Recognize. Rule-Out. Refer.

Biothreat Agent Bench Cards for the Sentinel Laboratory

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

LRN™ LABORATORY RESPONSE NETWORK

AMERICAN SOCIETY FOR MICROBIOLOGY

For questions, contact your designated LRN Reference Level Laboratory:

Idaho Bureau of Laboratories

(LRN Reference Level Laboratory Name)

208-334-2235

(Phone Number)

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APHL thanks the Sentinel Laboratory Partnerships and Outreach Subcommittee, the Public Health Preparedness and Response Committee, the American Society for Microbiology and the US Centers for Disease Control and Prevention for contributing their time and expertise to provide substantial guidance on the development of these bench cards.

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State-Specific Information

INSTITUTION / LRN REF LABORATORY: Idaho Bureau of Laboratories

Address: 2220 Old Penitentiary Rd

Phone Number: 208-334-2235

Website: www.statelab.idaho.gov

EMERGENCY NUMBERS

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Laboratory (after hours): 1-800-632-8000

Biothreat Coordinator: 208-334-0515

Epidemiology Dept. (business hours): 208-334-5939

Epidemiology Dept. (after hours): 1-800-632-8000

Duty Officer/Other On-Call: N/A

STATE / LOCAL PUBLIC HEALTH LABORATORY DEPARTMENTS

Microbiology: 208-334-0514

Virology: 208-334-0589

Serology: 208-334-0589

Specimen Receiving/Packaging: 208-334-0503

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TABLE OF CONTENTS

Safety
Safety Precautions 5
Preventing Aerosolization 6

Responding to a Biothreat Agent
Laboratory Response Network for Biological Threats 7
Responsibilities of the Sentinel Laboratory 8
Sentinel Laboratory Checklists 9
Biological Risk Assessments 10
Using BSL-3 Practices 11
Biothreat Agent Response Algorithm 12

Biothreat agent Identification
Gram Negative Bacilli/Cocobacilli Rule-Out Algorithm 13

Anthrax — *Bacillus anthracis*
Handling Instructions 14
Characterization 15
Rule-Out Algorithm 16

Anthrax — *Bacillus cereus* biovar *anthracis*
Characterization 17
Recommendations 18

Brucellosis — *Brucella* spp.
Handling Instructions 19
Characterization 20
Rule-Out Algorithm 21

Glanders — *Burkholderia mallei*
Handling Instructions 22
Characterization 23
Rule-Out Algorithm 24

Melioidosis — *Burkholderia pseudomallei*
Handling Instructions 22
Characterization 25
Rule-Out Algorithm 26

Tularemia — *Francisella tularensis*
Handling Instructions 27
Characterization 28
Rule-Out Algorithm 29

Plague — *Yersinia pestis*
Handling Instructions 30
Characterization 31
Rule-Out Algorithm 32

Appendix
Acronyms 33
Terms and Definitions 34
Identification Tests 37
Resources 39

Refer to the ASM Sentinel Laboratory Guidelines and consult with your LRN Reference Laboratory
for other suspect biothreat organisms not routinely seen in the Sentinel Laboratory, such as
***Clostridium botulinum*, novel influenza, Smallpox, *Staphylococcus aureus* enterotoxin B (SEB), *Coxiella burnetii*, etc.**

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Safety Precautions

Identification Systems

Use May Result in Exposure or Misidentification of Biothreat Agents

Using automated or manual identification systems (e.g., MALDI-TOF, Vitek, API 20 NE, Bactec) may result in exposure to dangerous pathogens, and could result in erroneous identification (e.g., *Bacillus anthracis* misidentified as *B. cereus*; *Yersinia pestis* misidentified as *Y. pseudotuberculosis,* etc.).

Filter Extract to Reduce Risk of Contamination or Exposure

If using automated identification systems for bacterial identification and the manufacturer provided an alternate tube extraction method (most common with MALDI-TOF), it is recommended that the resulting extract be filtered using a 0.2 µm (or less) filter. This additional step will reduce the risk of laboratory contamination with viable bacteria and spores.

Handling a Suspected Biothreat Agent

Use a Biological Safety Cabinet & BSL-3 Practices

As soon as a biothreat agent is suspected, perform all further work in a certified Class II BSC using BSL-3 practices and appropriate BSL-3 PPE.

Contact your LRN Reference Level Lab

If the agent cannot be ruled-out with tests listed within these bench cards, do not attempt further identification using commercial automated or kit identification systems. Contact your LRN Reference Level Laboratory to refer the isolate.

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Preventing Aerosolization

Aerosolization

Aerosolization can occur during any procedure which imparts energy into a microbial suspension, producing aerosols or droplets which may contain infectious organisms. Aerosols are very small particles that may remain suspended in the air and can be inhaled and retained in the lungs. Droplets are larger particles which can settle onto surfaces and gloves due to gravity. Droplets may also come into contact with the mucous membranes of the person performing the procedure.

Safety Precautions

Laboratory exposures can be decreased by working in a BSC using BSL-3 practices and appropriate BSL-3 PPE when a biothreat agent is suspected. Identified aerosol-generating procedure risks should be mitigated.

Examples of Aerosol Producing Procedures

- Opening culture plate, sniffing plates (Examining colony morphology/growth)
- Heat fixing a slide
- Dispensing pipette tips
- Centrifuge setup/run/unloading
- Vortexing
- Spills or splashes of liquid media
- Subculturing positive blood culture bottles
- Inoculation of media (plate or tube)
- Preparing samples for automated ID systems
- Open flames, sterilizing loops
- Sonicating
- Pipetting
- Catalase test
- Using automated and manual identification systems (e.g., MALDI-TOF, Vitek, API 20 NE, Bactec)

Your facility may identify additional aerosol generating procedures based on the laboratory's risk assessments.

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Laboratory Response Network for Biological Threats

The LRN-B was founded in 1999 by CDC, FBI and APHL to coordinate laboratory response to biological, chemical, radiological threats and other high priority public health emergencies, including emerging infectious diseases.

National Laboratories

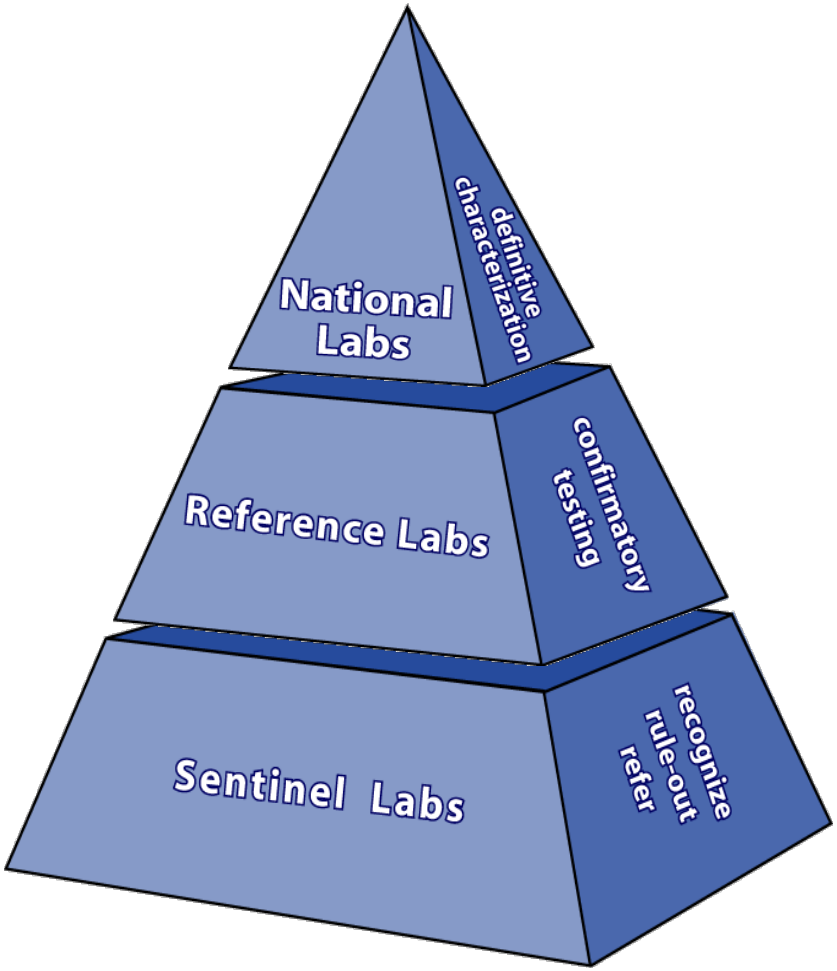
National labs, including the CDC, US Army Medical Research Institute of Infectious Diseases (USAMRIID), and the Naval Medical Research Center (NMRC), are responsible for specialized strain characterization, bioforensics, biothreat agent activity and handling of highly infectious biological agents.

Reference Laboratories

Reference labs, including state and local public health, military, veterinary, agriculture, food and water testing laboratories, are responsible for investigation and confirmatory testing. Facilities located in Australia, Canada, the United Kingdom, Mexico and South Korea serve as international reference laboratories.

Sentinel Laboratories

Sentinel labs, comprised of hospital-based and commercial laboratories, are responsible for the early detection and the rule-out or referral of potential biothreat agents.



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Responsibilities of the Sentinel Laboratory

A Sentinel Laboratory:

1. Is familiar with reportable disease guidelines in its jurisdiction, and has policies and procedures in place to refer clinical and diagnostic specimens or isolates suspected to contain agents of public health significance to the appropriate local or state public health laboratory.
2. Ensures sufficient personnel have met the applicable federal regulations for packaging and shipping of Category A and B infectious substances.
3. Has policies and procedures for the collection and referral of suspect biothreat agents or other emerging threat specimens and/or isolates to the appropriate LRN Reference Laboratory consistent with the ASM *Sentinel Level Clinical Laboratory Protocols and Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases*.
4. If a clinical core laboratory, provides their satellite facilities with written directions and training as needed for appropriate specimen collection and handling. Core laboratories should also provide satellite facilities with procedures for the recognition of the agents of bioterrorism and assure training at a level commensurate with the complexity of services offered at that facility.
5. Maintains the capability to perform the testing outlined in the ASM *Sentinel Clinical Laboratory Protocols* and must demonstrate annual competency by participation in proficiency testing or exercises, such as APHL, CDC and the College of American Pathologists Laboratory Preparedness Exercise (CAP LPX), state-developed proficiency/challenge sets, or other equivalent assessment.
6. Based on its risk assessment, has and utilizes a currently certified Class II or higher BSC when there is a risk of aerosol production or when working with a biological threat agent or other emerging threat organism is suspected.
7. Complies with the practices as outlined in the current edition of the *Biosafety in Microbiological and Biomedical Laboratories* guidelines and those detailed in the *Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories*.
8. Has a biosafety and biosecurity risk assessment policy and ensures that such risk assessments are routinely performed as part of their quality management program.
9. Complies with applicable US Occupational Safety and Health Administration regulations for bloodborne pathogens and has a respiratory protection program.
10. Complies with the applicable rules and regulations of the Federal Select Agent Program.
11. Has policies and procedures for secure storage of any remaining suspect biothreat or other emerging threat agent material retained within its facilities until it is transferred or destroyed.
12. Has policies and procedures for final decontamination/destruction of any remaining suspect biothreat or other emerging threat agent material within the required time-frame (e.g., primary specimens or subcultures retained within its facilities).

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RESPONDING TO A BIOTHREAT AGENT

Sentinel Laboratory Checklists

Laboratory Preparedness

Plans

- ☐ Institutional Emergency or Incident Response Plan
- ☐ Specific Bioterrorism Response Plan
- ☐ Institutional Risk Assessment Plan

Training

- ☐ Packaging and Shipping of Infectious Substances
- ☐ Rule-Out of Select/Biothreat Agents
- ☐ Select Agent Regulations
- ☐ Communications and Messaging

Proficiency Testing

- ☐ Proficiency test/exercise (e.g., CAP LPX)
- ☐ Maintain supplies for rule-out testing

Updates

- ☐ Review ASM's website for updated Sentinel Level Clinical Laboratory Protocols
- ☐ APHL trainings

If you have a:

Suspect BT Agent

- ☐ Follow rule-out procedures and conduct work in a BSC
- ☐ Initiate/maintain communication with departmental/hospital leadership and infection control
- ☐ Contact BT personnel at designated LRN Reference Level Laboratory
- ☐ Ship isolate to designated LRN Reference Level Laboratory
- ☐ Document courier transfer (e.g., institutional or commercial courier tracking number)
- ☐ Secure all potential biothreat agent(s) and residual samples
- ☐ Document personnel with access to potential biothreat agent(s) (*biosecurity*)
- ☐ Document personnel who have worked with suspect biothreat agent and those present in laboratory if exposure occurred (*biosafety)*

Confirmed BT Agent

- ☐ Follow directions from designated LRN Reference Level Laboratory for the destruction or transfer of all isolates/specimens
- ☐ Perform risk assessment review
- ☐ Document identification of biothreat agent(s) with APHIS/CDC Form 4
- ☐ Document disposition of biothreat agent(s) with APHIS/CDC forms:
  - Form 2 to transfer
  - Form 4 for destruction

Exposure to a BT Agent:

- ☐ Document any laboratory exposures with APHIS/CDC Form 3
- ☐ Work with designated LRN Reference Level Laboratory or health department for post-exposure prophylaxis

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Biological Risk Assessments

Biological Risk Assessment Goals

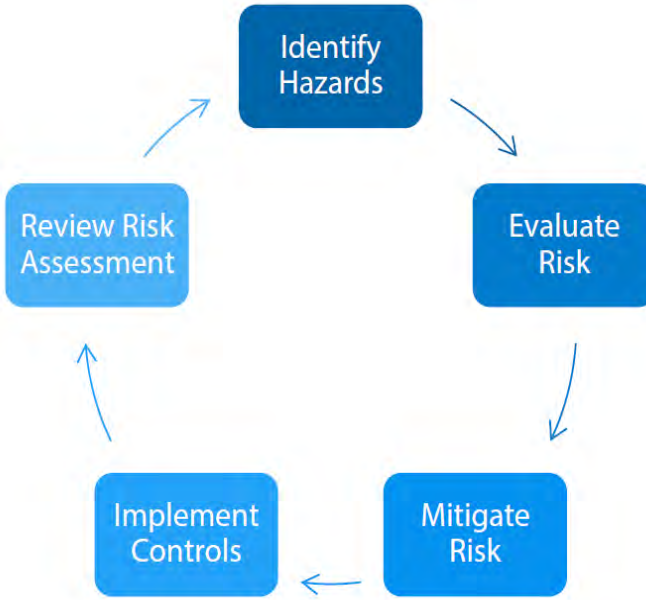
- Identify hazards associated with handling infectious agents in the laboratory.
- Identify and implement controls in order to minimize the risk of exposure to workers and the environment.
- In the clinical lab, focus is primarily on the prevention of laboratory acquired infections from:
  - Spills/splashes to mucous membranes
  - Inhalation of aerosols
  - Percutaneous inoculation from cuts, needle sticks, non-intact skin
  - Ingestion (e.g., contamination from surfaces, fomites to hands, etc.)

Conducting a Biological Risk Assessment

Risk assessments must be performed regularly based on procedure or agent, and when there are changes in agents, procedures, equipment or staff. Risks identified by the assessment should be prioritized and a mitigation plan should be established based on that prioritization.

Risk assessments require management involvement and support, knowledge of the hazards and understanding of the work, the environment and the staff. Ideally, they consist of a multidisciplinary team, depending on the work.

Consult with your LRN Reference Lab for guidance, and refer to APHL's Risk Assessment Best Practices for more information.



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Using BSL-3 Practices

BSL-3 Practices

- Restrict access to the laboratory.
- Wear additional PPE (solid-front gown, gloves and face/eye protection as a minimum) and respiratory protection (previously fit-tested for use).
- Laboratory personnel must demonstrate proficiency prior to handling pathogenic and potentially lethal agents, and must be supervised by scientists experienced and competent in handling the specific infectious agents present in the laboratory and associated procedures.
- Do not manipulate organisms or work in open vessels on the bench. All work must take place in a certified Class II or higher BSC, or other containment equipment. Tape plates shut.
- Evaluate all potential exposures immediately.
- Decontaminate all cultures, stocks and other potentially infectious materials prior to disposal by using an approved decontamination method, such as autoclaving or chemical disinfection. Decontamination would preferably take place within the laboratory.

When to Use BSL-3 Practices in a BSL-2 Laboratory

- When working with agents that can be transmitted via inhalation and are normally handled at BSL-3, but a BSL-3 laboratory is not readily available.
- When the laboratory director determines that BSL-3 practices are needed based on a risk assessment.
- When specific high-risk pathogenic organisms are suspected, such as *Brucella spp., Coccidioides* spp., *Blastomyces dermatitidis, Francisella tularensis, Histoplasma capsulatum, Mycobacterium tuberculosis*, MERS, SARS, highly pathogenic influenza, Tier 1 Select Agents, etc.

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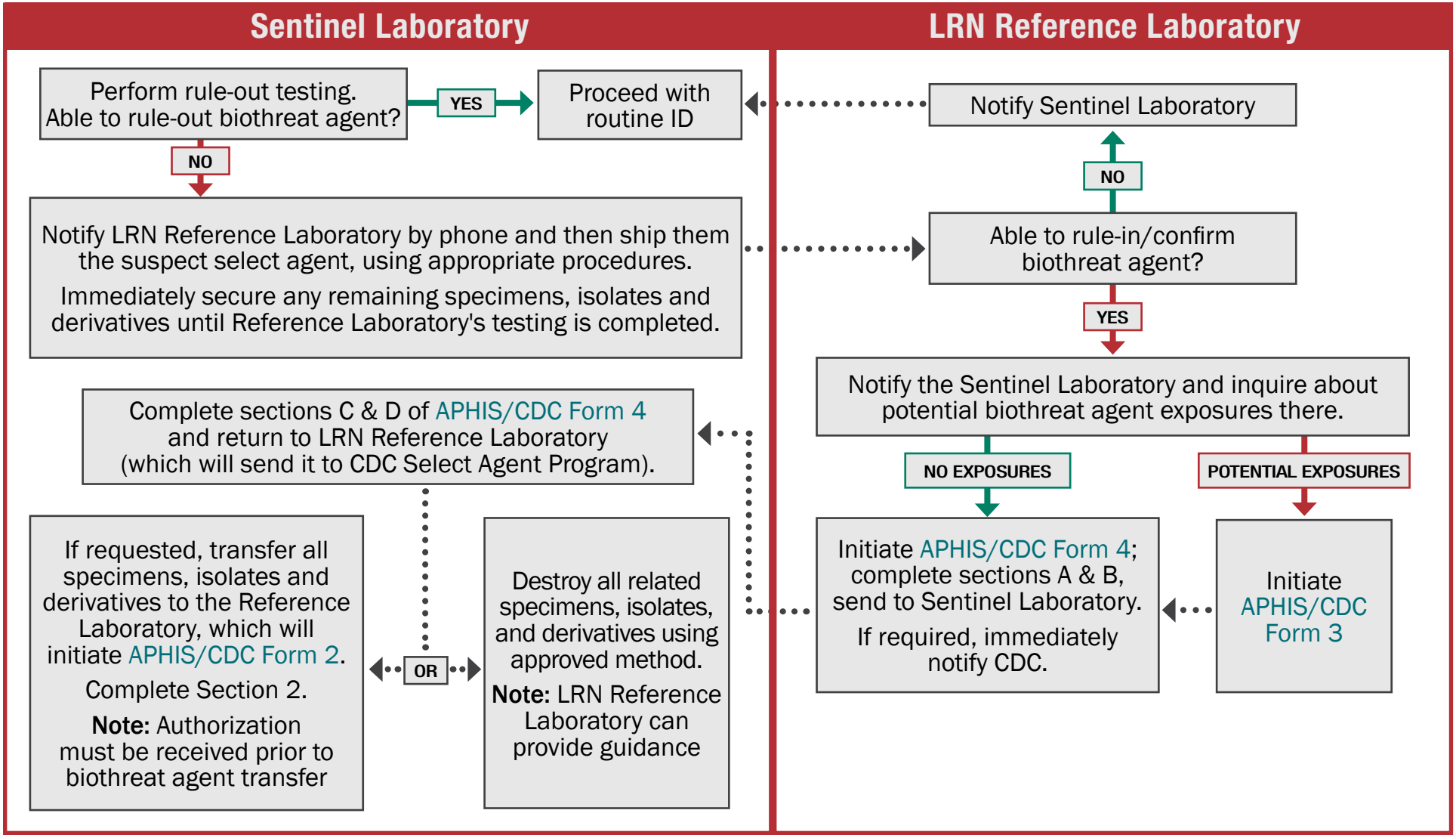
RESPONDING TO A BIOTHREAT AGENT

Biothreat Agent Response Algorithm



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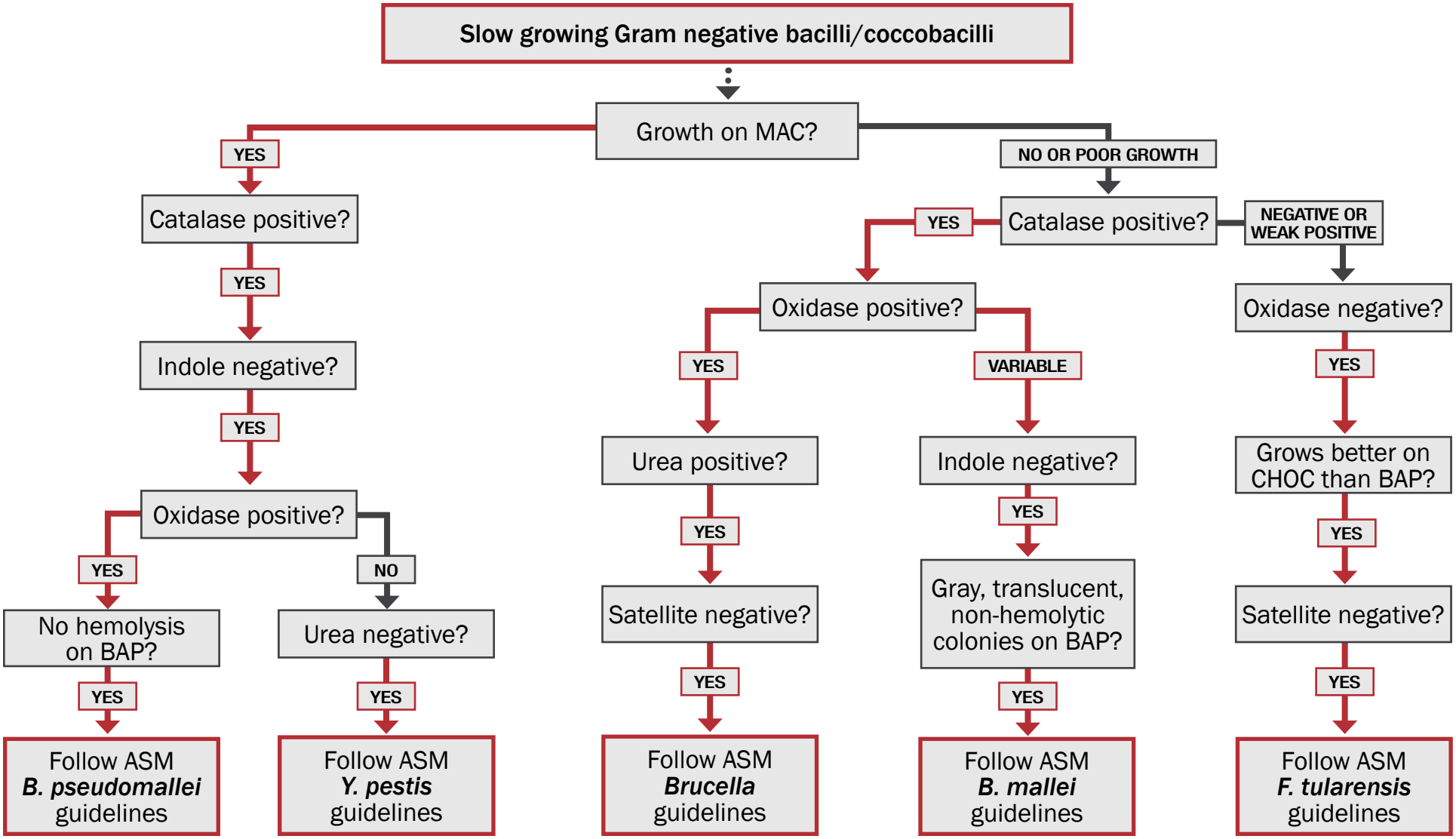
BIOTHREAT AGENT IDENTIFICATION

Gram Negative Bacilli/Coccobacilli Rule-Out Algorithm



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ANTHRAX — *Bacillus anthracis*

Handling Instructions

Safety

Patient specimens can be handled using **BSL-2 practices**.

As soon as *B. anthracis* is suspected, perform all further work within a Class II BSC using BSL-3 practices, especially when performing activities with a high potential for aerosol or droplet production.

Potential Lab Exposures

Ingestion, inhalation, inoculation and direct contact via skin abrasions and mucous membranes.

Specimen Collection			Ideal Time & Temp	
			Transport Within Facility	Storage
Cutaneous	Vesicular Stage	Collect fluid from intact vesicles on sterile swab(s). The organism is best demonstrated in this stage.	≤2 h RT	≤24 h RT
	Eschar Stage	Without removing eschar, insert swab beneath the edge of eschar, rotate and collect lesion material.	≤2 h RT	≤24 h RT
Gastrointestinal	Stool	Collect 5-10 g in a clean, sterile, leak proof container.	≤1 h RT	≤24 h 4°C
	Blood	Collect per institution's procedure for routine blood cultures.	≤2 h RT	Incubate per lab protocol
Inhalation	Sputum	Collect expectorated specimen into a sterile, leak proof container.	≤2 h RT	≤24 h RT
	Blood	Collect per institution's procedure for routine blood cultures.	≤2 h RT	Incubate per lab protocol

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ANTHRAX — *Bacillus anthracis*

Characterization

Gram Stain

- Large Gram positive rods (1-1.5 µm x 3-5 µm)
- Direct smears of clinical specimens:
  - Short chains (2-4 cells)
  - Capsule present
  - No spores present
- Smears from culture (BAP or CHOC):
  - Long chains
  - No capsule present
  - Spores in older cultures: oval, central to subterminal, no swelling of cell wall

Colony Morphology

- Grows well on BAP and CHOC
- Aerobic rapid growth as early as 4-8h
- Colonies 2-5 mm on BAP and CHOC at 24h
- No growth on MAC and EMB
- Flat or slightly convex with irregular edges that may have comma-like projections
- Ground-glass appearance
- Gamma hemolytic (non-hemolytic) on BAP
- Tenacious, sticky colonies, adheres to agar surface

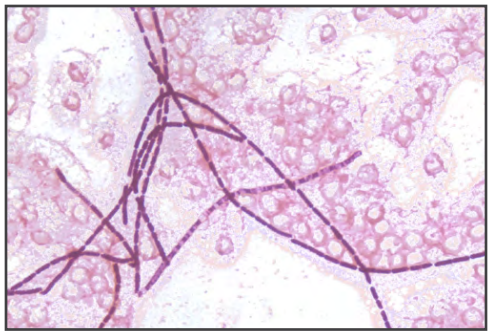
Biochemical/Test Reactions

- Catalase positive
- Non-motile

Common Misidentifications

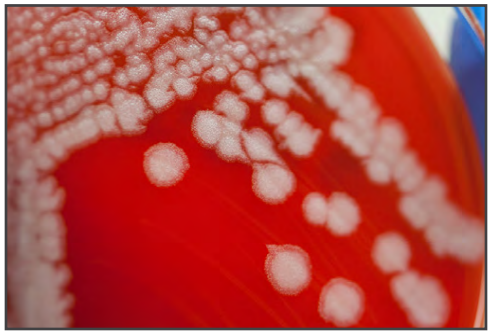
May not be identified in common automated ID systems, including MALDI-TOF, and possible misidentifications include *Bacillus megaterium* and other *Bacillus* species.

Note: *Bacillus cereus* Group includes *B. anthracis*, but automated ID systems may not alert microbiologist beyond this group identification.



Gram stain of blood culture

24h growth on BAP



Irregular-edged colonies

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ANTHRAX — *Bacillus anthracis*

Rule-Out Algorithm

SAFETY

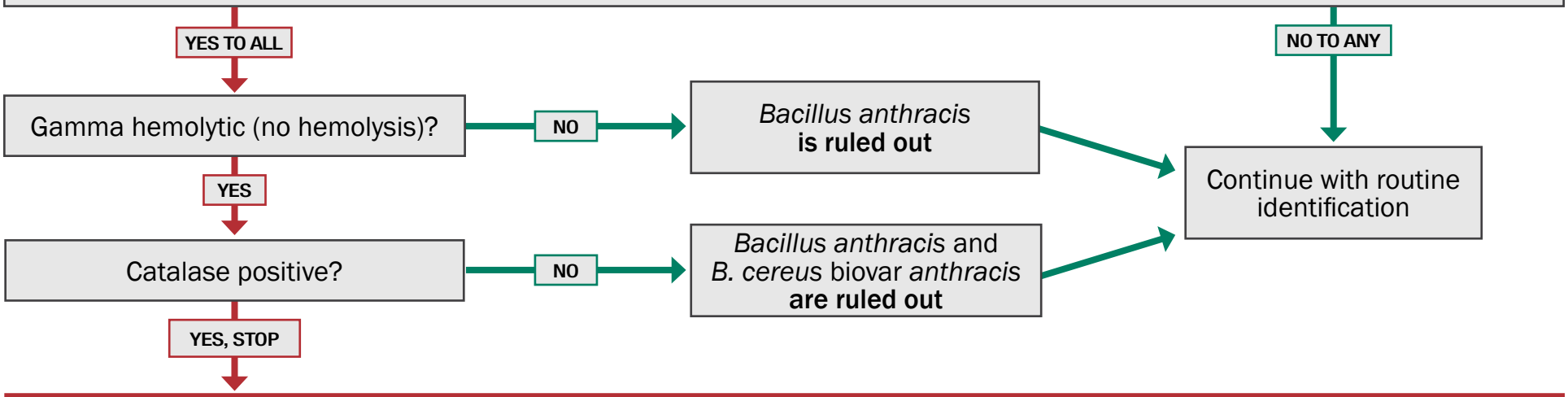
As soon as *B. anthracis* or *B. cereus* biovar *anthracis* is suspected **perform all further work in a Class II BSC using BSL-3 practices.** If *B. anthracis* or *B. cereus* biovar *anthracis* cannot be ruled out with the tests below, **do not attempt further ID** using commercial automated or kit identification systems.

Gram stain morphology

- ☐ Large, Gram positive rods?
  Note: Spores may be found in cultures grown in 5% CO_2 or ambient atmosphere but not usually observed in clinical samples.

Colony morphology

- ☐ Ground glass appearance?
- ☐ Non-pigmented, gamma hemolytic (no hemolysis) on BAP?
  Note: Some strains of *B. cereus* biovar *anthracis* may be weakly hemolytic after 48h
- ☐ No growth on MAC (or EMB)?



***B. anthracis* or *B. cereus* biovar *anthracis* not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *Bacillus anthracis* or *B. cereus* biovar *anthracis* submitted to LRN Reference Level Laboratory for confirmatory testing.

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ANTHRAX — *Bacillus cereus* biovar *anthracis*

Characterization

Characteristic	*B. anthracis*	*B. cereus*	*B. cereus* biovar *anthracis*	
			CI [1]	CA [2]
Hemolysis [3]	—	+	—	—
Motility [4]	—	+	+/—	
Gamma phage susceptibility [5]	+	—	—	—
Penicillin G [6]	S	R	S	R
Capsule [7]	+	Absent *in vitro*	+	+

[1] Côte d'Ivoire strains, from chimpanzees

[2] Cameroon strains, from gorillas or chimpanzees

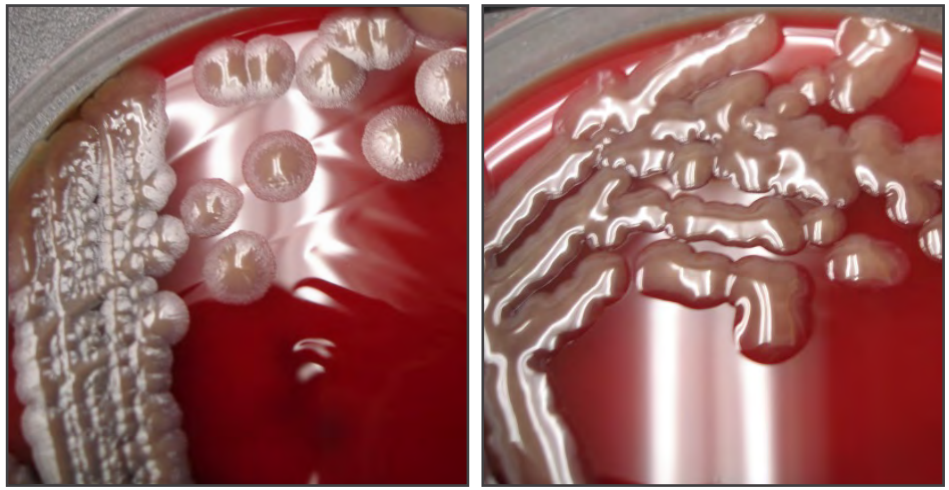
[3] Hemolysis
+.....beta hemolytic on sheep blood agar
–.....non-hemolytic

[4] Motility
+.....motile
–.....non-motile
+/– ...*B. cereus* biovar *anthracis* strains are usually **motile**, including those recovered from gorillas, chimpanzees, and elephants. *B. cereus* biovar *anthracis* goat strains from Democratic Republic of the Congo were **non-motile**.

[5] Gamma phage susceptibility
+.....susceptible
–.....resistant

[6] Penicillin G
S.....susceptible
R .....resistant

[7] Capsule
+.....Present



24 h growth on BAP, 5% CO_2 of CI (left) and CA (right) strains

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ANTHRAX — *B. anthracis* & *B. cereus* biovar *anthracis*

Recommendations

Sentinel-level laboratories should continue using the existing ASM Sentinel Level Clinical Laboratory Guideline for *B. anthracis* to rule out or refer isolates of *Bacillus* spp. that produce non-hemolytic colonies with a ground glass appearance and are non-motile. Until new guidelines are available, the following recommendations should be considered:

1. Suspect *Bacillus* spp. isolates that are large, catalase positive, Gram positive rods, and non-hemolytic at 24h incubation in ambient atmosphere or 5% CO_2 should be tested for motility. Isolates can appear weakly hemolytic upon extended incubation (48h) in ambient atmosphere and are more hemolytic in 5% CO_2 at 48h. Semi-solid medium is recommended for motility to ensure consistent results.

2. Suspect isolates should be investigated to determine if the isolate is significant regardless of motility. If the isolate was recovered from a sterile site or from a wound culture, follow the local public health guidelines to assess whether the public health lab or clinical lab should contact the patient's attending physician to determine the likely clinical significance (e.g., does the patient have an anthrax-like clinical syndrome?). Appropriate travel history should be obtained as well. If the isolate is deemed significant, the local LRN reference laboratory should be contacted to obtain guidance regarding the need to refer the isolate for confirmatory testing.

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BRUCELLOSIS — *Brucella* spp.

Handling Instructions

Safety

Patient specimens can be handled using **BSL-2 practices**.

As soon as *Brucella* spp. is suspected, perform all further work within a Class II BSC using BSL-3 practices, especially when performing activities with a high potential for aerosol or droplet production.

Potential Lab Exposures

Ingestion, inhalation, inoculation and direct contact via skin abrasions and mucous membranes. *Brucella* spp. have a very low infectious dose and laboratory workers can acquire brucellosis from direct exposure to samples or cultures.

Specimen Collection			Ideal Time & Temp	
			Transport Within Facility	Storage
Acute, Subacute or Chronic	Serum	Collect at least 1 mL acute phase specimen without anti-coagulant as soon as possible after disease onset. Collect a second, convalescent specimen 14-21 days after acute specimen collection.	~2 h RT	-20°C
	Blood	Collect per institution's procedure for routine blood cultures. **Note:** Slow-growing in automated blood culture systems, consider extended incubations up to 2-3 weeks.	≤2 h RT	Incubate per lab protocol
	Bone Marrow	Collect per institution's surgical or pathology procedure.	≤15 min RT	≤24 h 4°C
	Spleen or Liver	Collect tissue samples at least the size of a pea. Submit in sterile container. May add 1-2 drops of saline to keep moist.	≤1 h RT	≤24 h RT

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BRUCELLOSIS — *Brucella* spp.

Characterization

Gram Stain

- Faintly staining, not clustered, tiny Gram negative coccobacilli (0.4 µm-0.8 µm)
- May retain crystal violet stain and may be mistaken for Gram positive cocci

Biochemical/Test Reactions

- Catalase, oxidase and urea positive

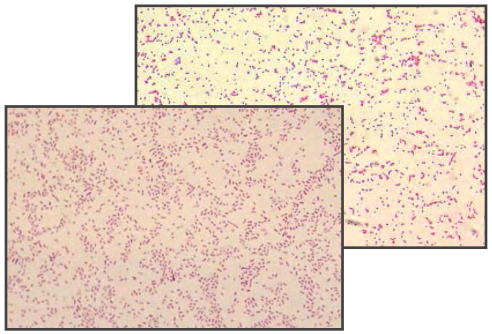
  Note: Oxidase may be variable and test should be performed on fresh cultures (18-24h)
- *S. aureus* streak negative (X & V Factor satellite test)

Colony Morphology

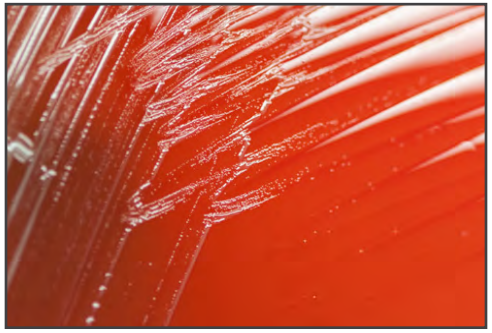
- Aerobic, slow growth
- Slow growth seen on BAP and CHOC (CO_2 may be required by some strains)
- Poor to variable growth on MAC. Pinpoint colonies may infrequently be observed with some strains after extended blood culture incubation (7 days)
- Non-mucoid
- Pinpoint colonies at 24h, and easily visible, discrete, white, non-hemolytic colonies at 48h (0.5 mm-1 mm)
- Colonies on BAP have no distinguishing features. They will appear as white, non-pigmented and non-hemolytic. Colonies will appear as raised and convex with an entire edge and shiny surface

Common Misidentifications

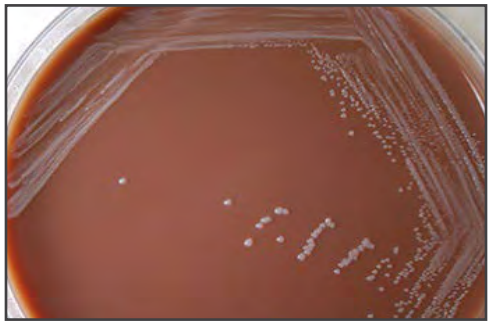
May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Moraxella* spp., *Micrococcus* spp., *Corynebacterium* spp., "slow growing" *Staphylococcus* spp., *Oligella ureolytica, Bordetella bronchiseptica, Haemophilus* spp., *Pasteurella* spp., *Psychrobacter phenylpyruvicus* and *Psychrobacter immobilis.*



Gram Stain



48h growth on BAP



72h growth on CHOC

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BRUCELLOSIS — *Brucella* spp.

Rule-Out Algorithm

SAFETY

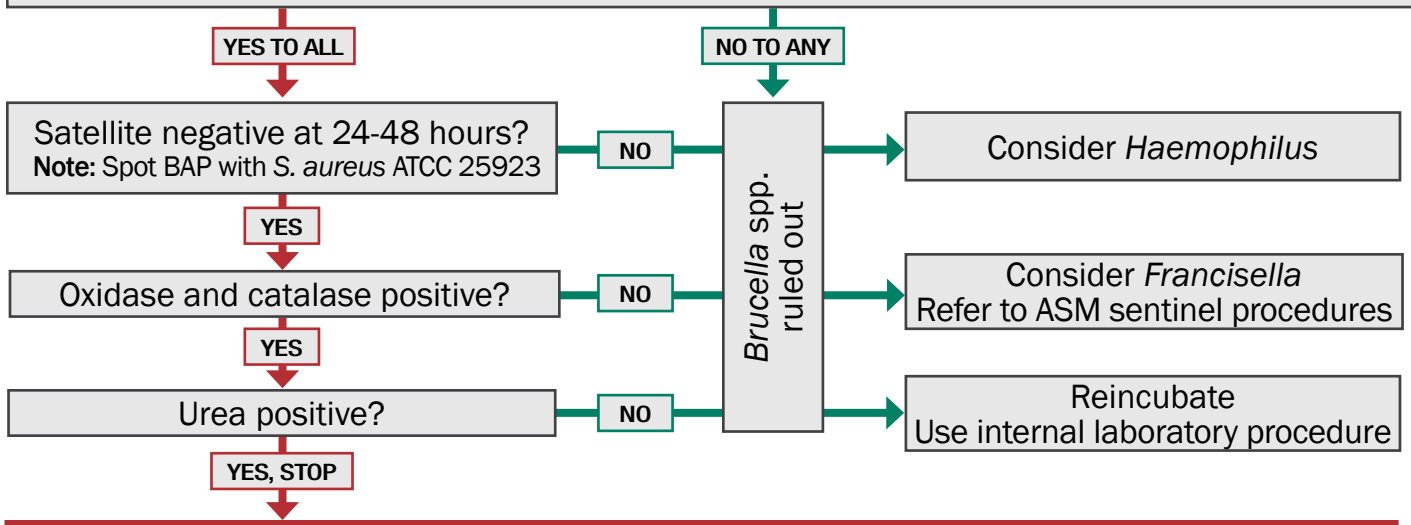
As soon as *Brucella* is suspected, perform **all further work in a Class II BSC using BSL-3 practices.** If *Brucella* spp. cannot be ruled out with tests below, **do not attempt further ID** using commercial automated or kit identification systems.

Gram stain morphology

- ☐ Faint staining, not clustered, tiny (0.4 x 0.8µm), Gram negative coccobacilli?
  Note: May retain crystal violet stain and be mistaken for Gram positive cocci

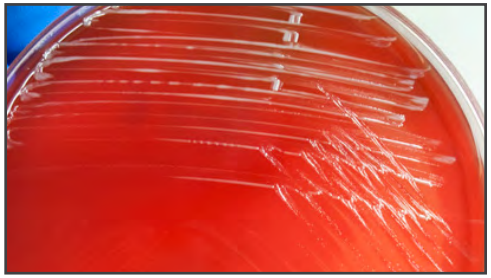
Growth

- ☐ Subculture positive aerobic blood culture to BAP, CHOC?
- ☐ Aerobic, slow, poorly growing colonies after 24h incubation in 5-10% CO_2 at 35°C?
  Note: Incubate plates for at least two additional days if no growth in 24h.
- ☐ Organism not growing on MAC?
- ☐ Slow growing in automated blood culture systems?
  Note: Consider extended incubations up to 2-3 weeks.



***Brucella* spp. not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *Brucella* spp. submitted to LRN Reference Level Laboratory for confirmatory testing.

24h growth on CHOC



48h growth on BAP

Revised 3/2018

GLANDERS — *Burkholderia mallei* & MELIOIDOSIS — *Burkholderia pseudomallei*

Handling Instructions

Safety

Patient specimens can be handled using **BSL-2 practices**.

As soon as *B. mallei* or *B. pseudomallei* are suspected, perform all further work within a Class II BSC using BSL-3 practices, especially when performing activities with a high potential for aerosol or droplet production.

Potential Lab Exposures

Ingestion, inhalation, inoculation, and direct contact via skin abrasions and mucous membranes.

Specimen Collection		Ideal Time & Temp	
		Transport Within Facility	Storage
Blood or Bone Marrow	Collect using standard automated blood culture system per institution's procedure for routine blood culture.	≤2 h RT	Delayed entry depends on instrument
Sputum/Bronchial	Collect into sterile leak proof container.	≤2 h RT	≤24 h 4 °C
Abscess Material and Wounds	Tissue aspirate, tissue fluid preferred to swab alternative.	≤2 h RT	≤24 h 4 °C
Urine	Collect at least 1 mL in leak proof container.	≤2 h RT	≤24 h 4 °C

Revised 3/2018

GLANDERS — *Burkholderia mallei*

Characterization

Gram Stain

- Small straight or slightly curved Gram negative coccobacilli (1.5 µm-3 µm x 0.5-1 µm) with rounded ends
- Cells arranged in pairs, parallel bundles, or the Chinese letter form

Colony Morphology

- Aerobic
- On BAP:
  - Pinpoint to small grey colonies at 24h that may become smooth, grey, and translucent at 48h with no distinctive odor
  - Non-hemolytic
- On MAC: No growth or pinpoint colorless colonies after 48h
- No pigment, even on Mueller Hinton agar
- No growth at 42˚C

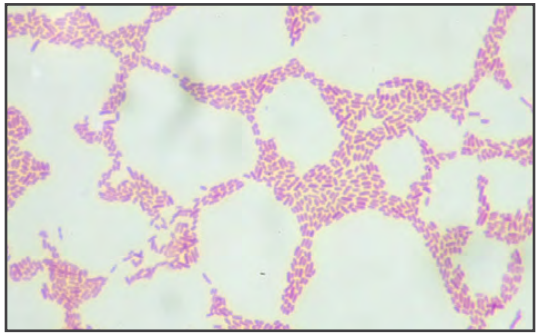
Biochemical/Test Reactions

- Catalase positive
- Oxidase variable; most are negative
- Spot indole negative
- Non-motile (Recommend tube test, not wet mount, due to potential aerosol production)
- Polymyxin B and colistin no zone, penicillin resistant, amoxicillin-clavulanate susceptible

Common Misidentifications

May not be identified in common automated ID systems, including MALDI-TOF, and possible misidentifications may include: *Burkholderia cepacia, Chromobacterium violaceum, Pseudomonas stutzeri, Bacillus* spp., *Pandoraea* spp., *Ralstonia* spp. other nonfermenting Gram negative bacilli.

Note: *B. pseudomallei* and *B. mallei* are arginine positive, unlike other *Burkholderia*; the arginine test may be in kit identification systems.



Gram Stain

24h growth on BAP

48h growth on BAP

Revised 3/2018

GLANDERS — *Burkholderia mallei*

Rule-Out Algorithm

SAFETY

As soon as *Burkholderia* is suspected, **perform all further work in a Class II BSC using BSL-3 practices.** If *B. mallei* cannot be ruled out with tests below, **do not attempt further ID** using commercial automated or kit identification systems.

Gram stain morphology

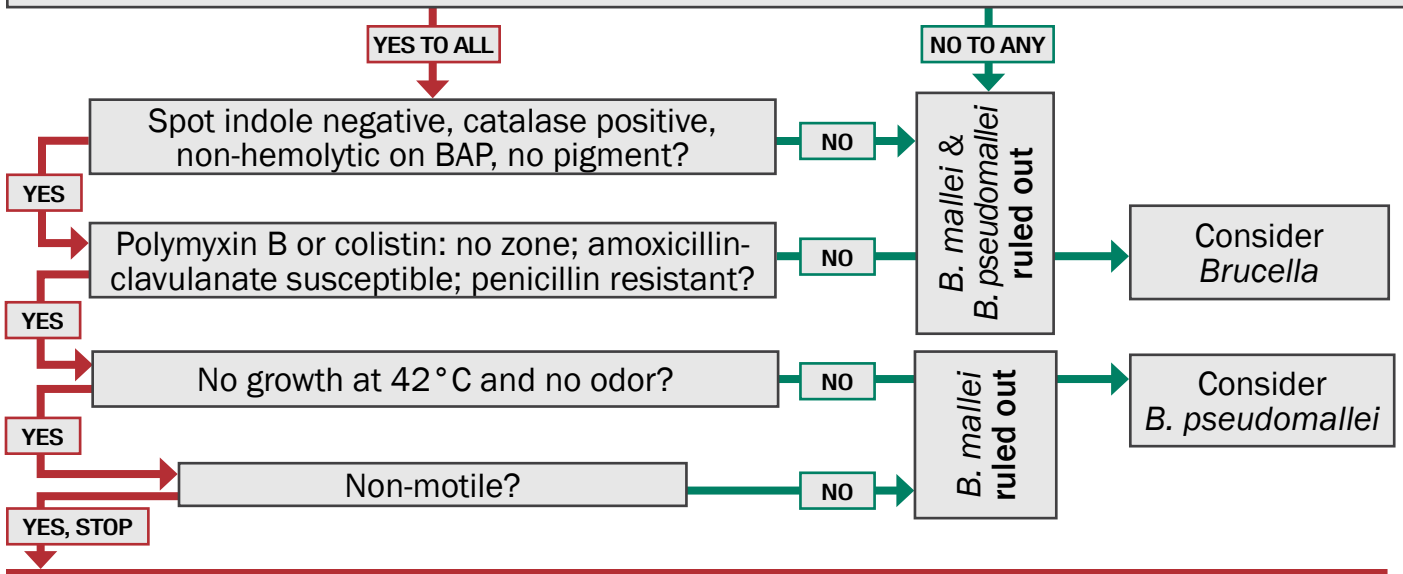
- ☐ Small straight or slightly curved Gram negative coccobacilli with rounded ends?
- ☐ Cells arranged in pairs, parallel bundles or the Chinese letter form?

Colony morphology

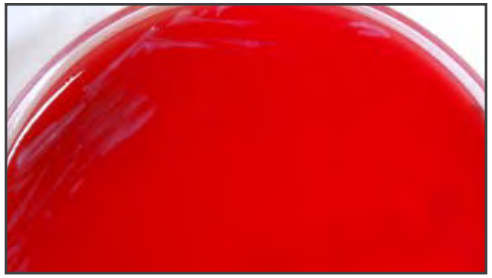
- ☐ Poor growth at 24h on all media?
- ☐ Better growth of grey, translucent colonies without pigment or hemolysis at 48h on BAP?
- ☐ Poor or no growth on MAC in 48h?
- ☐ No distinctive odor (from closed plate)?

Reactions

- ☐ Oxidase-variable?



***Burkholderia mallei* not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *Burkholderia mallei* submitted to LRN Reference Level Laboratory for confirmatory testing.



24h growth on BAP

48h growth on BAP

Revised 3/2018

MELIOIDOSIS — *Burkholderia pseudomallei*

Characterization

Gram Stain

- Straight, or slightly curved Gram negative rod (2-5 µm x 0.4-0.8 µm)
- Colonies may demonstrate bipolar morphology in direct specimens and peripheral staining in older cultures, which can mimic endospores

Colony Morphology

- Aerobic
- On BAP: small, smooth, creamy colonies in the first 1-2 days, that may gradually change in time to dry, wrinkled colonies (similar to *Pseudomonas stutzeri*)
- Poor growth at 24h, good growth at 48h
- Colonies are non-hemolytic and not pigmented on BAP or Mueller Hinton agar.
- Grows on MAC (may uptake pink dye)
- Distinctive musty, earthy odor is apparent without sniffing or opening plate
- Growth at 42˚C

Biochemical/Test Reactions

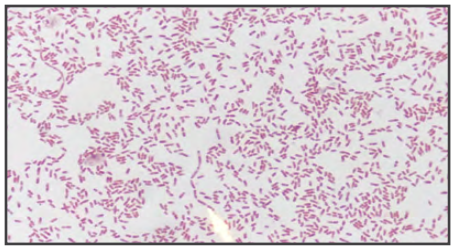
- Oxidase positive
- Spot indole negative
- Motile
  Note: Tube test, not wet mount, is recommended due to potential aerosolization
- Polymyxin B and colistin no zone, penicillin resistant, amoxicillin-clavulanate susceptible

Common Misidentifications

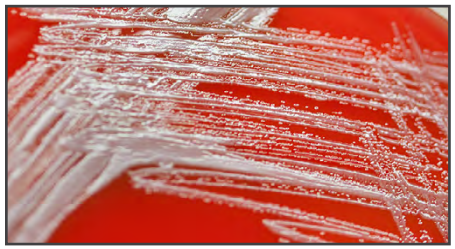
May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Burkholderia cepacia*, Chromobacterium violaceum, Pseudomonas aeruginosa, Pseudomonas stutzeri, S. maltophilia* and other nonfermenting Gram negative bacilli.

* *B. pseudomallei* is separated from *B. cepacia* by a susceptible amoxicillin-clavulanate test. Although rare in *B. pseudomallei*, resistance cannot rule out the identification.

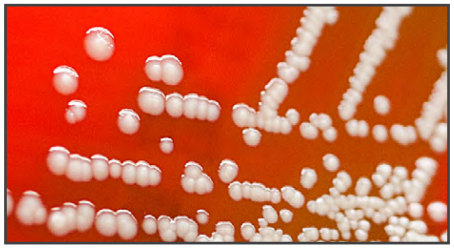
Note: *B. pseudomallei* and *B. mallei* are arginine positive, unlike other *Burkholderia;* arginine test may be in kit identification systems. Also, unlike *B. mallei, B. pseudomallei* grows at 42°C in 48h and is motile.



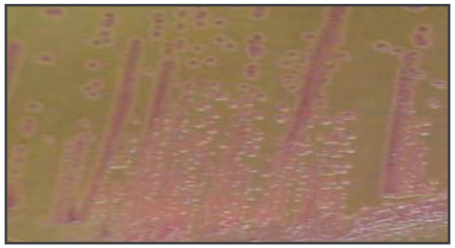
Gram Stain



24h growth on BAP



48h growth on BAP



48h growth on MAC

Revised 3/2018

MELIOIDOSIS — *Burkholderia pseudomallei*

Rule-Out Algorithm

SAFETY

As soon as *Burkholderia* is suspected, **perform all further work in a Class II BSC using BSL-3 practices.** If *B. pseudomallei* cannot be ruled out with tests below, **do not attempt further ID** using commercial automated or kit identification systems.

Gram stain morphology

- ☐ Gram negative rod, straight or slightly curved?

  Note: May demonstrate bipolar morphology at 24h and peripheral staining, like endospores, as cultures age.

Colony morphology

- ☐ Poor growth at 24h, but good growth of smooth, creamy colonies at 48h on BAP?

  Note: May develop wrinkled colonies in time

- ☐ Non-hemolytic?
- ☐ Strong musty/earthy odor (apparent without opening plate), growth on MAC in 48h?
- ☐ Non-pigmented on Mueller Hinton agar and BAP?

Reactions

- ☐ Oxidase positive, spot indole negative?

YES TO ALL → **NO TO ANY**

1. Growth on MAC? — NO → Rule out other agents such as *B. mallei, Brucella* and *Francisella*
   YES ↓
2. Oxidase positive and spot indole negative? — NO → Not *Burkholderia*
   YES ↓
3. Polymyxin B or colistin; no zone or growth on *B. cepacia* selective agars — NO → Not *Burkholderia*
   YES ↓
4. No hemolysis on BAP; not pigmented — NO → Consider *Chromobacterium violaceum* or indole-negative *Vibrio* spp.
   YES, STOP ↓

***Burkholderia pseudomallei* not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *Burkholderia pseudomallei* submitted to LRN Reference Level Laboratory for confirmatory testing.

24h growth on BAP

48h growth on BAP

Revised 3/2018

TULAREMIA — *Francisella tularensis*

Handling Instructions

Safety

Patient specimens can be handled using **BSL-2 practices**.

As soon as *F. tularensis* is suspected, perform all further work within a Class II BSC using BSL-3 practices, especially when performing activities with a high potential for aerosol or droplet production.

Potential Lab Exposures

Ingestion, inhalation, inoculation, and direct contact via skin abrasions and mucous membranes. *Francisella tularensis* has a very low infectious dose and laboratory workers can acquire Tularemia from direct exposure to samples or cultures.

Specimen Collection		Ideal Time & Temp	
		Transport Within Facility	Storage
Sputum or Throat	Collect routine throat culture using a swab or expectorated sputum collected into a sterile, leak proof container.	≤2 h RT	≤24 h 4°C
Bronchial or Tracheal Wash	Collect per institution's procedure in an area dedicated to collecting respiratory specimens under isolation or containment circumstances (i.e., isolation chamber or "bubble").	≤2 h RT	≤24 h 4°C
Blood	Collect per institution's procedure for routine blood cultures.	≤2 h RT	Incubate per lab protocol
Biopsy, Tissue, Scrapings, Aspirate or Swab	Submit in sterile container. For small tissue samples add several drops of sterile normal saline to keep tissue moist. For swabs, collect by obtaining firm sample of advancing margin of the lesion; place swab in transport package to keep moist with the transport medium inside packet.	≤2 h RT	≤24 h 4°C
Serum	Collect at least 1 mL without anticoagulant. Collect acute specimen as soon as possible after onset and a convalescent specimen >14 days after acute.	≤2 h RT	4°C

Revised 3/2018

TULAREMIA — *Francisella tularensis*

Characterization

Gram Stain

- Tiny, Gram negative coccobacilli (0.2-0.5 µm x 0.7-1.0 µm)
- Poor counterstaining with safranin (basic fuchsin counterstain may increase resolution)
- Pleomorphic
- Mostly single cells

Colony Morphology

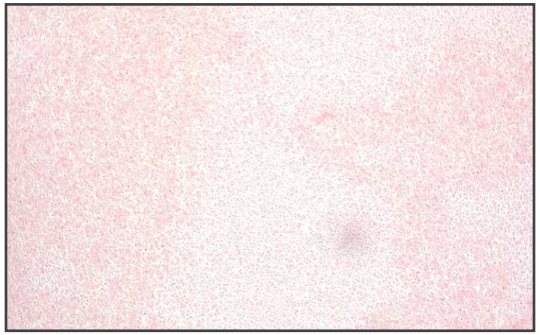
- Aerobic, fastidious
- No growth on MAC or EMB
- Scant or no growth on BAP; may grow on primary culture, not well on subculture
- Slow growing on CHOC, TM or BCYE: 1-2 mm after 48h
- Colonies are opaque, grey-white, butyrous with smooth and shiny surface

Biochemical/Test Reactions

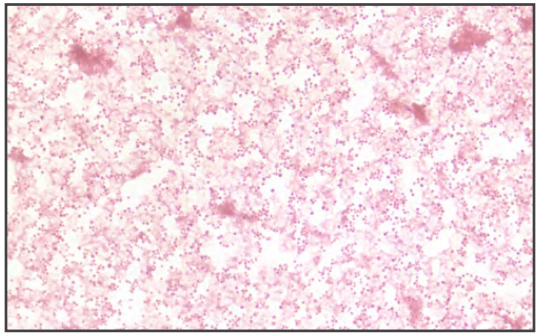
- Oxidase negative
- Catalase negative or weakly positive
- Satellite negative
- Beta-lactamase positive

Common Misidentifications

May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Aggregatibacter actinomycetemcomitans, Haemophilus influenzae, Oligella* spp. and *Psychrobacter* spp.



Gram Stain



Gram stain of a blood culture

48h growth on CHOC

Revised 3/2018

TULAREMIA — *Francisella tularensis*

Rule-Out Algorithm

SAFETY

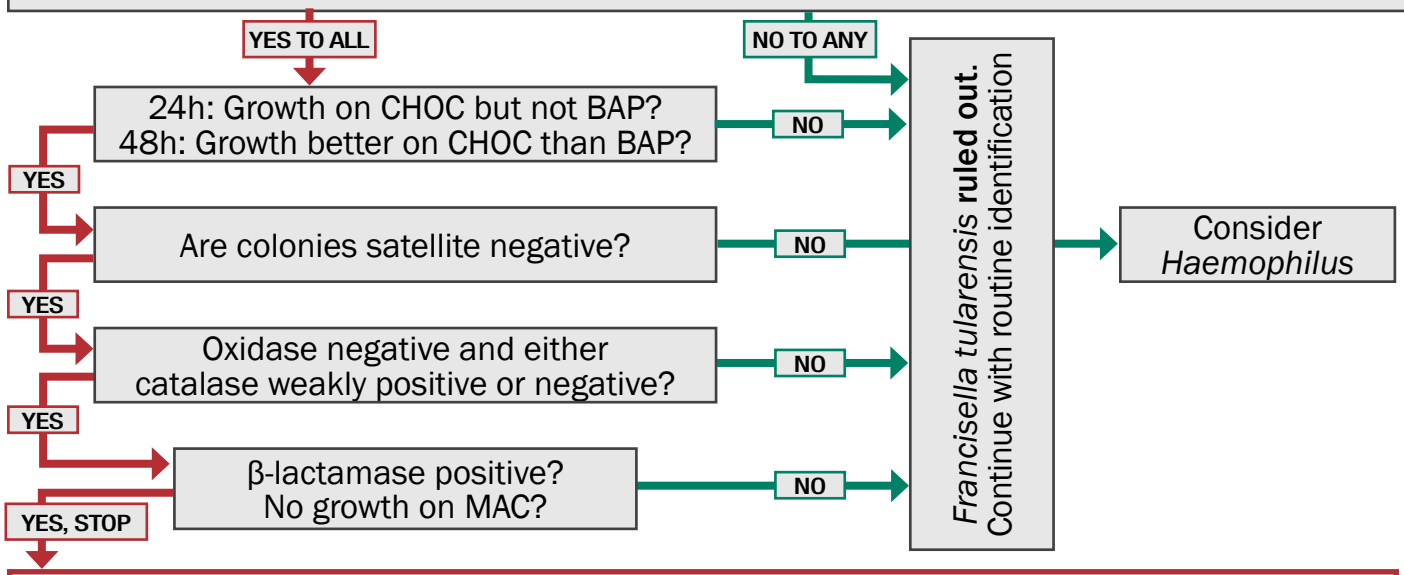
As soon as *Francisella* is suspected, **perform all further work in a Class II BSC** using BSL-3 practices. If *F. tularensis* cannot be ruled out with tests below, **do not attempt further ID** using commercial automated or kit identification systems.

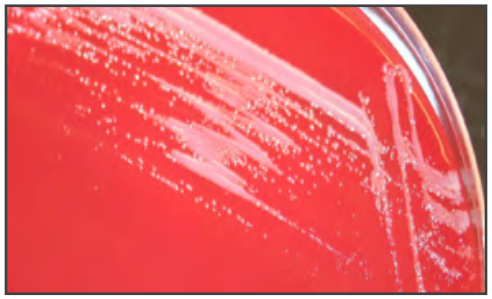
Gram stain morphology

- ☐ Pleomorphic?
- ☐ 0.2–0.5 µm by 0.7–1.0 µm faintly staining, Gram negative coccobacillus?
- ☐ Mostly single cells?

Colony morphology

- ☐ Aerobic and fastidious?
- ☐ No growth on MAC/EMB
- ☐ Scant to no growth on BAP after 48h? **Note:** may grow on primary BAP culture, but not on subculture.
- ☐ Slow growth on CHOC, TM or BCYE?
- ☐ 1-2 mm gray to grayish-white colonies on CHOC after 48h
- ☐ Colonies opaque, grey-white, butyrous with smooth and shiny surface?





48h growth on BAP

48h growth on CHOC

***Francisella tularensis* not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *F. tularensis* submitted to LRN Reference Level Laboratory for confirmatory testing.

Revised 3/2018

PLAGUE — *Yersinia pestis*

Handling Instructions

Safety

Patient specimens can be handled using **BSL-2 practices.**

As soon as *Y. pestis* is suspected, perform all further work within a Class II BSC using BSL-3 practices, especially when performing activities with a high potential for aerosol or droplet production.

Potential Lab Exposures

Ingestion, inhalation, inoculation, and direct contact via skin abrasions and mucous membranes.

Specimen Selection			Ideal Time & Temp	
			Transport Within Facility	Storage
Pneumonic	Sputum or Throat	Collect routine throat culture using a swab or expectorated sputum collected into a sterile, leak proof container	≤2 h RT	≤24 h 4°C
	Bronchial or Tracheal Wash	Collect per institution's procedure in an area dedicated to collecting respiratory specimens under isolation or containment circumstances (i.e., isolation chamber or "bubble")	≤2 h RT	≤24 h 4°C
Septicemic	Blood	Collect per institution's procedure for routine blood cultures	≤2 h RT	Incubate per lab protocol
Bubonic	Tissue or Aspirate	Submit in sterile container, may add 1-2 drops of saline to keep moist	≤2 h RT	≤24 h 4°C

Revised 3/2018

PLAGUE — *Yersinia pestis*

Characterization

Gram Stain

- Plump Gram negative rods (0.5 x 1-2 µm) seen mostly as single cells or pairs, and may demonstrate short chains in liquid media
- May exhibit bipolar, "safety-pin" appearance that is not seen on Gram stain, may be exhibited by Giemsa stain or Wright's stain

Colony Morphology

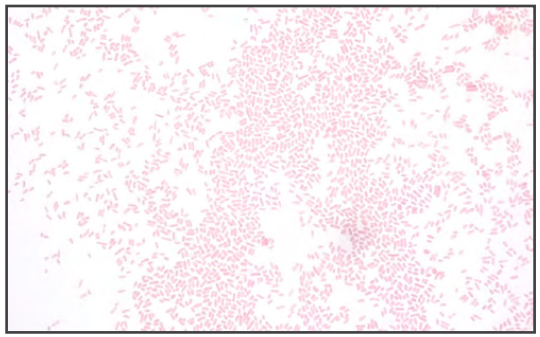
- Facultative anaerobe
- Slow growing at 35˚C, better growth at 25-28˚C
- Grey-white, translucent pinpoint colonies at 24h, usually too small to be seen
- On BAP:
  - After 48h: colonies approximately 1-2 mm in diameter, gray-white to slightly yellow and opaque
  - Older cultures (~96h): "Fried egg" or "hammered copper" appearance (under magnification)
  - Little to no hemolysis
- Lactose non-fermenter at 48h on MAC or EMB

Biochemical/Test Reactions

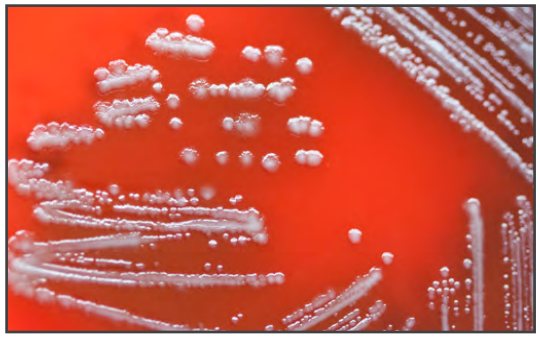
- Catalase positive
- Oxidase, urease (at 35˚C) and indole negative

Common Misidentifications

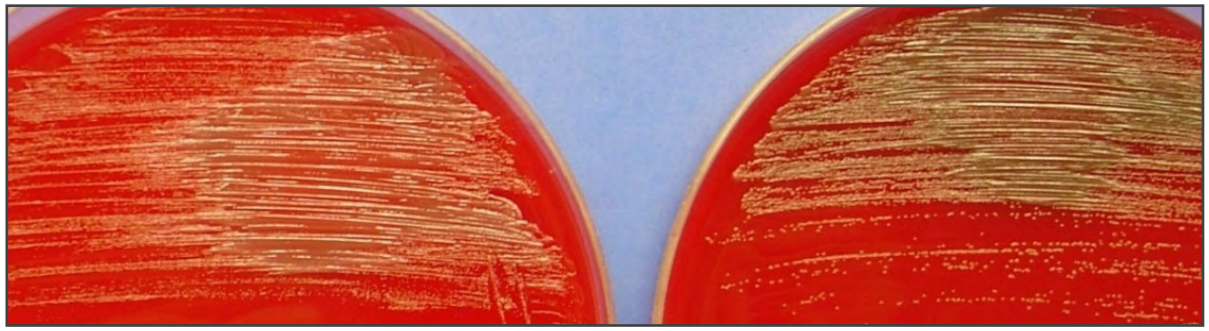
May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Shigella* spp., H_2S(-) *Salmonella* spp., *Acinetobacter* or *Pseudomonas* spp. and *Yersinia pseudotuberculosis.*



Gram Stain



48h growth on BAP



24h growth on BAP at 25°C (left) and 35°C (right)

Revised 3/2018

PLAGUE — *Yersinia pestis*

Rule-Out Algorithm

SAFETY

As soon as *Yersinia* is suspected, **perform all further work in a Class II BSC using BSL-3 practices.** If *Y. pestis* cannot be ruled out with tests below, **do not attempt further ID** using commercial automated or kit identification systems.

Gram stain morphology

- ☐ Gram-negative plump rods, 0.5 x 1-2 µm?
  Note: Seen mostly as single cells or pairs, and may demonstrate short chains in liquid media.

Colony morphology

- ☐ Facultative anaerobe?
- ☐ Slow growing at 35°C with better growth at 25-28°C?
- ☐ Either pinpoint colonies or no growth on BAP after 24h
- ☐ Colonies are 1-2 mm, gray-white to slightly yellow and opaque on BAP after 48h?
- ☐ Non-lactose fermenter on MAC/EMB?
- ☐ "Fried egg" or "hammered copper" on BAP in older cultures (~96h), when magnified?
- ☐ Little to no hemolysis on BAP?

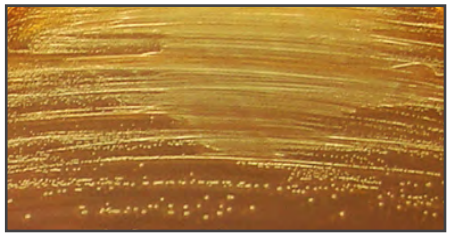
YES TO ALL → Oxidase negative, catalase positive and indole negative?

YES → Urease negative at 35°C?

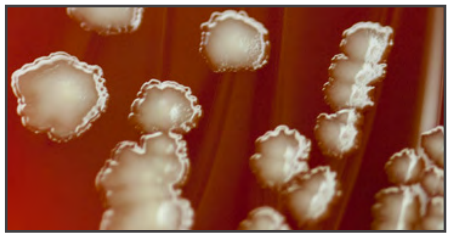
YES, STOP → ***Yersinia pestis* not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *Y. pestis* submitted to LRN Reference Level Laboratory for confirmatory testing.

NO → ***Yersinia pestis* ruled out.** Continue routine identification

NO TO ANY → ***Yersinia pestis* ruled out.** Continue routine identification



48h growth on MAC



Fried egg appearance at 96h (magnified)

Revised 3/2018

Acronyms

APHL ..... Association of Public Health Laboratories

ASM ..... American Society for Microbiology

BAP ..... Blood agar plate

BCYE ..... Buffered Charcoal Yeast Extract

BSC ..... Biological safety cabinet

BSL ..... Biosafety Level (1 - 4)

BT ..... Biothreat

CDC ..... Centers for Disease Control and Prevention

CHOC ..... Chocolate agar

EMB ..... Eosin Methylene Blue agar

LRN ..... Laboratory Response Network

MAC ..... MacConkey agar

MALDI TOF .... Matrix Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometer

NF ..... Non-fermentor

PPE ..... Personal Protective Equipment

RT ..... Room Temperature

TM ..... Thayer Martin agar

TTC ..... 2,3,5-Triphenyltetrazolium chloride

Revised 3/2018

APPENDIX

Terms and Definitions

Administrative controls
Changes in work procedures such as written safety policies, work practices, rules, supervision, schedules and training with the goal of reducing the duration, frequency and severity of exposures to hazardous materials or situations.

Aerobic
Requiring oxygen.

Aerosolization
The generation of liquid droplets or particles, five microns or less in diameter, that can be inhaled and retained in the lungs.

Anaerobic
Requiring the absence of oxygen.

Antimicrobial
An agent that kills microorganisms or suppresses their growth and multiplication.

Antiseptic
A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

Barriers
Any method used to separate workers, the outside community and the environment from hazardous material; includes primary and secondary barriers.

Barriers, Primary
Specialized laboratory equipment with engineering controls designed to protect against exposure to hazardous laboratory materials, including, but not limited to, biologic safety cabinets, chemical fume hoods, enclosed containers, bench shields, animal cages, and engineered sharps injury-protection devices (e.g., safety needles, safety scalpels, and sharps containers).

Barriers, Secondary
Facility design and construction features to include, but not be limited to, directional air flow, entrance airlocks, controlled-access zones, HEPA-filtered exhaust air, facility controls, decontamination equipment, eyewash stations, protective showers, and sinks for hand washing.

Biohazardous materials
Infectious agents or hazardous biologic materials that present a risk or potential risk to the health of humans, animals, or the environment. The risk can be direct through infection or indirect through damage to the environment. Biohazardous materials include certain types of recombinant DNA, organisms and viruses infectious to humans, animals, or plants (e.g., parasites, viruses, bacteria, fungi, prions, and rickettsia), and biologically active agents (e.g., toxins, allergens, and venoms) that can cause disease in other living organisms or cause significant impact to the environment or community.

BSL-1
Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment.

BSL-2
Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. (Most Sentinel Laboratory facilities fall under the definition of BSL-2).

BSL-3
Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.

Revised 3/2018

APPENDIX

Terms and Definitions

BSL-4
Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission.

Containment
Methods used to shield or protect personnel, the immediate work environment, and the community from exposure to hazardous, radiologic, chemical, or biologic materials.

Decontamination
The removing of chemical, biologic, or radiologic contamination from, or the neutralizing of it on, a person, object, or area. Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

Disinfectant
A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

Disinfection
A physical or chemical process of reducing or eliminating microorganisms from a surface or space, but not necessarily spores.

Droplet nuclei
The residue of dried droplets of infectious agents that is easily inhaled and exhaled and can remain suspended in air for relatively long periods or be blown over great distances.

Droplet spread
The direct transmission of an infectious agent by means of the aerosols produced in sneezing, coughing, or talking that travel only a short distance before falling to the ground.

Engineering controls
Refers to methods to remove a hazard or place a protective barrier between the worker and the workplace hazard, which usually involves building design elements and specialized equipment.

Exposure
Having come into contact with a cause of, or possessing a characteristic that is a determinant of, a particular health problem.

Fomite
An inanimate object that can be the vehicle for transmission of an infectious agent (e.g., bedding, towels or surgical instruments).

Incident
An unexpected event that causes or has the potential to cause loss, injury, illness, unsafe conditions, or disruptions to normal procedures.

Incubation period
The time interval from exposure to an infectious agent to the onset of symptoms of an infectious disease.

Infection
Invasion of the body tissues of a host by an infectious agent, whether or not it causes disease.

Medical surveillance
Monitoring of a person who might have been exposed to an infectious, chemical, radiologic, or other potentially causal agent, for the purpose of detecting early symptoms.

Mitigate
To correct identified deficiencies and to make a hazard less severe. This includes corrective actions taken as a result of an inspection or audit, or after an incident.

Mode of transmission
The manner in which an agent is transmitted from its reservoir to a susceptible host.

Revised 3/2018

APPENDIX

Terms and Definitions

Personal protective equipment (PPE)
Items worn by laboratory workers to prevent direct exposure to hazardous materials, including gloves, gowns, aprons, coats, containment suits, shoe covers, eye and face shields, respirators, and masks.

Risk
The probability that an event will occur (e.g., that a person will be affected by, or die from, an illness, injury, or other health condition within a specified time or age span).

Risk assessment
A process to evaluate the probability and consequences of exposure to a given hazard, with the intent to reduce the risk by establishing the appropriate hazard controls to be used.

Risk factor
An aspect of personal behavior or lifestyle, an environmental exposure, or a hereditary characteristic that is associated with an increase in the occurrence of a particular disease, injury, or other health condition.

Routes of exposure
Paths by which humans or other living organisms come into contact with a hazardous substance. Three routes of exposure are breathing (inhalation), eating or drinking (ingestion), and contact with skin (dermal absorption).

Sharps
Items capable of cutting or piercing human skin. Examples include hypodermic needles, syringes (with or without attached needles), Pasteur pipettes, scalpel blades, suture needles, blood vials, needles with attached tubing, and culture dishes (regardless of presence of infectious agents). Also included are other types of broken or unbroken glassware that have been in contact with infectious agents (e.g., used microscope slides and cover slips).

Sterilization
The use of physical or chemical process to completely destroy or eliminate all classes of microorganisms and spores.

Symptom
Any indication of disease noticed or felt by a patient.

Transmission (of infection)
Any mode or mechanism by which an infectious agent is spread to a susceptible host. Airborne transmission is the transfer of an agent suspended in the air (considered a type of indirect transmission). Direct transmission is the immediate transfer of an agent from a reservoir to a host by direct contact or droplet spread. Indirect transmission is the transfer of an agent from a reservoir to a host either by being suspended in air particles (airborne), carried by an inanimate objects (vehicleborne), or carried by an animate intermediary (vectorborne).

TTC
2,3,5-Triphenyltetrazolium chloride, indicator dye within motility test medium.

Universal precautions
Guidelines recommended by CDC for reducing the risk for transmission of bloodborne and other pathogens in hospitals, laboratories, and other institutions in which workers are potentially exposed to human blood and body fluids. The precautions are designed to reduce the risk for transmission of microorganisms from both recognized and unrecognized sources of infection in hospitals, laboratories, and other institutions to the workers in these facilities.

Virulence
The ability of an infectious agent to cause severe disease, measured as the proportion of persons with the disease who become severely ill or die.

Zoonosis
An infectious disease that is transmissible from animals to humans.

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APPENDIX

Identification Tests

Arginine Dihydrolase (Decarboxylase)

Look for pink/purple color change

Uninoculated Base

Positive Base

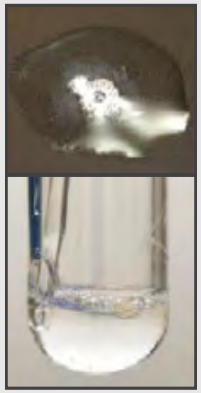
Negative Positive
Controls

NF Base Positive

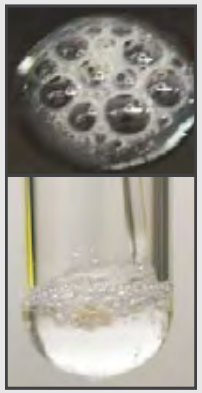
Catalase

3% Hydrogen peroxide: look for bubbles

Negative



Weak Positive



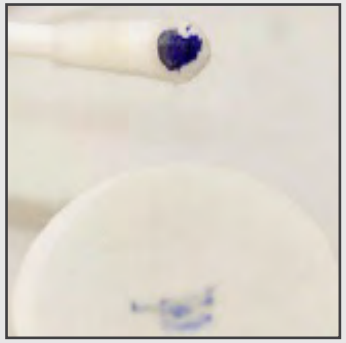
Positive

Safety Note: Recommended to perform this test in a BSC, covered petri dish or tube to contain aerosols

Oxidase

Tetramethyl reagent: look for purple color change

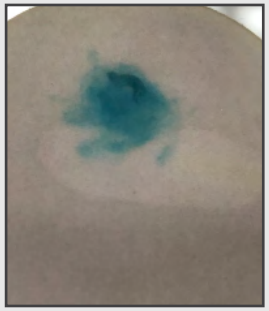
Negative



Positive

Spot Indole

Look for color change, varies by reagent; Cinnamaldehyde preferred

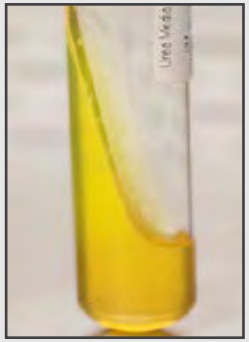


Cinnamaldehyde: positive is blue

Benzaldehyde: positive is pink

Urea

Look for pink color change



Negative

Positive

Revised 3/2018

APPENDIX

Identification Tests

Motility

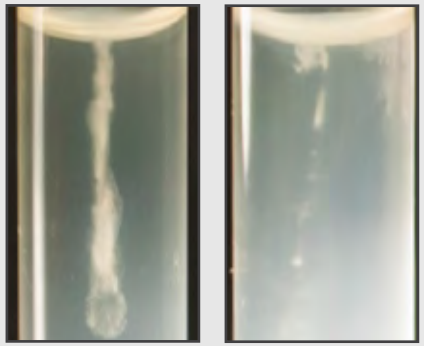
Negative (Non-motile)
Growth only in line of inoculum; no fuzziness or spreading; media is clear

Intermediate
Start to see growth outside line of inoculum (appears fuzzy), media still clear

Positive (Motile)
Distinct growth outside line of inoculum into the media, which is not clear

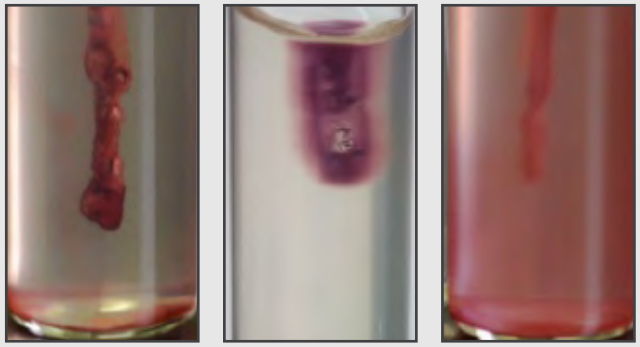
Safety Note: Avoid wet mount motility tests, which are hazardous due to the potential for creating an aerosol. Perform a tube motility test instead, and always in a BSC.

No Additives



Negative Positive

With 2,3,5-Triphenyltetrazolium chloride (TTC)



Negative Intermediate Positive

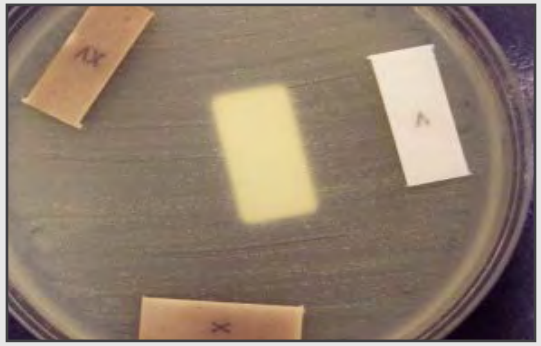
TTC: Colorless medium dye, turns red when reduced by bacteria. Inhibits some bacteria; look for growth away from line of inoculum.

X/V Factor Satellite Test

Use *Staphylococcus aureus*-streaked media or X and V growth factor-impregnated discs

Negative
Growth is not isolated to area immediately adjacent to *S. aureus* streak or X and V factors

Positive (Satellite)
Growth occurs only along *S. aureus* streak/ X and V factors



Negative: *Brucella* growing across entire plate

Positive: *Haemophilus* growing only around the *Staphylococcus aureus* streak

Revised 3/2018

APPENDIX

Resources

APHL

Public Health Preparedness & Response Program
aphl.org/programs/preparedness/Pages/default.aspx

Lab Biosafety & Biosecurity Resources
aphl.org/programs/preparedness/Biosafety-and-Biosecurity/Pages/BB-Resources.aspx

National Laboratory Training Network (NLTN)
aphl.org/training/Pages/overview.aspx

State Public Health Laboratories Emergency Contact Directory
aphl.org/programs/preparedness/Crisis-Management/Pages/Emergency-Lab-Contacts.aspx

Training Department
aphl.org/training/Pages/default.aspx

ASM

Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases
(Includes sentinel laboratory definition & emergency contacts)

asm.org/index.php/guidelines/sentinel-guidelines

CDC

Biosafety in Microbiological and Biomedical Laboratories (5th Edition)
cdc.gov/biosafety/publications/bmbl5/

Federal Select Agent Program
selectagents.gov

Federal Select Agent Program Forms
selectagents.gov/forms.html

Morbidity and Mortality Weekly Report,"Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories."
cdc.gov/mmwr/preview/mmwrhtml/su6101a1.htm

CDC TRAIN
cdc.train.org/DesktopShell.aspx

New York State Dept. of Health, Wadsworth Center

Basic Select Agent Flow Chart & Evaluation (B-SAFE) Bench Cards

- health.ny.gov/guidance/oph/wadsworth/final_card.pdf
- health.ny.gov/guidance/oph/wadsworth/

State Hygienic Laboratory at the University of Iowa

Education/Training Resources
shl.uiowa.edu/edtrain/index.xml

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Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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