



Bench Guide for Bioterrorism Agents

**Wisconsin State Laboratory of Hygiene 24/7 Emergency Messaging Center:
608-263-3280**

Front Cover

WARNING!
PERFORM **ALL**
ADDITIONAL
WORK IN A BSC
ONCE A
BIOTERRORISM
AGENT IS
SUSPECTED.

WARNING!
MANIPULATE
CULTURES NO
MORE THAN
NECESSARY
ONCE A
BIOTERRORISM
AGENT IS
SUSPECTED.

WARNING!
USE OF
AUTOMATED ID
SYSTEMS IS **NOT**
RECOMMENDED
IF A
BIOTERRORISM
AGENT IS
SUSPECTED.

Warnings

BIOTERRORISM AGENT WORKUP TABLE

Gram Stain Morphology	Growth			Rule out
	Sheep Blood Agar	Chocolate Agar	MAC/EMB	
Gram Positive Rod				
Large gram positive rods. Subterminal spores may be present with no significant swelling of the cell. Capsule may be visualized as a clear area around the bacillus. (Page 3).	Good growth at 24 hrs. 2-8 mm slightly convex colonies with irregular borders, "Medusa head" shape, ground glass color. No or very little hemolysis. (Page 6)	See SBA	NG	<i>B. anthracis</i> (Page 6,7)
Gram Negative Bacilli				
Gram negative rod, "enteric-like". Single, pairs, short chains. "Safety pin" stain may occasionally be seen. (Page 4)	No or very little detectable growth at 24 hours. Gray-white to slightly yellow and opaque, nonhemolytic colonies 1-2 mm in diameter appear after 48 hrs. Colonies have a "fried egg" appearance at 4x magnification.	See SBA	Small nonlactose fermenting colonies at >48 hrs.	<i>Y. pestis</i> (Page 8)
Gram Negative Coccobacilli				
Gram negative coccobacillus, small, lightly stained. (Page 5)	Very little/no growth at 24 hrs. Poor growth at 48 hrs. Colonies are nonpigmented and nonhemolytic; appear as punctate colonies after 48 hrs.	Better growth than on SBA (<i>F. tularensis</i>) >48 hrs. 1-2 mm diameter, blue-gray to gray in color, flat in elevation with an entire, smooth and shiny surface.	Some strains may grow (Brucella); NG (<i>F. tularensis</i>)	<i>Brucella</i> (Page 9) and/or <i>F. tularensis</i> (Page 10)

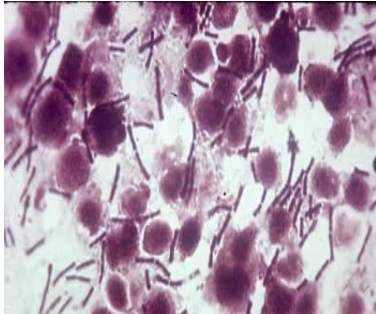
MAC/EMB = MacConkey Agar / Eosin-methylene blue Agar

WORK UP ALL SLOW GROWING, GRAM NEGATIVE ORGANISMS IN A CLASS 2 BSC.

Summary

GRAM POSITIVE (+) ROD

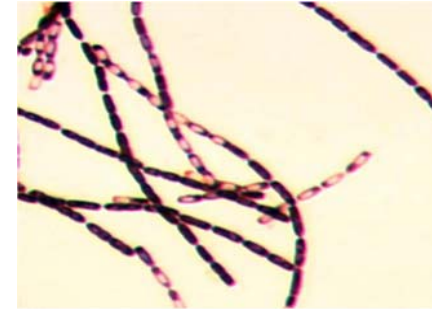
(large; single or in chains; central to subterminal spores may be present in cultures with no significant swelling of the cell)



***B. anthracis*, Gram stain.**
Direct smear from
cutaneous lesion
(CDC)



***B. anthracis* from culture.**
Single cells & short chains,
(<http://www.state.sd.us/doh/Lab/BT/bacillusGram.htm>)



***B. anthracis*, Gram stain.**
with spores, 1000X
(ASM)

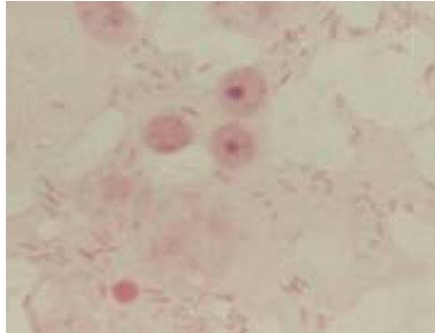
REFER TO *B. ANTHRACIS*

Wisconsin State Laboratory of Hygiene 24/7 Emergency Messaging Center: 608-263-3280

G+ Rod

GRAM NEGATIVE (-) ROD

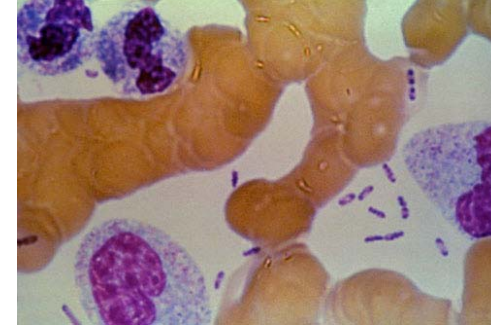
(Medium-sized; plump; mostly single cells; pairs or short chains from liquid media; stains well; bipolar “safety pin” stain may occasionally be seen)



Y. pestis, Gram stain.
1000X
(ASM)



Yersinia pestis,
Gram stain
(CDC Public Health Image
Library)



Y. pestis,
Wright stain, Bipolar staining
(CDC Public Health Image
Library)

Note: Bipolar staining reported with other enteric bacteria, e.g., Pasteurella spp.

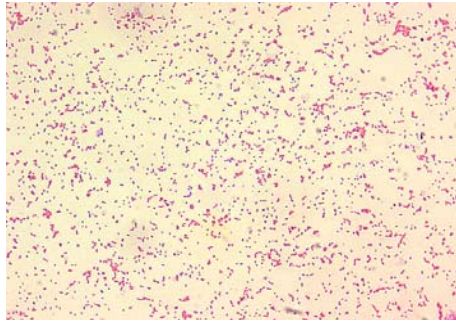
REFER TO *Y. PESTIS*

Wisconsin State Laboratory of Hygiene 24/7 Emergency Messaging Center: 608-263-3280

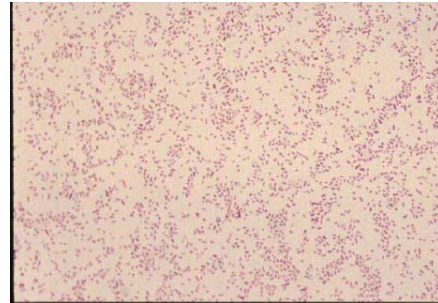
G- Rod

GRAM NEGATIVE (-) COCCOBACILLUS

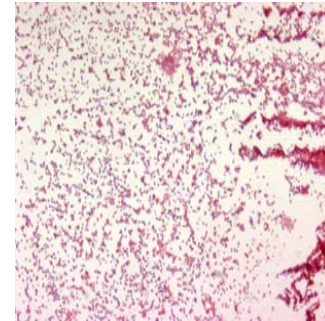
(tiny, stains poorly)



Brucella spp.
(CDC Public Health Image Library
Courtesy of Larry Stauffer, Oregon
State Public Health Laboratory)



Brucella
Gram stain
(ASM)



F. tularensis
(CDC Public Health Image Library
Courtesy of Larry Stauffer, Oregon
State Public Health Laboratory)

REFER TO *F. TULARENSIS* and *BRUCELLA SPP.*

Wisconsin State Laboratory of Hygiene 24/7 Emergency Messaging Center: 608-263-3280

G- Coccobacilli

B. ANTHRACIS-1

GRAM POSITIVE (+) ROD



Growth on Sheep Blood Agar
Rapid, non-pigmented, flat, slightly convex,
irregular edge, comma projections,
ground-glass surface,
tenacious (beaten egg white)



Hemolysis

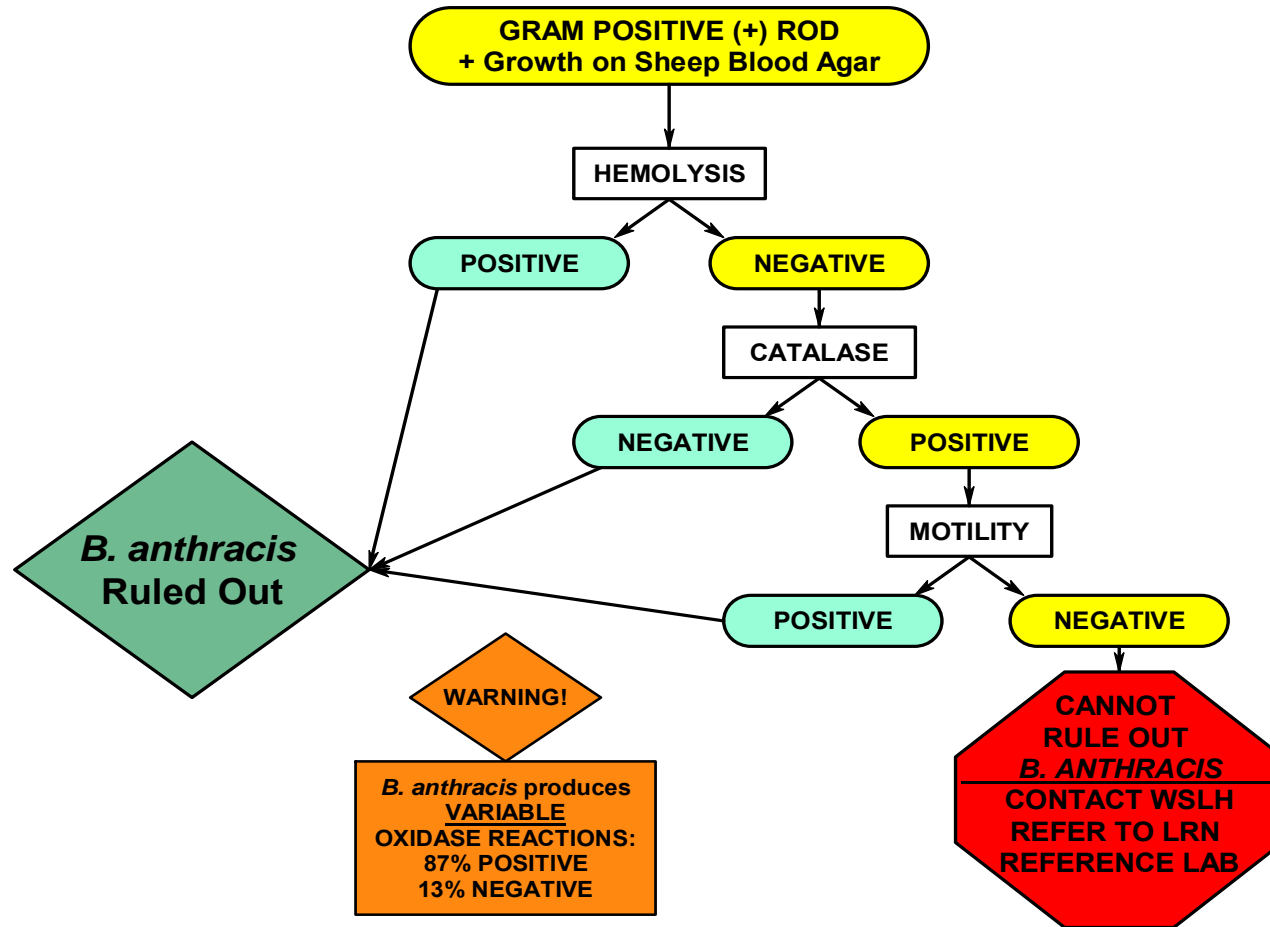
No Hemolysis

***B. anthracis*
Ruled Out**

REFER TO Pg 7-*B. ANTHRACIS-2*

B. anthracis -1

B. ANTHRACIS - 2



***B. anthracis* - 2**

Y. PESTIS

GRAM NEGATIVE (-) ROD
SLOW-GROWING, PINPOINT COLONIES, Gray-White to Opaque
on Sheep Blood Agar after 24 hours



Sheep Blood Agar- 48 hr

NO

YES

Proceed with
Routine ID
Procedures

OXIDASE: NEGATIVE
CATALASE: POSITIVE
UREASE: NEGATIVE
INDOLE: NEGATIVE

NO -
Features
NOT present

WARNING!

YES -
Features
present

Y. pestis
Ruled Out.
Continue ID

Automated ID Systems
May Key Out As
H₂S-negative Salmonella,
Shigella, Acinetobacter,
Y. pseudotuberculosis

**CANNOT
RULE OUT
Y. PESTIS
CONTACT WSLH
REFER TO LRN
REFERENCE LAB**

Wisconsin State Laboratory of Hygiene 24/7
Emergency Messaging Center: 608-263-3280

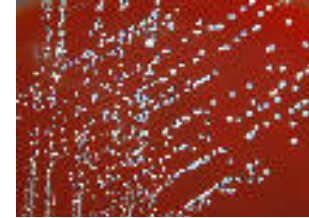
Y. pestis

BRUCELLA spp.

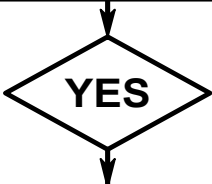


Sheep Blood Agar-48 hr

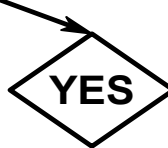
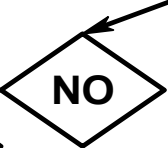
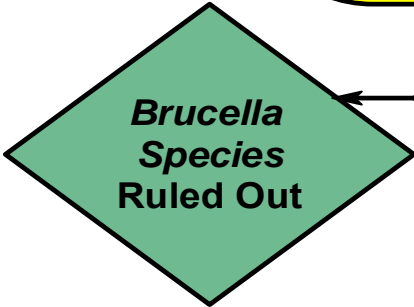
**GRAM NEGATIVE TINY COCCOBACILLI
POOR GROWTH on Sheep Blood Agar after 48 hr
Non-pigmented, moist, convex, non-hemolytic**



Sheep Blood Agar-48 hr



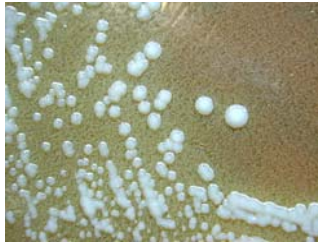
**X & V Factors NOT required.
OXIDASE: POSITIVE
CATALASE: POSITIVE
UREASE: POSITIVE**



Wisconsin State Laboratory of Hygiene 24/7
Emergency Messaging Center: 608-263-3280

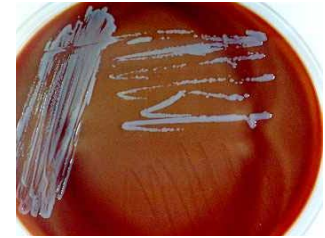
Brucella

F. TULARENSIS



Cysteine Heart Agar – 72 hr.

**GRAM NEGATIVE COCCOBACILLI
TINY, STAINS POORLY,
POOR GROWTH on Sheep Blood Agar after 48 hours
Blue-white to gray, flat, smooth, shiny, pleomorphic**



Chocolate Agar-72 hr

YES

**X & V Factors Not Required
OXIDASE: NEGATIVE
CATALASE: WEAK POSITIVE
B-lactamase: POSITIVE
UREASE: NEGATIVE**

NO

**F. tularensis
Ruled Out**

YES

**CANNOT
RULE OUT
F. TULARENSIS
CONTACT WSLH
REFER TO LRN
REFERENCE LAB**

**Wisconsin State Laboratory of Hygiene 24/7
Emergency Messaging Center: 608-263-3280**

F. tularensis

SPOT TESTS OF SELECTED ORGANISMS (adapted from CDC)

Organism	Gram Stain Morphology	Growth			Hemolysis	Motility	Oxidase	Catalase	Indole	Urease
		SBA	Choc	MAC/EMB						
<i>Bacillus anthracis</i>	Gram positive broad rod	+	+	—	—	—	+ V	+	—	—
<i>Brucella spp.</i>	Gram negative coccobacillus	+	+	V	—	—	+	+	—	+
<i>Francisella tularensis</i>	Gram negative coccobacillus	—	+	—	—	—	—	+	—	—
<i>Yersinia pestis</i>	Gram negative rod	+	+	+	—	—	—	+	—	—

+ = $\geq 90\%$ positive; — = $\leq 10\%$ positive; + V = most species/strains positive; —V = most species/strains negative; V, variable = 11-89% positive

SBA = Sheep Blood Agar; **Choc** = Chocolate Agar; **MAC/EMB** = MacConkey Agar / Eosin-methylene blue Agar

Spot Tests

BIOSAFETY and the PRIORITY AGENTS OF BIOTERRORISM (adapted from CDC)

Agent	Biosafety Level		Specimen Exposure/Risk	Recommended Precautions for Sentinel Laboratories	
	Specimen Handling	Culture Handling			
<i>Bacillus anthracis</i>	2	2	Blood, skin lesion exudates, CSF, pleural fluid, sputum, rarely urine & feces.	BSL2: Activities involving clinical material collection & diagnostic quantities of infectious cultures.	BSL3: Activities with high potential for aerosol or droplet production.
<i>Brucella spp.</i>	2	3	Blood, bone marrow, CSF, tissue, semen, occasionally urine.	BSL2: Activities limited to collection, transport & plating of clinical material.	BSL3: <u>All</u> activities involving manipulations of cultures.
<i>Clostridium botulinum</i>	2	2	Toxin may be present in food specimens, clinical material (serum, gastric & feces), & environmental samples (soil, surface water). TOXIN IS EXTREMELY POISONOUS!	BSL2: Activities with materials known or potentially containing toxin must be handled in a BSC (Class II) with lab coat, disposable gloves, & face shield (as needed).	BSL3: Activities with high potential for aerosol or droplet production.
<i>Francisella tularensis</i>	2	3	Skin lesion exudates, respiratory secretions CSF, blood, & urine. Tissues from infected animals & fluids from infected arthropods.	BSL2: Activities limited to collection, transport & plating of clinical material.	BSL3: All activities involving manipulations of cultures.
<i>Yersinia pestis</i>	2	2	Bubo fluid, blood, sputum, CSF, feces, urine.	BSL2: Activities involving clinical material collection & diagnostic quantities of infectious cultures	BSL3: Activities with high potential for aerosol or droplet production.

Safety