UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

Specimen Collection and Submission Manual

Special Pathogens Laboratory

Diagnostic Systems Division

Fort Detrick, Maryland 21702

Revised FEB 2024
Supersedes previous version dated JUNE 2016

This manual is designed to provide detailed instructions for submission of samples that will be analyzed in the SPL. Tests that are not listed may require special preparation or advance notice. Please notify the SPL in advance for tests not listed in this manual. Please call the laboratory manager to obtain additional information.

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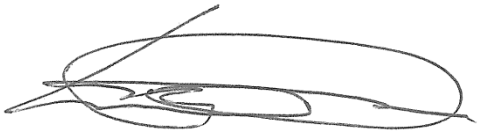
INTRODUCTION

1. This manual has been prepared to assist the Special Pathogens Laboratory (SPL) customer base. It contains information about procedures performed in the SPL. Included are information about specimen types and special instructions about particular tests and procedures. Changes to this handbook will be published as needed in the form of revised pages and distributed through normal channels.

2. Your comments, suggestions, and assistance in regards to the improvement of this manual or of services in general will be appreciated and can be addressed to me, my managerial and supervisory staff, or any other member of the SPL.



William Dorman
Technical Manager
Special Pathogens Laboratory

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Disclaimer:

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army or the Department of Defense. The mention of specific commercial entities does not imply endorsement by the US Army or the Department of Defense.

SPECIAL PATHOGENS LABORATORY CONTACT LISTING

Commercial Area Code (301), DSN 343

USAMRIID EMERGENCY HOTLINE	(888) 872-7443
USAMRIID Security (24hrs), SPL after hours notification	301-619-2257
USAMRIID Commander	301-619-2772
Division Chief	301-619-4721
Laboratory Manager	301-619-4291
Technical Manager	301-619-3318
Laboratory Technician	301-619-4496

Normal hours of operation are Monday through Friday 0730 to 1630. Support during non-duty hours is available by prior arrangement.

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TECHNICAL GUIDELINES AND GENERAL INFORMATION

Acceptable Specimen Volumes

Test entries list minimum acceptable specimen volumes. The minimum volume is defined as the absolute minimum needed to run a validated test algorithm. If there is insufficient specimen volume for testing, there may be delays, and the request may be referred to management for testing approval using modified procedures. If modified procedures are required to complete testing, the client will be notified before testing.

Accreditation/Licensure

SPL maintains current licenses, permits, and registrations required by federal regulations. For clinical microbiology samples, the SPL is CLIP (Clinical Laboratory Improvement Program) certified and COLA accredited. Clinical samples may be tested using a variety of in vitro diagnostic products (IVD), as well as assays classified under an Emergency Use Authorization (EUA), as Laboratory Developed Tests (LDT), or as Research Use Only (RUO) assays. Please contact the SPL with any questions concerning testing methods. For nonclinical, environmental samples, the SPL adheres to ISO 17025 standards as a sample testing laboratory. For additional information or copies of certificates, please contact the SPL.

General Bacterial Isolation Requirements

SPECIMEN	CONTAINER OR TRANSPORT DEVICE	PROCEDURE
Throat	Sterile Swab	Follow local, approved medical treatment facility collection procedures.
Nasopharynx	Flexible sterile calcium alginate swab	Follow local, approved medical treatment facility collection procedures.
Urine	Sterile screw-capped, leak proof, container or sterile urine container	A clean catch mid-stream sample is preferred. Follow local, approved medical treatment facility collection procedures.
Superficial wounds and abscesses	Sterile swab	Follow local, approved medical treatment facility collection procedures.
Rectal / Oral	Sterile swab	Follow local, approved medical treatment facility collection procedures.
Blood	Blood culture bottles (aerobic and/or anaerobic) or isolator tubes	Follow local, approved medical treatment facility collection procedures.

Crisis Contingency Plan

SPL maintains a corporate contingency plan for crisis recovery and business continuation. The purpose of this plan is to ensure prompt recovery of SPLs' critical business functions in the event of a crisis affecting any aspect of our continued patient care service. In the event of a local, regional, or national crisis that adversely affects timely delivery of specimens to SPL facilities, SPL will expeditiously initiate specific client-notification procedures to provide clients with necessary information and instruction on prearranged transportation and testing alternatives.

Health Insurance Portability and Accountability Act (HIPAA)

SPL is committed to complying with privacy and security standards promulgated in the Health Insurance Portability and Accountability Act (HIPAA). MEDCOM has implemented policies, processes, and procedures designed to ensure compliance with the standards. Compliance is continuously monitored for effectiveness. Workforce training is completed annually.

SPL complies with security standards by ensuring that systems, policies, and procedures meet or exceed all required and addressable implementation specifications. Internet and interface connectivity is encrypted and/or password protected, and electronic access is limited to authorized entities. Breaches of protected health information (PHI) or other confidential business information are reviewed and reported to the Department of Health and Human Services (DHHS) as necessary.

Inappropriate Submissions

All specimens should be collected, labeled, transported, and processed according to this procedure. Review the appropriate container type, volume, and special handling requirements needed for analysis before the specimen is collected. Due to the nature of typical specimens submitted to the SPL, less than optimal specimens may be tested upon consultation with SPL management. If any of the guidelines for these processes are not met, the specimen may be rejected or the test may be canceled. SPL will contact the client for resolution. The following list represents some possible causes for specimen rejection or test cancellation:

- Inappropriate specimen type
- Insufficient volume for analysis
- Improperly labeled specimen
- Inappropriate specimen container
- Improper specimen transport
- Specimen has leaked in transit
- Specimen has been submitted in incorrect or expired transport media
- Incomplete test request
- Test order without a specimen
- Specimen without a test order
- No specimen type provided
- No source provided*
- Compromised specimen (e.g., hemolysis, lipemic, or clotted specimens)

* The source of specimen, when appropriate, must be included on the paper or electronic request. The source of specimen is required for all infectious disease testing, including PCR tests.

Laboratory Result Reporting

SPL communicates laboratory results to clients by several means, including printed reports, email, and verbal results. Clients may request a phone notification or fax report by written notification when tests are ordered.

Preliminary results may be offered for infectious diseases and other tests in which a final report follows. Final results are generated at the completion of testing and may contain updated information from the preliminary result. When critical results are obtained, results are called to the physician or requesting lab.

Critical Values/Normal Values

SPL's testing laboratories operate Monday through Friday 0730 - 1630. Critical results are reported as soon as testing has been completed and a critical value has been identified. SPL reports critical values immediately to the contact(s) provided by our client(s) in accordance with the Laboratory Accreditation Program Inspection Checklists.

The normal value for most infectious agent detection is "not-detected." A confirmed detected/identified agent will be reported immediately to the client as well as federal agencies; as appropriate.

Referral Testing

One of SPL's service goals is to support clients by providing comprehensive service for all laboratory testing. To accomplish this goal, SPL may on occasion, select additional resources to perform additional tests not performed at SPL. Primary referral resources are typically intrinsic to USAMRIID and share the same aspects of service, quality, reliability, and turnaround time.

Every effort is made to test specimens at SPL, although the referral services may not fall under accredited services. Those tests not performed by SPL are clearly identified in the sample report.

Shipment Address

Samples to be shipped by commercial carrier should be addressed to:

USAMRIID
Special Pathogens Laboratory
Attn: Dorman/Tostenson
1425 Porter Street
Fort Detrick MD 21702-5011

It is highly recommended that SPL personnel or the USAMRIID Command be notified before shipment.

Samples should be clearly and legibly marked for special testing and appropriate chain of custody procedures maintained. Each sample should be placed in a water-tight receptacle with a screw cap closure. It is recommended that the screw cap be reinforced with adhesive tape. Liquid specimens should be placed individually in a second plastic vial or zip-top bag to prevent leakage. Absorbent material sufficient to absorb the entire content of the primary receptacle is placed between the primary and secondary packaging; for liquid specimens placed in a plastic vial, the absorbent material should be placed between the plastic vial and another secondary packaging material. The secondary packaging should be of material that prevents leakage.

For transportation, all samples must be packaged in an International Air Transportation Association (IATA) or Department of Transportation 49, Code of Federal Regulation 173 approved container, accordingly.

Additional guidance on packing and shipping infectious substances can be found through American Society for Microbiology: http://www.asm.org/images/pdf/Clinical/pack-ship-7-15-2011.pdf

LIST OF TESTS

Arbovirus - Detection in surveillance and clinical samples

Methodology: culture, molecular, and immunological methods
Tests May Include: West Nile virus (WNV), Eastern equine encephalitis virus, Venezuelan equine encephalitis virus, yellow fever virus, dengue virus, chikungunya virus, and Zika virus
Specimen: see specimen requirements
Shipping: ship cold on wet ice or ice packs.
Turnaround: within 10 days of specimen receipt

Note: See page 22 for additional information regarding the collection of Arbovirus.

Bacillus anthracis - Detection in clinical samples and environmental samples

Methodology: culture, biochemical testing, JBAIDS and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: see specimen requirements
Shipping: ship cold on wet ice or ice packs.
Turnaround: 2-3 days

Note: The Laboratory Response Network and JBAIDS PCR kit is FDA cleared for the identification of *Bacillus anthracis* from whole blood, blood culture samples and isolated organism grown on agar plates. See page 15 for additional information regarding the collection of this organism.

Bacillus anthracis - Identification/confirmation of referred isolate

Methodology: biochemical testing, PCR and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: actively growing pure culture on suitable medium
Shipping: ship at room temperature.
Turnaround: within 1-2 days of specimen receipt

Note: This organism has been designated as a Tier 1 select agent (Select Agent Regulation, 42 CFR, 73, Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Bacteria, Aerobic - Identification

Methodology: biochemical testing, typing or grouping if appropriate, sequencing of 16S ribosomal DNA if indicated
Specimen: actively growing pure culture on suitable medium
Shipping: ship at room temperature.
Turnaround: 1 – 3 weeks

Bacteria, anaerobic- identification

See: *Clostridium botulinum*, *Clostridium perfringens*
Comments: anaerobic bacteria other than *Clostridium* spp. not identified at SPL

***Brucella* spp.** - detection in clinical samples

Methodology: culture and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: see specimen requirements
Shipping: ship cold on wet ice or ice packs.
Turnaround: negative results available after 7-21 days of incubation

Note: See page 16 for additional information regarding the collection of this organism.

***Brucella* spp.** - identification/confirmation of referred isolate

Methodology: biochemical testing and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: actively growing pure culture on suitable medium
Shipping: ship at room temperature.
Turnaround: within 1 week of specimen receipt

Note: *B. abortus, B. melitensis*, and B. *suis* have been designated as select agents (Select Agent Regulation, 42 CFR, 73, Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Burkholderia mallei - detection in clinical samples and environmental samples
Methodology: culture, biochemical testing and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: see specimen requirements
Shipping: ship cold on wet ice or ice packs.
Turnaround: 4-7 days

Note: See page 17 for additional information regarding the collection of *Burkholderia* species.

Burkholderia mallei - identification/confirmation of referred isolate

Methodology: biochemical testing, PCR and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: actively growing culture on suitable medium, see specimen requirements
Shipping: ship at room temperature.
Turnaround: 4 working days

Note: This organism has been designated as a Tier 1 select agent (Select Agent Regulation, 42 CFR, 73, Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Burkholderia pseudomallei - detection in clinical samples

Methodology: culture, biochemical testing, PCR and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: see specimen requirements
Shipping: ship cold on wet ice or ice packs.
Turnaround: 4-7 days

Burkholderia pseudomallei - identification/confirmation of referred isolate

Methodology: biochemical testing, PCR and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: actively growing culture on suitable medium, see specimen requirements
Shipping: Ship at room temperature.
Turnaround: 4 working days

Note: This organism has been designated as a Tier 1 select agent (Select Agent Regulation, 42 CFR, 73, Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Clostridium botulinum - detection in clinical and environmental samples and food

Methodology: culture
Specimen: see specimen requirements, pages 18-19
Shipping: see shipping requirements, page 19
Turnaround: within 10 working days of specimen receipt

Clostridium botulinum - identification/confirmation of referred isolate

Methodology: biochemical testing, toxin testing
Specimen: actively growing pure culture on suitable medium
Shipping: ship at room temperature.
Turnaround: 1-2 weeks

Note: This organism has been designated as a Tier 1 Select Agent (Select Agent Regulation, 42 CFR, 73, Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Clostridium botulinum **toxin** - detection in clinical and environmental samples and food

Methodology: toxin neutralization assay, mouse bio-assay
Specimen: see specimen requirements
Shipping: see specimen requirements
Turnaround: within 7 working days of specimen receipt

Note: This toxin has been designated as a Tier 1 Select Agent (Select Agent Regulation, 42 CFR, 73, Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

See pages 18-19 for additional information regarding the collection of this organism.

Clostridium perfringens - detection in stool or implicated food

Methodology: culture
Specimen: stool in ParaPak C&S, modified Carey-Blair or equivalent - fill to line (approximately 5 ml)
Implicated food - minimum of 10 g in original container or transferred to sterile container using sterile instruments.
Shipping: ship stool at room temperature, for food, ship cold on wet ice or ice packs..
Turnaround: within 5 working days of specimen receipt

Clostridium perfringens - identification/confirmation of referred isolate

Methodology: biochemical testing, toxin testing
Specimen: actively growing pure culture on suitable medium
Shipping: ship at room temperature.
Turnaround: Within 5 working days of specimen receipt

***Coxiella burnetii* -** detection of DNA in clinical samples

Methodology: Laboratory Response Network Protocols
Tests May Include: PCR or immunoassays
Specimen: tissue or bone marrow (100 mg); Whole EDTA blood or serum (0.5 ml) Nasopharyngeal or throat swab, dry or in transport medium; Sputum, bronchial/tracheal washings (0.5 ml); Lesion exudates
Shipping: ship cold on wet ice or ice packs.
Turnaround: 2 days

Note: This organism has been designated as a Tier 1 Select Agent (Select Agent Regulation, 42 CFR, 73, Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Francisella tularensis - detection in clinical samples

Methodology: culture and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: see specimen requirements
Shipping: ship cold on wet ice or ice packs.
Turnaround: negative results available after 5-7 days of incubation

Note: The JBAIDS PCR kit is FDA cleared for the identification of *Francisella tularensis* from whole blood, blood culture samples and isolated organism grown on agar plates. See page 20 for additional information regarding the collection of this organism.

Francisella tularensis - identification/confirmation of referred isolate

Methodology: culture and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: actively growing pure culture on suitable medium
Shipping: ship at room temperature.
Turnaround: 3-7 days

Note: This organism has been designated as a Tier 1 select agent (Select Agent Regulation, 42 CFR, 73, Interim Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – detection in clinical samples

Methodology: molecular
Specimen: If possible collect 3 specimen types (lower respiratory, upper respiratory, and serum) CDC Interim Guidelines for Clinical Specimens from PUI for MERS-CoV (http://www.cdc.gov/coronavirus/mers/guidelines-clinical-specimens.html)
Shipping: ship cold on wet ice or ice packs. For delays exceeding 72 hours, ship frozen on dry ice.
Turnaround: 1-2 days

Note: The CDC MERS-CoV Real-time RT-PCR is a FDA Emergency Use Authorization (EUA) assay. (http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM400989.pdf)

Ricin toxin - detection in non-clinical samples

Methodology: immunological and molecular methods
Specimen: food, soil, water, environmental surface wipe
Shipping: ship cold on wet ice or ice packs.
Turnaround: 1-2 days

Staphylococcal enterotoxin A & B - detection in non-clinical samples

Methodology: immunological and molecular methods
Specimen: food, soil, water, environmental surface wipe
Shipping: ship cold on wet ice or ice packs.
Turnaround: 1-2 days

Vaccinia virus - detection in clinical samples

Methodology: culture
Specimen: roof of lesion in a sterile container swab of lesion, dry or in transport medium. Contact lab for details. Vesicular fluid sample in viral transport medium.
Shipping: ship cold on wet ice or ice packs.
Comments: These tests are recommended for patients exhibiting acute, generalized, vesicular or pustular rash illness. For details on evaluating patients see the CDC poster "Evaluating Patients for Smallpox" (http://emergency.cdc.gov/agent/smallpox/diagnosis/evalposter.asp)
Shipping: ship cold on wet ice or ice packs.
Turnaround: 1 – 3 weeks

Vaccinia virus - detection of DNA in clinical samples

Methodology: PCR (Laboratory Response Network protocols)
Specimen: roof of lesion in a sterile container swab of lesion, dry or in transport medium. Contact lab for details. Touch-prep (slide) of vesicular fluid.
Shipping: ship cold on wet ice or ice packs.
Turnaround: 1-2 days
Comments: These tests are recommended for patients exhibiting acute, generalized, vesicular or pustular rash illness. For details on evaluating patients see the CDC poster "Evaluating Patients for Smallpox" (http://emergency.cdc.gov/agent/smallpox/diagnosis/evalposter.asp)

Varicella zoster virus - detection of DNA in clinical samples

Methodology: PCR (Laboratory Response Network protocols)
Specimen: roof of lesion in a sterile container swab of lesion, dry or in transport medium. Contact lab for details. Touch-prep (slide) of vesicular fluid.
Shipping: ship cold on wet ice or ice packs.
Turnaround: 1-2 days
Comments: These tests are recommended for patients exhibiting acute, generalized, vesicular or pustular rash illness. For details on evaluating patients see the CDC poster "Evaluating Patients for Smallpox" (http://emergency.cdc.gov/agent/smallpox/diagnosis/evalposter.asp)

Variola virus- detection in clinical samples
Restrictions: If smallpox is suspected, call SPL for instructions.

Viral hemorrhagic fevers – detection in clinical samples

Methodology: culture, serology, and molecular
Tests May Include: Ebola, Marburg, CCHF, RVF, Hantavirus, Lassa, Junin, Machupo
Specimen: see specimen requirements page 23.
Shipping: ship cold on wet ice or ice packs.
Turnaround: 1-2 days

Note: See page 22 for additional information regarding the collection of these viruses.

Note: The Ebola EZ1 Real-time RT-PCR is a FDA Emergency Use Authorization (EUA) assay. (http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM418802.pdf)

***Yersinia pestis* -** detection in clinical samples

Methodology: culture, JBAIDS and Laboratory Response Network protocols
Tests May Include: biochemical, molecular, and immunological methods
Specimen: see specimen requirements
Shipping: ship cold on wet ice or ice packs.
Turnaround: 5 days

Note: The JBAIDS PCR kit is FDA cleared for the identification of *Yersinia pestis* from whole blood, sputum, blood culture samples and isolated organism grown on agar plates. See page 21 for additional information regarding the collection of this organism.

***Yersinia pestis* -** identification/confirmation of referred isolate

Tests May Include: biochemical, molecular, and immunological methods
Specimen: actively growing pure culture on suitable medium
Shipping: ship at room temperature.
Turnaround: 2-3 days

Note: This organism has been designated as a Tier 1 select agent (Select Agent Regulation, 42 CFR, 73, Interim Final Rule). Special handling criteria apply. Please contact the laboratory for special instructions.

Diagnostic Specimen Requirements for *Bacillus anthracis*

Type of Infection	Specimen type	Minimum Volume	Collection Comments
Cutaneous anthrax	Vesicle Swab	2 swabs	Vesicle should be unroofed and 2 sterile, dry swabs should be soaked in the vesicular fluid.
	Vesicle Aspirate	1 ml	An aspirate of the fluid is also an appropriate specimen.
	Eschar Swab	2 swabs	Roll swabs beneath the edge of the eschar without removing it.
	Fresh/Frozen Tissue	1 punch biopsy	
Gastro-intestinal anthrax	Stool	5 g	If unable to obtain stool, obtain rectal swab by inserting swab 1 inch beyond anal sphincter.
	Rectal Swab	1 swab	
Inhalation anthrax	Nasal Swab	1 swab	For epidemiologic purposes only. Useful only within 24 hours of exposure.
	Sputum	1 ml	If patient has a productive cough, this is the specimen of choice in the early course of the disease.
	Tracheal Aspirates, Bronchoalveolar Lavage, etc.	1 ml	
Meningitis	CSF	1 ml	Centrifuge ≥1 ml of fluid
Blood	3.2% Sodium Citrate Blood (Blue Top)	1 ml	For molecular testing.
	Serum/Plasma	2 ml	
	Blood Culture	Refer to manufacturer's recommendation	Collect appropriate blood volume and number of sets per submitting lab's protocol. Collect prior to antibiotic use if possible. Most likely to be positive in later stages of disease.
	Blood in Sodium Polyanethol Sulfonate (SPS)	8 ml	Blood collected in SPS may be inoculated into blood culture bottles upon receipt at SPL.
Other	Pleural Fluid	1 ml	
Environmental	Swab	1 swab	

Diagnostic Specimen Requirements for *Brucella* spp.

Specimen Type	Minimum Volume	Collection Comments
Blood culture	Refer to manufacturer's recommendation	Collect appropriate blood volume and number of sets per submitting lab's protocol. Collect prior to antibiotic use if possible. Multiple specimens increase possibility of obtaining a positive culture.
Blood in sodium polyanethol sulfonate (SPS)	8 ml	Blood collected with SPS may be inoculated into blood culture bottles upon receipt at SPL.
Bone marrow blood culture in bottle fluid	1 ml	Collect appropriate bone marrow volume per manufacturer's recommendation. Some blood culture systems are appropriate for bone marrow.
Bone marrow in sodium SPS	1 ml	Bone marrow collected in SPS may be inoculated into culture bottles upon receipt at SPL.
Abscess material	1 ml	Collect as needed based on clinical presentation. Appropriate postmortem specimen.
Lymph node, liver/spleen biopsy	1-5 g	Collect as needed based on clinical presentation. Appropriate postmortem specimen.
Synovial fluid, CSF, other body fluids	1 ml	Collect as needed based on clinical presentation. Appropriate postmortem specimen.
Whole blood	1 ml	3.2% sodium citrate blood (blue top)
Nasal swab	1 swab	For epidemiologic purposes only. Useful only within 24 hours of exposure.
Serum: **Acute and Convalescent**	2 ml	**Acute-phase** specimen should be collected ASAP after onset of disease. **Convalescent-phase** specimen should be collected >14 days after the acute specimen.
Environmental	1 swab	

Diagnostic Specimen Requirements for *Burkholderia* spp.

Specimen Type	Minimum Volume	Collection Comments
Abscess material, tissues	1 ml	Collect tissues and fluids rather than swabs, when possible. Collect as needed based on clinical presentation. Appropriate postmortem specimen.
CSF, other body fluids	1 ml	Collect as needed based on clinical presentation. Appropriate postmortem specimen.
Sputum	1 ml	
Skin swab	1 swab	
Urine	1 ml	Collect a midstream clean-catch or a catheterized specimen.
Blood culture	Refer to manufacturer's recommendation	Collect appropriate blood volume and number of sets per submitting lab's protocol. Collect prior to antibiotic use if possible.
Blood in sodium polyanethol sulfonate (SPS)	8 ml	Blood collected in SPS may be inoculated into blood culture bottles upon receipt at SPL.
Throat or Nasal swab	1 swab	For epidemiologic purposes only. Useful only within 24 hours of exposure.
Whole blood	1 ml	3.2% sodium citrate blood (Blue top)
Serum	1 ml	Collect in serum separator tube (SST™) or red top tube. For molecular testing.
Environmental	1 swab	

Collection and Transport of Diagnostic Samples for Botulism Testing

Suspected Food borne Botulism

Acceptable Specimens	Required Volume/Comments	Toxin Assay (T) or Culture (C) Performed
Serum (priority sample type)	5 ml (less results in incomplete testing)	T
Gastric contents	20 ml, anaerobic transport system	T, C
Vomitus	20 ml, anaerobic transport system	T, C
Stool	25-50 g (walnut-size) collected before anti-toxin treatment.	T, C
Sterile water enema	Collect using a minimal amount of water, before anti-toxin treatment.	T, C
Implicated consumed food – commercial or home-prepared	Leave foods in their original containers, if possible, or transfer to sterile, leak-proof containers. Empty containers with remnants of food are acceptable.	T, C
Unopened home-prepared food from the batch consumed by the patient	Leave foods in their original containers.	T, C
Unopened commercial products	Products are referred immediately to the FDA.	T, C

Suspected Infant Botulism

Acceptable Specimens	Required Volume/Comments	Toxin Assay (T) or Culture (C) Performed
Stool (priority sample type)	As above.	T, C
Sterile water enema	As above.	T, C
Serum	1 ml	T
Rectal Swab	1 swab (culture only)	C
Potential Sources	Include honey, opened formula other foods/liquids fed to the infant. Environmental sampling is discouraged.	C

Suspected Wound Botulism

Acceptable Specimens	Required Volume/Comments	Toxin Assay (T) or Culture (C) Performed
Serum (priority sample type)	5 ml (less results in incomplete testing)	T
Wound swab	Anaerobic transport system	C
Tissue or exudate	Anaerobic transport system	C
Stool	To rule out food borne botulism. Collect as above	T,C

Suspected Intentional Toxin Release

Acceptable Specimens	Required Volume/Comments	Toxin Assay (T) or Culture (C) Performed
Clinical material	Serum, stool, sterile water enema as above.	T
Food	As above.	T
Environmental swabs	Send in individual clean, dry containers	T

Shipping Requirements (Botulism)

Notify the laboratory in advance.

- Collect and transport clinical samples in sterile, leak-proof containers.
- Leave foods in their original containers, if possible, or place in sterile leak-proof, unbreakable containers. Place each container in a separate sealed plastic bag to prevent cross-contamination during shipping. Label completely.
- Ship by the most rapid means available.
- Store and ship specimens in anaerobic transport systems at room temperature.
- Store and ship all other specimens cold on wet ice or ice packs.
- Freezing should be avoided as it decreases recovery of *C. botulinum* and may decrease toxin activity. However, if a delay of more than several days cannot be avoided, freeze samples for storage and ship frozen on dry ice.

Diagnostic Specimen Requirements for *Francisella tularensis*

Type of Infection	Specimen type	Minimum Volume	Collection Comments
Pulmonary	Sputum, throat swab, tracheal aspirates, bronchoalveolar lavage, etc.	1 ml	
	Nasal swab	1 swab	For epidemiologic purposes only. Useful only within 24 hours of exposure.
Ulceroglandular	Ulcer scraping, biopsy, or swab (eye)	1 g 1 swab	Specimen from advancing edge of the lesion not central necrotic area, which is usually secondarily infected.
Glandular	Lymph node aspirate, tissue	1ml 1-5g	
Septicemia	Blood culture	Refer to manufacturer's recommendation	Collect appropriate blood volume and number of sets per submitting lab's protocol. Collect before antibiotic use if possible. Most likely to be positive in later stage of disease.
	Blood in sodium polyanethol sulfonate (SPS)	8 ml	Blood collected in SPS may be inoculated into blood culture bottles upon receipt at SPL.
Meningitis	CSF	1 ml	Centrifuge ≥1 ml of fluid
Misc/Other	Whole blood	1 ml	3.2% sodium citrate blood (Blue top)
	Serum/plasma	2 ml	**Acute-phase** specimen should be collected ASAP after onset of disease. **Convalescent-phase** specimen should be collected >14 days after the acute specimen.
Postmortem	Lymph, lung, liver, spleen tissue, bone marrow, CSF	1-5g 1 ml	
Environmental	Swab	1 swab	

Diagnostic Specimen Requirements for *Yersinia pestis*

Type of Infection	Specimen type	Minimum Volume	Collection Comments
Bubonic Plague	Lymph node (bubo) aspirate	2 ml	
Septicemic Plague	Blood culture	Refer to manufacturer's recommendation	A series of three venipuncture specimens taken 15-30 minutes apart is most effective. Collect before antibiotic use if possible.
	Blood in sodium polyanethol sulfonate (SPS)	8 ml	Blood collected in SPS may be inoculated into blood culture bottles upon receipt at SPL.
Pneumonic Plague	Sputum	1 ml	"Bloody" sputum is a hallmark of this disease.
	Tracheal aspirates, bronchoalveolar wash, etc.	1 ml	Bronchial or tracheal aspirates are the specimens of choice.
	Nasal/Throat swab	1 swab	For epidemiologic purposes only. Useful only within 24 hours of exposure.
Misc/Other	Whole blood	1 ml	3.2% sodium citrate blood (Blue top)
	Serum/plasma	2 ml	**Acute-phase** specimen should be collected ASAP after onset of disease. **Convalescent-phase** specimen should be collected >14 days after the acute specimen.
Postmortem	Lymph and lung tissue, bone-marrow	1-5 g 1ml	

Diagnostic Specimen Requirements for Viruses

Type of Infection	Specimen type	Minimum Volume	Collection Comments
Arbovirus	0-24 hr Nasal swabs, induced respiratory secretions	1 swab or 1ml	Place swab or fluid in viral transport media.
	0-72 h Serum; throat swabs, CSF	1 swab or 1ml	If submitting for culture, place in viral transport media.
	> 6 days Serum; tissue	1ml 1-5g	Serum for IgM; Pathology samples plus brain.
Viral Hemorrhagic Fevers	0-24 hr Nasal swabs, induced respiratory secretions	1 swab or 1ml	Place swab or fluid in viral transport media.
	24-72 h Serum	1ml	If submitting for culture, place in viral transport media.
	> 6 days Serum; tissue	1ml 1-5g	Serum for IgM; Pathology samples plus adrenal gland.
Environmental	Swab	1 swab	

Environmental samples can be collected to determine the nature of a bio-aerosol either during, shortly after, or considerably after an event. The sooner that the environmental sample is taken, in conjunction with early post-exposure clinical samples, the better it will be to help identify the agent.

Samples taken well after an event may allow identification of the agent used. While this information would likely be too late for useful prophylactic treatment, when combined with other information, may be used in the prosecution of war crimes or other criminal proceeding (special chain of custody procedures apply to forensic or criminal cases). All known particulars (i.e. what, where, when, how, etc.) of the sample collection should be documented both in writing and with pictures. The types of samples taken can be extremely variable. Some of the possible samples are:

- Aerosol collections in buffer solutions
- Soil
- Swabs (Dacron or macro-foam; cotton has been shown to be PCR inhibitory)
- Dry powders
- Container of unknown substance
- Vegetation
- Food / Water
- Dead animals
- Human remains

Substances collected will depend greatly on the situation. At a minimum, anything that appears to be contaminated can be sampled with swabs or with clean, inert absorbent paper or cloth. Samples should ideally be double bagged in Ziploc or similar resealable bags (the outside of the inner bag decontaminated with dilute bleach before placing in the second bag) labeled with time and place of collection along with any other pertinent data. As always, please contact the SPL with any questions regarding sample collection or submission.

Acronyms/Definitions

The following terms and acronyms, associated with the Special Pathogens Laboratory (SPL), are used throughout this manual. These are defined here to provide clarity of meaning.

Chain of Custody (CoC) Chain of custody refers to the act or acts undertaken by the SPL to guard and trace the flow of samples and documents from the time of receipt through permanent archiving or destruction. The SPL accomplishes chain of custody through a series of standard operating procedures. External CoC may be initiated at time of collection, processing and/or shipment.

Clinical Laboratory Improvement Program (CLIP) Program derived by the Department of Defense that allows military owned clinical laboratories to meet the Clinical Laboratory Improvement Amendments (CLIA) of 1988 standards, with certain exceptions to meet military operational requirements.

Health Insurance Portability and Accountability Act of 1996 (HIPAA) The HIPAA Privacy Rule provides federal protections for personal health information held by covered entities and gives patients an array of rights with respect to that information. At the same time, the privacy rule is balanced so that it permits the disclosure of personal health information needed for patient care and other important purposes.

In Vitro Diagnostic Product (IVD) In vitro diagnostic products are those reagents, instruments, and systems intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body. IVD are classified based on the level of regulatory control that is necessary to ensure efficacy and safety.

ISO/IEC 17025 ISO/IEC 17025 was first issued in 1999 by the International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC). It is the single most important standard for calibration and testing laboratories around the world. Laboratories that are accredited to this international standard have demonstrated that they are technically competent and able to produce precise and accurate test and/or calibration data..

Joint Biological Agent Identification and Diagnostic System (JBAIDS) JBAIDS is the United States DoD standard platform used to identify biological agents in a dual purpose role: for diagnostic applications in a clinical setting and for environmental and food sample confirmatory testing. The ruggedized JBAIDS is an open platform that analyzes 32 samples and is deployed in field hospitals, mobile analytical labs, shipboard medical labs, food and water safety test centers, research labs, and other mobile scenarios.

Laboratory Developed Tests (LDT) LDT is a type of in vitro diagnostic test that is designed, manufactured and used for in-house pathology and diagnostic purposes within a single laboratory.

Laboratory Response Network (LRN) The LRN is a collaborative effort within the US federal government involving the Association of Public Health Laboratories and the Centers for Disease Control and Prevention (CDC). Most state public health laboratories participate as reference laboratories of the LRN. These facilities support hundreds of sentinel laboratories in local hospitals throughout the United States and can provide sophisticated confirmatory diagnosis and typing of biological agents that may be used in a bioterrorist attack or other bio-agent incident. The LRN was established in 1999.

Polymerase Chain Reaction (PCR) The PCR is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Protected Health Information (PHI) The privacy rule protects all *"individually identifiable health information"* held or transmitted by a covered entity or its business associate, in any form or media, whether electronic, paper, or oral. The privacy rule calls this information "protected health information."

Research Use Only (RUO) RUO is a US Food and Drug Administration (FDA) term referring to a product, device, test or procedure, which is not approved for diagnostic purposes.

Sodium polyanethol sulfonate (SPS) SPS tubes are used for blood culture specimen collection in microbiology. Eight gentle tube inversions will prevent the blood from clotting. The blood can remain in the SPS tube before it has to be transferred to a blood culture bottle.

Special Pathogens Laboratory (SPL) Special Pathogens Laboratory receives and analyzes clinical, environmental and biological material for the presence of biological threat agents and disease causing agents. The laboratory works within the chain of command of the Diagnostic Systems Division at USAMRIID.

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)
USAMRIID is a basic and applied research facility located at Fort Detrick, Maryland, whose mission is to support military readiness through defensive medical research and development.

*"The **Specimen Collection and Submission Manual** is a comprehensive reference guide of testing services, shipping information and submission requirements. Efforts have been made to be concise and current with all information, but as testing procedures and regulations change, some information may not be current. Many of these tests are only available with prior approval from SPL. It is impossible to address all situations in this guide, so please contact us with any questions."*