Select Agent Identification Chart

Select Agent	*Bacillus anthracis*		*Yersinia pestis*		*Francisella tularensis*		*Brucella* species		*Burkholderia mallei*		*Burkholderia pseudomallei*	
Illness	Anthrax		Plague		Tularemia		Brucellosis, Malta fever		Glanders		Meliodosis	
Signs and Symptoms	Cutaneous: eschar, edema Gastrointestinal: nausea, fever, abdominal pain, bloody diarrhea Inhalational: initial fever, malaise, some improvement, then abrupt respiratory distress		Fever, chills, headache, coughing up blood, buboes, sepsis		Ulceroglandular disease, sudden onset, chills, head cold, pharyngitis, cough, chest pain or tightness, possible skin lesions (cutaneous)		Acute: fever, sweats, headache, anorexia Chronic: mimic miliary TB		Cutaneous: nodules w/ localized lymphadenitis Systemic: broncho or lobar pneumonia, lesions in liver and spleen		Acute: pneumonia, high fever, dyspnea, chest pain Subacute: low grade fever, malaise, anorexia, weight loss Chronic: mimic miliary TB (Vietnamese time bomb)	
Zoonotic	Yes		Yes		Yes		Yes		Yes		Yes	
Transmission	Inhaling spores (e.g. from wool) or consuming undercooked meat from infected animals		Fleas, rodents, prairie dogs		Ticks, mosquitoes, fleas, contaminated hay, water, infected carcasses, infected animals, aerosolized particles, rabbits		Cattle (*B. abortus*), goats, sheep, camels (*B. melitensis*), pigs (*B. suis*), dogs (*B. canis*)		Equines (horses, mules, donkeys)		Soil, water (direct contact with), or aerosols from environmental sources	
Growth Media	Rec. Tests		Rec. Tests		Rec. Tests		Rec. Tests		Rec. Tests		Rec. Tests	
SBA		Growth 18 hr, ground glass		Growth 24-48 hr, gray-white to opaque		Scant to no growth 48-72 hr**		Growth 48-72 hr, white colonies		Growth 48 hr, gray, translucent colonies		Growth 24 hr, white colonies, wrinkled colonies > 72 hr
CHOC	*	Growth 18 hr	*	Growth 24-48 hr	*	Growth 48-72 hr, gray to grayish white colonies	*	Growth 48-72 hr	*	Growth 48 hr	*	Growth 24 hr
MAC		No growth		Growth 48 hr, clear, non-lactose fermenter		No growth		No growth		Poor to no growth 48 hr		Growth 24 hr
Gram stain, morphology, & size	*	Positive Large rods, spores may be present 1-1.5 x 3-5 µm	*	Negative Plump rods 1-2 x 0.5 µm	*	Negative Coccobacillus, faintly staining 0.2-0.5 x 0.7-1.0 µm	*	Negative, Coccobacillus, faintly staining 0.4 x 0.8 µm		Negative Coccobacillus or small rod 1.5-3 x 0.5-1 µm		Negative Straight or slightly curved rods 2-5 x 0.4-0.8 µm
Hemolysis	*	Non-hemolytic		Non-hemolytic		Non-hemolytic		Non-hemolytic	*	Non-hemolytic	*	Non-hemolytic
Oxidase		+	*	-	*	-	*	+		Variable, most -	*	+
Catalase	*	+	*	+	*	+ (weak)	*	+	*	+	*	+
Urease		-	*	-		-	*	+		variable		variable
Motility	*	-		-		-		-	*	-	*	+
Indole		-	*	-		-		-	*	-		-
Satellite		NR		NR	*	-	*	-		NR		NR
Growth @ 25° C		NR	*	+		NR		NR		NR		NR
Growth @ 42° C		NR		NR		NR		NR	*	-	*	+
Polymyxin B or Colistin		NR		NR		NR		NR	*	resistant	*	resistant
Amoxicillin clavulanate		NR		NR		NR		NR	*	susceptible	*	susceptible
Penicillin		NR		NR		NR		NR	*	resistant	*	resistant
β-lactamase		NR		-	*	+		-		NR		NR
ID panel misidentification				*Shigella, Y. pseudotuberculosis, Acinetobacter*		*Haemophilus influenzae, Aggregatibacter*		*Roseomonas gilardii, Kodcuria* spp.		*Burkholderia* spp., *Ralstonia picketii*		*Chromobacterium violaceum, B. cepacia*
Occurrence	Endemic in the USA		Endemic in Idaho		Endemic in Idaho		Endemic in Idaho		Not endemic in USA		Not endemic in USA	

NR: Not a recommended test for identification of specific select agent

**Initial culture may be positive on SBA; subsequent passages negative for growth on SBA

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Culture Growth, Gram Stain, and Biochemical Tests

Bacillus anthracis



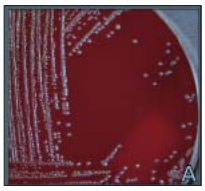
B. anthracis colonies at 24 h on SBA


Comma-shaped colonies


Tenacity on SBA

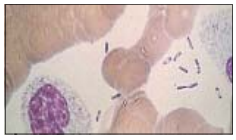

Gram stain

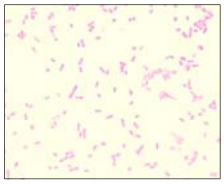
Yersinia pestis



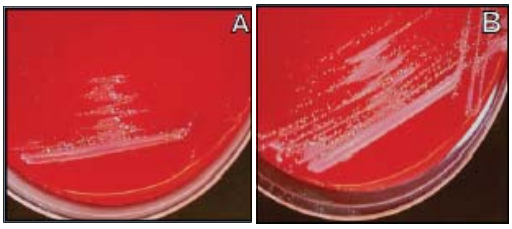
Yersinia pestis growth on SBA at (A) 48 h, (B) 72 h, (C) 96 h, (D) 96 h "Fried egg"

Giemsa stain (100X from blood culture): note *Y. pestis* bipolar appearance

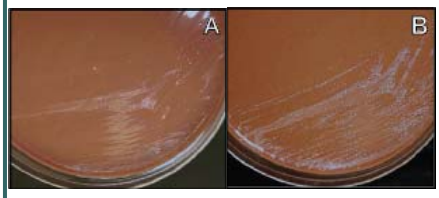

Gram stain: note that bipolar staining may be poor



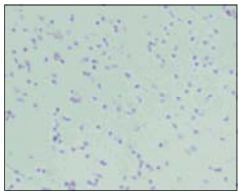
Francisella tularensis



Growth on SBA (A) 48 h, (B) 72 h



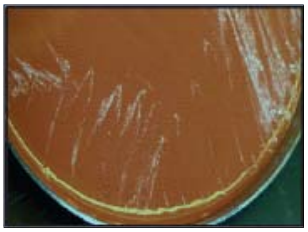
Growth on CHOC (A) 48 h, (B) 72 h

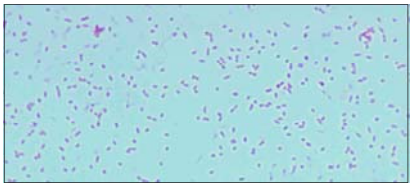

Gram stain

Brucella species

Growth on SBA at 48 h

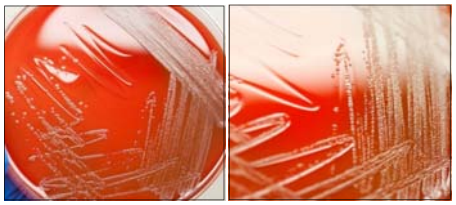

Growth on CHOC at 48 h

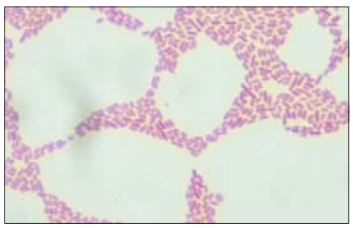

Gram stain



Burkholderia mallei

Growth on SBA at 48 h

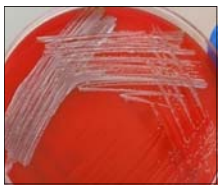

Gram stain


Motility


Non-Motile

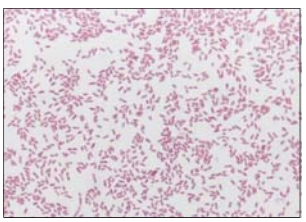
Burkholderia pseudomallei

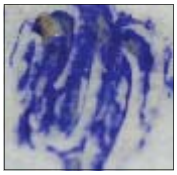
Growth on SBA at 24


Growth on SBA at 48 h



Gram stain


Oxidase


Positive

Motility


Motile

Images produced by New York State Department of Health—Wadsworth Center, 2007, ASM Sentinel Level Clinical Laboratory Guidelines, and Idaho Bureau of Laboratories

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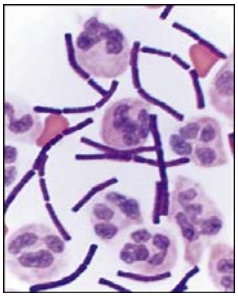
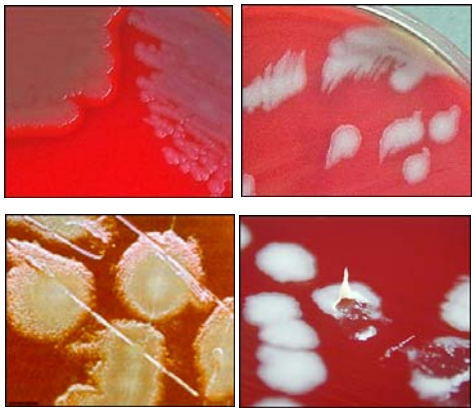
Bacillus anthracis Rule-Out Flowchart

Major Characteristics of *Bacillus anthracis*
Gram stain morphology: Large, Gram-positive rods
Spores may be found in cultures grown in 5% CO_2 or upon extended incubation but not usually in clinical specimens.
Colony morphology: Ground glass appearance, no hemolysis or pigment on SBA, no growth on MAC



Gram-positive bacillus



B. anthracis on SBA

Beta-hemolytic (i.e., exhibits complete clearing of blood cells on SBA)?

Yes

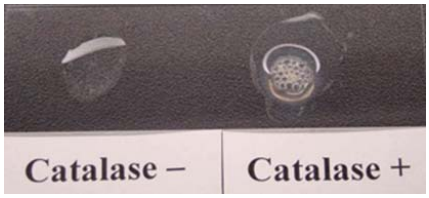
No

Catalase positive?

No

Bacillus anthracis is ruled out. Continue with routine identification.

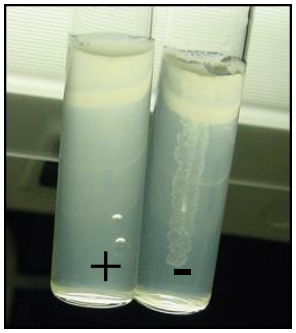
Yes



B. anthracis is catalase positive.

Motile?

Yes

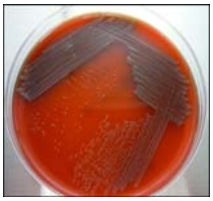


B. anthracis is non-motile.

No

Bacillus anthracis not ruled out.
Call Idaho Bureau of Laboratories (208-334-2235) to notify LRN-B personnel and send suspected agent according to applicable shipping regulations.
Report: Possible *Bacillus anthracis* submitted to LRN Reference Laboratory.

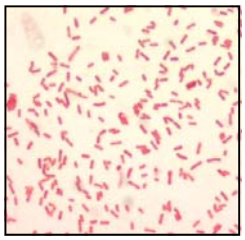
Yersinia pestis Rule-Out Flowchart



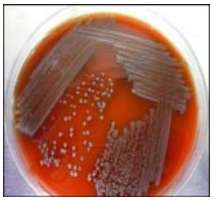
24 hours on SBA, 35°C

Major Characteristics of *Yersinia pestis*

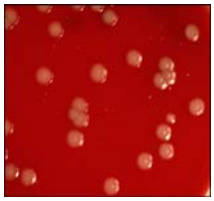
Gram stain morphology: Gram-negative, plump rods, 0.5 x 1-2 µm
Colony morphology: Slow growing, pinpoint (1-2 mm), gray-white to opaque colonies on SBA after 24 hours; non-lactose fermenter on MAC/EMB; grows well at 25-28°C and 35-37°C.
Specimen is blood, sputum, or lymph node aspirate.



Gram-negative bacillus



48 hours on SBA, 35°C

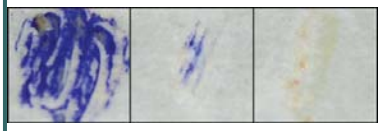


72 hours on SBA, 35°C

Oxidase negative?
Catalase positive?
Indole negative?
Urease negative?

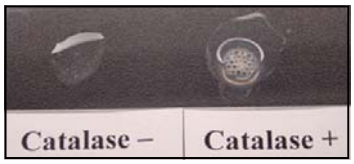
No → *Yersinia pestis* is ruled out. Continue with routine identification.

Yes to all



Y. pestis is oxidase negative.

Yersinia pestis not ruled out.
Call Idaho Bureau of Laboratories (208-334-2235) to notify LRN-B personnel and send suspected agent according to applicable shipping regulations.
Report: Possible *Yersinia pestis* submitted to LRN Reference Laboratory.



Y. pestis is catalase positive.

Y. pestis is urease negative.

Warning: Some of the automated identification systems do not identify *Y. pestis* adequately. *Y. pestis* has been falsely identified as *Y. pseudotuberculosis*, *Shigella*, H_2S-negative *Salmonella*, *Acinetobacter*, and *Pseudomonas* spp.

Differentiation of other important *Yersina* species

***Yersinia* species**	**Oxidase**	**Catalase**	**Urea**	**Indole**
Y. pseudotuberculosis	Negative	Positive	Positive	Negative
Y. enterocolitica	Negative	Positive	Positive	Variable
Y. frederiksenii	Negative	Positive	Positive	Positive
Y. kristensenii	Negative	Positive	Positive	Variable
Y. ruckeri	Negative	Positive	Negative	Negative
Y. pestis	**Negative**	**Positive**	**Negative**	**Negative**

Revised 6/2014 Idaho Bureau of Laboratories, www.statelab.idaho.gov, 208-334-2235

IDAHO DEPARTMENT OF HEALTH & WELFARE

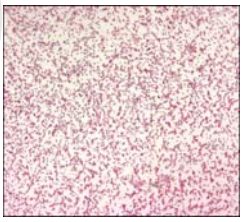
Francisella tularensis Rule-Out Flowchart

Major Characteristics of *Francisella tularensis*

Gram stain morphology: Aerobic, pleomorphic, minute (0.2 to 0.5 x 0.7 to 1.0 µm), faintly staining, Gram-negative coccobacillus

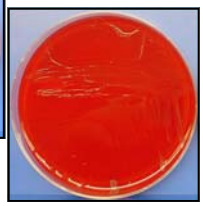
Colony morphology: No growth on MAC, scant to no growth on SBA after >48 hours
Produces 1-2 mm gray to grayish-white colonies on CHOC after >48 hours.

Safety: Perform all work in a Class II biosafety cabinet using BSL-3 precautions.



Gram-negative coccobacillus

24 hours on SBA, 35°C



48 hours on SBA, 35°C

Oxidase negative and catalase weakly positive or negative?

No → *Francisella tularensis* is ruled out. Continue with routine identification.

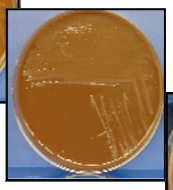
Yes

β -lactamase positive? No growth on MAC?

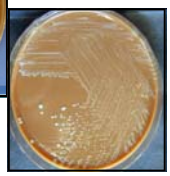
No → *Francisella tularensis* is ruled out. Continue with routine identification.

Yes to both

24 hours on CHOC, 35°C



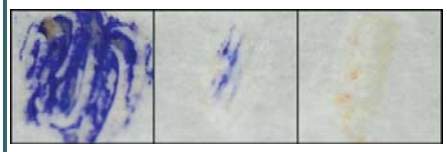
48 hours on CHOC, 35°C



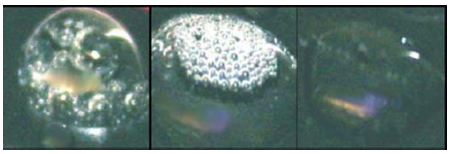
72 hours on CHOC, 35°C

Does the organism satellite around *S. aureus* on SBA?

Yes → *Francisella tularensis* is ruled out. Continue with routine identification.



F. tularensis is oxidase negative.



F. tularensis is weakly positive or negative on the catalase test.

No

Francisella tularensis not ruled out.
Call Idaho Bureau of Laboratories (208-334-2235) to notify LRN-B personnel and send suspected agent according to applicable shipping regulations.

Report: Possible *Francisella tularensis* submitted to LRN Reference Laboratory.

Warning: Automated identification systems may key out as non-*F. tularensis* (e.g., *Haemophilus influenzae* and *Aggregatibacter* spp.

Brucella spp. Rule-Out Flowchart

Major Characteristics of *Brucella* species

Gram stain morphology: Small (0.4 x 0.8 µm), Gram-negative coccobacillus

THINK BRUCELLA

Colony morphology: Poor growth after 24 hours incubation on SBA and CHOC.

Incubate plates for at least 2 additional days if no growth in 24 hours.

Organism does **not** grow on MAC.

Safety: Perform ALL further work in a Class II biosafety cabinet using BSL-3 precautions when *Brucella* spp. is suspected.

Growth at 24 hours on SBA, 35°C

Growth at 48 hours on SBA, 35°C

Gram-negative coccobacillus

Oxidase and catalase positive?

No

Yes to both

Urease positive?

No

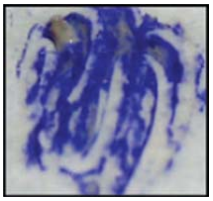
Brucella spp. is ruled out. Continue with routine identification.

Yes

Does the organism satellite around *S. aureus* on SBA?

Yes

No



Brucella spp. is oxidase positive.

Brucella spp. is urease positive.

Brucella spp. not ruled out.

Call Idaho Bureau of Laboratories (208-334-2235) to notify LRN-B personnel and send suspected agent according to applicable shipping regulations.

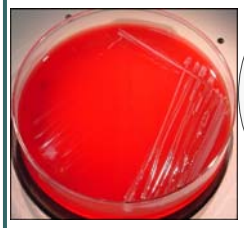
Report: Possible *Brucella* spp. submitted to LRN Reference Laboratory.

Antimicrobial therapy: Rifampin or Streptomycin plus Doxycycline

Differentiation of Brucella from other urease positive, oxidase positive, Gram-negative coccobacilli

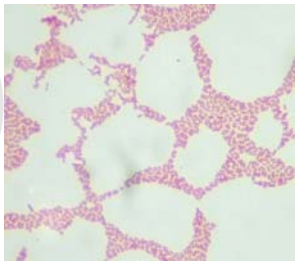
	Brucella spp.	*Psychrobacter immobilis*	*Paracoccus yeei*	*Psychrobacter phenylpyruvicus*	*Methylobacterium* spp.	*Oligella urolytica*	*Bordetella bronchiseptica, B. hinzii, Cupriavidus pauculus*	*Haemophilus* spp.
Gram stain morphology	Tiny coccobacillus, stains faintly	Coccobacillus/ bacillus	Cocci in packets	Coccobacillus/ bacillus, retains crystal violet	Vacuolated bacilli	Tiny coccobacillus	Bacillus	Tiny coccobacillus
Catalase	+	+	+	+	+	+	+	v
Oxidase	+	+	+	+	+	+	+	v
Urease	+	v	+	+	v	+	+	v
Motility	-	-	-	-	-	+ (delayed)	+	-
SBA distinctions	-	Prefers 20°C, odor of roses	Mucoid	-	Pink, mucoid	-	-	No growth
MAC at 48 hr	-	-	-	-	-	-	+	-

Burkholderia mallei Rule-Out Flowchart



Growth at 24 hours on SBA, 35°C

Major Characteristics of *Burkholderia mallei*
Gram stain morphology: Gram-negative coccbacilli or small rod
Colony morphology: Poor growth at 24 hours; better growth of gray, translucent colonies without pigment or hemolysis at 48 hours on SBA; poor or no growth on MAC at 48 hours; no distinctive odor

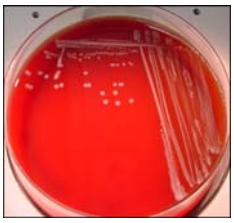


Gram-negative coccobacillus

Indole negative, catalase positive, non-hemolytic, poor growth or no growth on MAC?

No → *Burkholderia mallei* is ruled out. Continue with routine identification.

Yes

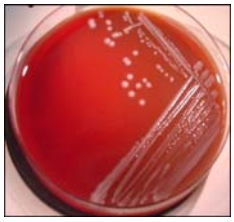


Growth at 48 hours on SBA, 35°C

Polymyxin B or colistin resistant (no zone)
Amoxicillin-clavulanate susceptible
Penicillin resistant

No → *Burkholderia mallei* is ruled out. Continue with routine identification.

Yes

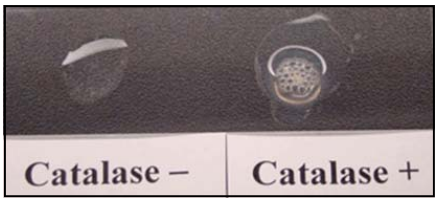


Growth at 72 hours on SBA, 35°C

Scant or no growth at 42°C

No → *Burkholderia mallei* is ruled out. Continue with routine identification.

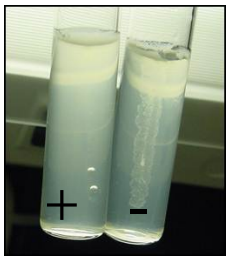
Yes



B. mallei is catalase positive.

Non-motile?

No → *Burkholderia mallei* is ruled out. Continue with routine identification.



B. mallei is non-motile.

Yes

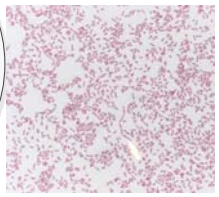
Burkholderia mallei is not ruled out.
Call Idaho Bureau of Laboratories (208-334-2235) to notify LRN-B personnel and send suspected agent according to applicable shipping regulations.
Report: Possible *Burkholderia mallei* submitted to LRN Reference Laboratory.
Additional screening test: *B. mallei* is arginine positive, unlike many other *Burkholderia* spp.

Burkholderia pseudomallei Rule-Out Flowchart

Major Characteristics of *Burkholderia pseudomallei*

Gram stain morphology: Gram-negative rod, straight or slightly curved; may demonstrate bipolar morphology at 24 hours and peripheral staining, like endospores, when cultures are older

Colony morphology: Moderate growth at 24 hours, good growth of white colonies at 48 hours on SBA; may develop wrinkled colonies in time; non-pigmented; non-hemolytic; often demonstrates strong characteristic musty, earthy odor; growth on MAC at 48 hours.



Gram-negative bacillus

Growth at 24 hours on SBA, 35°C

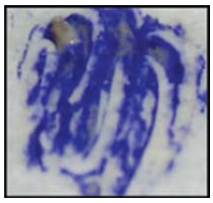
Growth at 48 hours on SBA, 35°C

Growth at 72 hours on SBA, 35°C

Growth on MAC? — No → Rule out other agents, such as *Burkholderia mallei*, *Brucella* spp. and *Francisella tularensis*.

Yes

Oxidase positive, catalase positive, and indole negative? — No →

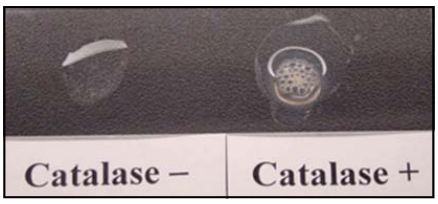


B. pseudomallei is oxidase positive.

Yes to all

Polymyxin B or colistin resistant (no zone) or Growth on B. cepacia selective agars — No → *Burkholderia pseudomallei* is ruled out. Continue with routine identification.

Yes to one



B. pseudomallei is catalase positive.

No hemolysis on SBA White to creamy yellow colonies on SBA — No → *Burkholderia pseudomallei* is ruled out. Consider *Chromobacterium violaceum* or indole-negative *Vibrio* spp.

Yes

Burkholderia pseudomallei is not ruled out.
Call Idaho Bureau of Laboratories (208-334-2235) to notify LRN-B personnel and send suspected agent according to applicable shipping regulations.

Report: Possible *Burkholderia pseudomallei* submitted to LRN Reference Laboratory.

Additional screening test: B. *pseudomallei* is arginine positive, unlike other *Burkholderia* spp. Unlike *B. mallei*, *B. pseudomallei* grows at 42°C at 48 hours and is motile.