

Louisiana Office of Public Health Laboratories	
Test Name	Rule out Anthrax
PHL Location	Office of Public Health Laboratory Baton Rouge
CPT Code	N/A
Synonyms	BT Test, Bioterrorism Test, Clinical Rule Out
Brief Description of Test	<p>Prior notification requested. Contact Infectious Disease Epidemiology at 800-256-2748 or BT Program Advisor at 225-219-5241.</p> <p>To rule out or confirm bacterial isolates as <i>Bacillus anthracis</i> or to rule out or confirm presence/absence of <i>Bacillus anthracis</i> in direct patient specimens.</p> <p><i>B. anthracis</i> is suspected and cannot be ruled out if the isolate fulfills the following characteristics:</p> <ul style="list-style-type: none"> • Direct Gram staining reveals large Gram-positive rods • Grows on BAP as non-pigmented, odorless, white colonies having a ground glass appearance with edges that are slightly undulate (“Medusa heads”; comma shaped) • Does not grow on MAC (or EMB) • Non-hemolytic on BAP • Catalase – positive • Motility – negative
Possible Results	<p>Direct Sample Testing Presumptive Positive Equivocal Inconclusive Negative</p> <p>Culture Isolate Testing Confirmed Positive Equivocal Negative</p>
Reference Range	Negative
Specimen Type	Direct Sample – clinical specimens require prior approval

	A. Collection and Transport of Clinical Specimens for Laboratory Rule-Out Testing*	
	Cutaneous Vesicular (early) stage Eschar (late) stage	<ul style="list-style-type: none"> • Unroof vesicle and aspirate fluid or collect with two sterile swabs (dacron) • Insert swab (dacron) beneath the edge of the eschar, rotate swab or obtain an aspirate • Transport specimens at room temperature
	Gastrointestinal	<ul style="list-style-type: none"> • Stool (> 5 grams...pecan size), collect and transport in a leak proof sealed container • Collect blood (late stage of infection) directly into an appropriate blood culture bottle (aerobic and anaerobic) • Transport specimens and bottles at room temperature
	Inhalational	<ul style="list-style-type: none"> • Sputum • Blood: collect directly into an appropriate blood culture bottle (aerobic and anaerobic) • Cerebral Spinal Fluid only if signs of meningitis occur • Transport specimens and bottles at room temperature
	Postmortem Tissue	<ul style="list-style-type: none"> • Tissue pieces should be collected and kept moist • Transport in sterile container at room temperature within 1 hour of collection
	Culture Isolate Actively growing, pure culture inoculated in/on unexpired media	
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:	1mL of blood, 0.1g of tissue, 5mL of sputum or solids approximately the size of a pea	
Collection Instructions	<p>According to the 5th Edition of the BMBL, unless you are working with high concentrations of this organism or performing procedures that produce aerosols, <i>B. anthracis</i> can be handled using BSL-2 practices. Do not process nonclinical (environmental or animal) specimens in hospital or commercial reference laboratories; restrict processing to human clinical specimens only. Nonclinical specimens should be directed to the designated LRN Reference Laboratory.</p> <p>All patient specimens can be handled using BSL-2 practices. BSL-3 precautions, wearing gloves and gown and working in a certified Class II biosafety cabinet (BSC) are recommended when performing activities having a high potential for aerosol production. Subcultures should be performed in a BSC and plates should be taped/shrink sealed, and incubated in 5 – 10% CO₂. All additional testing should be performed only in the BSC while wearing gloves to prevent acquiring infection through the skin.</p> <p>Decontamination of laboratory surfaces is easily accomplished using a fresh solution of 10% bleach. In addition, pipettes, needles, plastic loops, and microscopic slides should be soaked in 10% bleach or 10 – 30% formalin for 24h before being autoclaved. Phenolics are not sporicidal at the usual working dilutions</p> <p>For swab samples, use nylon, polyester (e.g. Dacron), rayon or foam swabs instead of cotton.</p>	

	<p>Label specimen with Patient Name and a 2nd Unique Identifier such as a chart number or medical record number. DOB is not considered unique.</p> <p>Complete a LAB Form 93 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, 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 93 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>Culture may be shipped ambient. 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>If inhibitors are present in a DNA extraction, PCR assays may produce a false negative result.</p> <p>A false negative result may occur if a sample is improperly collected, transported or handled. False negative results may occur if inadequate numbers of organisms are present in the sample.</p> <p>Data suggest that clinical specimens collected subsequent to initiation of</p>

	<p>antimicrobial treatment may not be positive for <i>B. anthracis</i> due to reduction of <i>B. anthracis</i> organisms and DNA. Whenever possible, specimens collected prior to administration of antimicrobial agents should be used to determine infection with <i>B. anthracis</i>.</p> <p>Real-time PCR results are considered presumptive unless a pure culture isolate exhibiting colony morphology and Gram stain characteristics (Gram positive rods) consistent with <i>B. anthracis</i> was used to generate the result.</p>
Interfering Substances	N/A for direct culture testing
References	<p>Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases – <i>Bacillus anthracis</i> http://www.asm.org/images/PSAB/LRN/Anthrax316.pdf</p> <p>Laboratory Response Network</p>
Additional Information	If <i>B. anthracis</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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