to perform. Reagents on site allow for testing capacity of 150 specimens. Rule-in procedures for VZV, Vaccinia, Variola virus are available. Approximately 500 tests can be performed with reagents on site. Cooperative agreements with facilities housing electron microscopes is being developed.

3. Reassignment of laboratory staff to handle the influx of specimens to meet laboratory testing capacity would be developed depending on available staff and the change in workload. The state lab has 2 employees that could be diverted to perform Sample Receiving tasks. There are currently 6 Microbiologists trained in rapid testing methods for smallpox rule-out protocols. There are 3 additional surge capacity Microbiologists that could be reassigned if necessary. The Virology section could be requested to perform the VZV DFA rule-out test if necessary.

4. Contacts at the CDC are currently designated as the CDC Emergency Duty officer and the Rapid Response and Advanced Technology Laboratory staff. FBI contact is the Hazardous Materials Response Unit. All contacts have 24 hour/7 day a week coverage. The Utah Department of Health has 24 hour/7 day a week coverage with a pager system. A determination of how to proceed with a suspected case of smallpox will be determined by a conference call consultation with the State of Utah Public Health Laboratory, CDC, and FBI.

5. All Microbiologists and support staff that could be exposed to smallpox in the course of their duties have been registered with the CDC for potential vaccination.

6. A call-down list within the Utah Department of Health has been developed to inform the Bureau Director, Division Director, and Epidemiology counterparts of test status and results. The current test algorithm requires confirmation of smallpox testing at the CDC. Final results will be reported via secure fax, email, phone lines or by mail. Contacts at the CDC and FBI have been identified for reporting results in a timely and secure fashion.

7. A gap has been identified in the Health Alert Network that can be rectified by upgrading electronic contact hardware. Web-based data interface would allow the test results to be disseminated securely and quickly to the appropriate parties. However, this capacity does not currently exist.

8. Laboratory security is provided by the University of Utah Police. A lockdown procedure is being developed at the state lab to prevent entry of unauthorized personnel during an emergency. No plan has been drafted to consider staff rotations to allow rest and feeding of laboratory staff during a prolonged response.

9. Plans are developed to continue to improve relationships with the Level A and sub-level A laboratories in Utah, to provide training for rule-out identifications of bioterrorism agents, safely package, handle and ship specimens, and refer specimens for testing at higher level laboratories.

SMALLPOX Pages from the
Microbiology Client Services Manual

State of Utah Public Health Laboratory

46 North Medical Drive

Salt Lake City, UT 84113-1105

Phone: 801-584-8400 FAX: 801-584-8486

Utah Department of Health

MICROBIOLOGY CLIENT SERVICES MANUAL
State of Utah Public Health Laboratory

GENERAL INSTRUCTIONS

CONTACT US:

ADDRESS, PHONE, FAX, and WEBSITE

State of Utah Public Health Laboratory
46 North Medical Drive
Salt Lake City, UT 84113-1105
Phone: 801-584-8400
FAX: 801-584-8486
Webpage: [HTTP://health.utah.gov/els/microbiology](http://health.utah.gov/els/microbiology)

KEY PERSONNEL

Billing

Bob Anderson

Environmental (Water) Microbiology

Sanwat Chaudhuri, Ph.D. -- Section Chief

Microbiology Bureau

Barbara Jepson, MPA, MT(ASCP) -- Bureau Director

Dan Andrews, MS, MT(ASCP) -- Section Chief of Bacteriology,

Food Bacteriology, Mycobacteriology, Parasitology

Jana Coombs, BS, M/SV (ASCP) -- Section Chief of Newborn Screening

June Pounder, Ph.D. -- Section Chief of Molecular Biology, and
Bioterrorism coordinator

Tom Sharpton, MS, SM(ASCP) -- Section Chief of Immunology, Virology

Technical Services

Chris Peper, MT(ASCP) -- Section Chief

REPORTING:

You must supply your correct Customer ID Code to receive test results.

Some mail services and couriers are taking a week or more to get your samples to us.

If you are having problems with turn around time for results, check your delivery method.

See individual test for specific reporting criteria and methods.

REQUISITIONS:

Blank request forms with your customer ID code are available from Technical Services
(also see Appendix B for blank forms WITHOUT the customer ID).

All information must be provided. Incomplete requisitions cannot be processed.

SPECIMEN LABELING: See individual requirements under specific test.

*****NOTE: Specimen containers from the State of Utah Public Health Lab have an outdate printed on the label. Do not collect any sample in an outdated container.**

Call Technical Services at 801-584-8204 for a new container.

We do not supply blood collection tubes.

MICROBIOLOGY CLIENT SERVICES MANUAL
State of Utah Public Health Laboratory

LAB TEST – Bioterrorism

TEST	Variola virus (Smallpox)
METHOD	N/A
AVAILABLE	All Clients – Contact UDOH Epidemiology prior to submitting test specimens: (801)538-6191
PATIENT PREP	N/A
SPECIMEN	Microscope slide touch preps, scabs, dried vesicular fluid, vesicular swabs, vesicular tissue
COLLECT IN	Refer to Level A Manual for Agents of Bioterrorism
PROCESSING	Refer to Level A Manual for Agents of Bioterrorism
TRANSPORT	Refer to Level A Manual for Agents of Bioterrorism
TIME CRITICAL	Should be received at our laboratory as soon as possible
LABEL	Patient’s full name or unique ID number, and date of collection
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	24 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	Refer to the Smallpox Specimen Information link on the Microbiology website (health.utah.gov/els/microbiology)
CONTACT	(801)584-8449: Kim Christensen, or (801)584-8595: Barbara Jepson.

BIOTERRORISM TEST REQUEST FORM STATE OF UTAH PUBLIC HEALTH LABORATORY 46 NORTH MEDICAL DRIVE SALT LAKE CITY, UTAH 84113-1105 TELEPHONE: (801) 584-8400 FAX: (801) 584-8486	FOR LABORATORY USE ONLY LAB#: DATE STAMP:
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TESTING WILL NOT BE PERFORMED UNLESS SLIP IS COMPLETELY FILLED OUT. PLEASE PRINT CLEARLY FOR ACCURACY.

PATIENT INFORMATION:			
Patient Name (Last, First): _____			
Patient ID #:	DATE OF BIRTH (mm/dd/yy) ____/____/____	AGE: _____	SEX: M F

PROVIDER INFORMATION:	Physician: _____	SPECIMEN COLLECTION DATE
Provider Code:	Provider Phone: _____	(MM/DD/YY)
	Provider Email: _____	____/____/____
	Secure Fax #: _____	

SPECIMEN SOURCE/SITE:

Blood

Serum

Urine

CSF

Skin

Bronchial Wash

Tissue (specify): _____

Fluid (specify): _____

Feces

Wound/Abscess

Throat

Swab (specify): _____

Sputum

Lesion

Scab

Isolate (source): _____

Environmental (specify): _____

Food (specify): _____

Other (specify): _____

TEST ORDERED:

Bacillus anthracis

Burkholderia spp.

Brucella spp.

Clostridium botulinum culture & toxin

Coxiella burnetii

Francisella tularensis

Orthopox virus

Ricin toxin (non-clinical)

Staphylococcus Enterotoxin B (non-clinical)

Vaccinia virus

Varicella zoster virus

Variola virus

Yersinia pestis

Other (specify): _____

STATE OF ORIGIN OF PATIENT/SAMPLE

ADDITIONAL INFORMATION

(List pertinent information including presumptive ID)
