A Case Study on Brucella

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Disclosure

- Quest employee (2014-present)

Simple Background Info

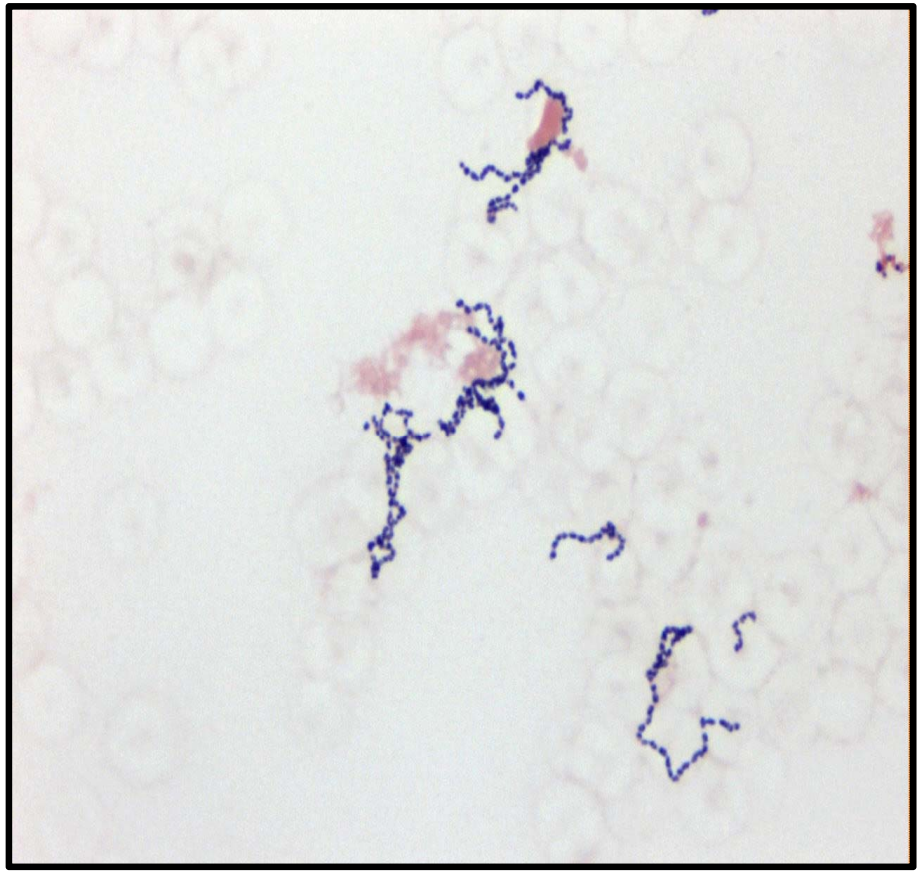
Saturday, October 27, 2018, late evening

Calendar Reference

October - November 2018 Time Frame						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
21	22	23	24	25	26	27 10:00 PM
28	29	30 11:00 AM	31	1	2	3

“Dr. Rank, We Have a Weird Bug…”

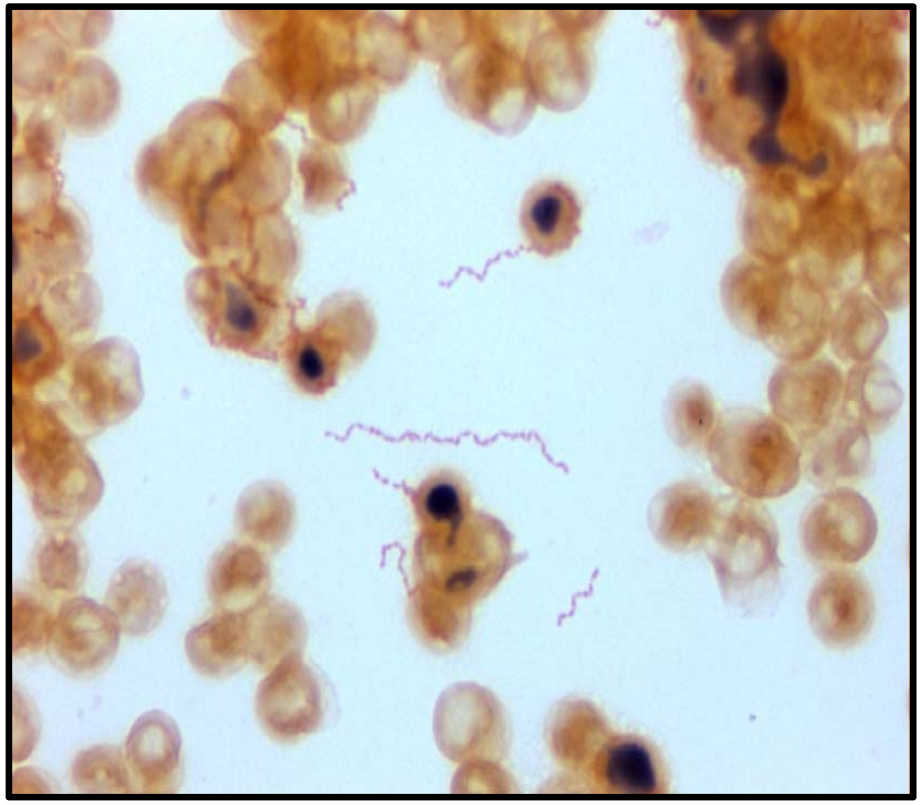
Another View



"Not So Fast…"

- So, we have a bacteremic patient and we need to see what grows on the plates.
- We waited that day for the subcultures to grow.
- So on Day 2, nothing appeared to be growing, so we re-stained the bottle contents for another look at the gram stain.

Day 2: A Confounding View



“…And It’s Not Growing Well…”

- BAP : No growth
- MAC: No growth
- Chocolate: Slight growth, not great, but something less than a “visible” haze.

What Would You Do, At This Point?

- Wait another day for the plate growth?
- Direct from the bottle Identification?
- Polymerase Chain Reaction test?
- Whole Genome Sequencing?

Vitek-MS

No ID

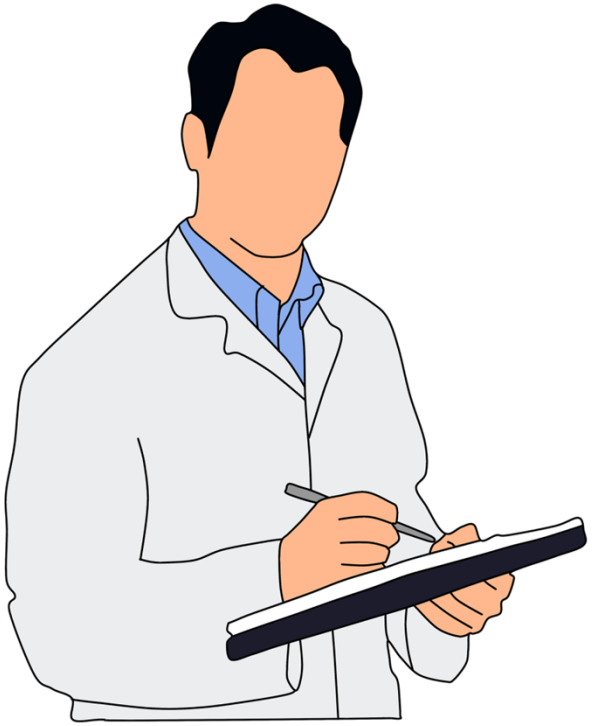
“OK. Patient Information?”

- Female
- 6 years old

Surmising…

- Gram Negative Rod bacteremia
- 6 years old
- Blood culture only
- What would be your next step?

“What’s Missing?”



Second Time Frame

October - November 2018 Time Frame						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
21	22	23	24	25	26	27 10:00 PM
28	29	30 11:00 AM	31	1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24

Brucella abortus BR-51 (16S)

- Immediately notified the physician group
- State Departments of Health (DOH) notifications:
  - New York DOH, resident lives in NY
  - New Jersey DOH, because isolated in NJ
  - Irvine (Orange County) DOH, CA
  - because ID performed in San Juan Capistrano
  - CDC for final confirmation
- Cattle vaccination strain
- categorized as a Bio-Terrorism agent

Review of Patient's Additional Orders

(Test abbreviations defined below)

- CRP = 15.6 (Ref <8.0)
- EBV IgG, IgM = Negative
- EBNA = Negative
- Influenza A, B = Negative
- *B. pertussis* DNA = Not Detected
- Group A *Streptococcus*, probe = Not Detected

CRP = C-Reactive Protein; EBV = Epstein-Barr Virus; NA = nucleolar antigen

NJ Assessment of Exposure Risk

- What were you doing when…?
- How close were you to the plate when…?
- Did you sniff the plate…?
- Did you mouth pipette…
- Did you experience a spray onto your…?
- Did you centrifuge, vortex, make a slide, open a culture, pick colonies, subculture, inoculate, set up an instrument…..?

"Biosafety Risk Assessment Worksheet". NJ PHEL. 2019.

Excellent Sources

APHL Best Risk Assessment Practices

- https://www.aphl.org/programs/preparedness/Documents/APHL%20Risk%20Assessment%20Best%20Practices%20and%20Examples.pdf

Assessing Laboratory Risk Level and PEP (CDC)

- https://www.cdc.gov/brucellosis/laboratories/risk-level.html

*APHL = Association of Public Health Laboratories

*PEP = Post-Exposure Prophylaxis

High Risk or Low Risk?

Specimen handling	Exposure scenario	PEP	Follow-up/ monitoring
Routine clinical specimen (eg, blood, serum, cerebrospinal fluid)	Person who manipulates a routine clinical specimen (eg, blood, serum, cerebrospinal fluid), resulting in contact with broken skin or mucous membranes, regardless of working in a certified Class II biosafety cabinet, with or without appropriate personal protective equipment (ie, gloves, gown, eye protection).	Doxycycline 100mg twice daily, and rifampin 600 mg once daily, for three weeks. For patients with contraindications to doxycycline or rifampin: TMP-SMZ, in addition to another appropriate antimicrobial, should be considered. Two antimicrobials effective against *Brucella* should be given. Pregnant women should consult their obstetrician. **Note:** RB51 is resistant to rifampin in vitro, and therefore this drug should not be used for PEP or treatment courses.	Regular symptom watch (eg, weekly) and daily self-fever checks* through 24 weeks post- exposure, after last known exposure. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks post-exposure, after last known exposure. **Note:** no serological monitoring currently available for RB51 and *B. canis* exposures in humans.
Enriched material (eg, a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (eg, amniotic fluid, placental products)	Person who manipulates (or is ≤ 5 feet from someone manipulating) enriched material (eg, a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (eg, amniotic fluid, placental products), outside of a certified Class II biosafety cabinet.		
	Person who manipulates enriched material (eg, a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (eg, amniotic fluid, placental products), within a certified Class II biosafety cabinet, without appropriate personal protective equipment (ie, gloves, gown, eye protection).		
	All persons present during the occurrence of aerosol-generating events** (eg, centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes) with manipulation of enriched material (eg, a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (eg, amniotic fluid, placental products) on an open bench.		

"Assessing Laboratory Risk Level and PEP". https://www.cdc.gov/brucellosis/laboratories/risk-level.html

Laboratory Risk Assessment and Post-Exposure Prophylaxis (PEP): Minimal (but not zero) Risk

Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Routine clinical specimen (e.g., blood, serum, cerebrospinal fluid)	Person who manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) in a certified Class II biosafety cabinet, with appropriate personal protective equipment (i.e., gloves, gown, eye protection).	None	N/A

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Laboratory Risk Assessment and Post-Exposure Prophylaxis (PEP): Minimal (but not zero) Risk

Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Routine clinical specimen (e.g., blood, serum, cerebrospinal fluid)	Person present in the lab while someone manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) in a certified Class II biosafety cabinet, or on an open bench where manipulation did not involve occurrence of aerosol- generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes).	None	May consider symptom watch for following scenarios: 1. Person who manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) on an open bench with or without appropriate personal protective equipment (i.e., gloves, gown, eye protection), or in a certified Class II biosafety cabinet without appropriate personal protective equipment. 2. Person present in the lab while someone manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) on an open bench, resulting in occurrence of aerosol-generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes).

"Assessing Laboratory Risk Level and PEP". https://www.cdc.gov/brucellosis/laboratories/risk-level.html

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Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Enriched material (e.g., a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Person who manipulates enriched material (e.g., a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products) in a certified Class II biosafety cabinet, with appropriate personal protective equipment (i.e., gloves, gown, eye protection).	None	May consider symptom watch for following scenarios: 1. Person who manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) on an open bench with or without appropriate personal protective equipment (i.e., gloves, gown, eye protection), or in a certified Class II biosafety cabinet without appropriate personal protective equipment. 2. Person present in the lab while someone manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) on an open bench, resulting in occurrence of aerosol-generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes).

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Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Enriched material (e.g., a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Person present in the lab while someone manipulates enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products) in a certified Class II biosafety cabinet.	None	May consider symptom watch for following scenarios: 1. Person who manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) on an open bench with or without appropriate personal protective equipment (i.e., gloves, gown, eye protection), or in a certified Class II biosafety cabinet without appropriate personal protective equipment. 2. Person present in the lab while someone manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid) on an open bench, resulting in occurrence of aerosol-generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes).

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Laboratory Risk Assessment and Post-Exposure Prophylaxis (PEP): LOW Risk

Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Enriched material (e.g., a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Person present in the lab at a distance of greater than 5 feet from someone manipulating enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products), on an open bench, with no occurrence of aerosol-generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes).	May consider if immunocompromised or pregnant. Discuss with health care provider (HCP). Note: RB51 is resistant to rifampin in vitro, and therefore this drug should not be used for PEP or treatment courses.	Regular symptom watch (e.g., weekly) and daily self-fever checks through 24 weeks post- exposure, after last known exposure. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks post- exposure, after last known exposure. Note: no serological monitoring currently available for RB51 and B. canis exposures in humans.

"Assessing Laboratory Risk Level and PEP". https://www.cdc.gov/brucellosis/laboratories/risk-level.html

Laboratory Risk Assessment and Post-Exposure Prophylaxis (PEP): HIGH Risk

Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Routine clinical specimen (e.g., blood, serum, cerebrospinal fluid)	Person who manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid), resulting in contact with broken skin or mucous membranes, regardless of working in a certified Class II biosafety cabinet, with or without appropriate personal protective equipment (i.e., gloves, gown, eye protection).	1. Doxycycline 100mg twice daily, and rifampin 600 mg once daily, for three weeks. 2. For patients with contraindications to doxycycline or rifampin: TMP-SMZ, in addition to another appropriate antimicrobial, should be considered. Two antimicrobials effective against Brucella should be given. 3. Pregnant women should consult their obstetrician.	1. Regular symptom watch (e.g., weekly) and daily self-fever checks through 24 weeks post-exposure, after last known exposure. 2. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks post-exposure, after last known exposure.

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Laboratory Risk Assessment and Post-Exposure Prophylaxis (PEP): HIGH Risk

Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Person who manipulates (or is ≤ 5 feet from someone manipulating) enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products), outside of a certified Class II biosafety cabinet.	1. Doxycycline 100mg twice daily, and rifampin 600 mg once daily, for three weeks. 2. For patients with contraindications to doxycycline or rifampin: TMP-SMZ, in addition to another appropriate antimicrobial, should be considered. Two antimicrobials effective against Brucella should be given. 3. Pregnant women should consult their obstetrician.	1. Regular symptom watch (e.g., weekly) and daily self-fever checks through 24 weeks post-exposure, after last known exposure. 2. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks post-exposure, after last known exposure.

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Laboratory Risk Assessment and Post-Exposure Prophylaxis (PEP): HIGH Risk

Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	Person who manipulates enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products), within a certified Class II biosafety cabinet, without appropriate personal protective equipment (i.e., gloves, gown, eye protection).	1. Doxycycline 100mg twice daily, and rifampin 600 mg once daily, for three weeks. 2. For patients with contraindications to doxycycline or rifampin: TMP-SMZ, in addition to another appropriate antimicrobial, should be considered. Two antimicrobials effective against Brucella should be given. 3. Pregnant women should consult their obstetrician.	1. Regular symptom watch (e.g., weekly) and daily self-fever checks through 24 weeks post-exposure, after last known exposure. 2. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks post-exposure, after last known exposure.

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Laboratory Risk Assessment and Post-Exposure Prophylaxis (PEP): HIGH Risk

Specimen Handling	Exposure Scenario	PEP	Follow-Up/Monitoring
Enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products)	All persons present during the occurrence of aerosol-generating events (e.g., centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes) with manipulation of enriched material (e.g., a Brucella isolate, positive blood bottle) or reproductive clinical specimen (e.g., amniotic fluid, placental products) on an open bench.	1. Doxycycline 100mg twice daily, and rifampin 600 mg once daily, for three weeks. 2. For patients with contraindications to doxycycline or rifampin: TMP-SMZ, in addition to another appropriate antimicrobial, should be considered. Two antimicrobials effective against Brucella should be given. 3. Pregnant women should consult their obstetrician.	1. Regular symptom watch (e.g., weekly) and daily self-fever checks through 24 weeks post-exposure, after last known exposure. 2. Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks post-exposure, after last known exposure.

"Assessing Laboratory Risk Level and PEP". https://www.cdc.gov/brucellosis/laboratories/risk-level.html

CDC Risk Level and PEP: Low Risk

Enriched material (eg, a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (eg, amniotic fluid, placental products)

- Person present in the lab at a distance of **greater than 5 feet** from someone manipulating enriched material (eg, a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (eg, amniotic fluid, placental products), **on an open bench, with no occurrence of aerosol-generating events** (eg, centrifuging without sealed carriers, vortexing, sonicating, spillage/splashes).

CDC Risk Level and PEP: Low Risk

PEP

- May consider if immunocompromised or pregnant. Discuss with health care provider (HCP). Note: RB51 is resistant to rifampin in vitro, and therefore this drug should not be used for PEP or treatment courses.

Follow-up/Monitoring

- Regular symptom watch (eg, weekly) and daily self-fever checks through 24 weeks postexposure, after last known exposure.
- Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks postexposure, after last known exposure. Note: no serological monitoring currently available for RB51 and B. canis exposures in humans.

CDC Risk Level and PEP: High Risk

Routine clinical specimen (eg, blood, serum, CSF.

- resulting in contact with broken skin or mucous membranes,

Enriched material (eg, a *Brucella* isolate, positive blood bottle) or reproductive clinical specimen (eg, amniotic fluid, placental products)

- **≤ 5 feet from someone manipulating enriched material**
- within a certified Class II biosafety cabinet, without appropriate personal protective equipment (ie, gloves, gown, eye protection).

CDC Risk Level and PEP: High Risk

PEP

- **Doxycycline 100mg twice daily and rifampin 600 mg once daily, for three weeks.**
- For patients with **contraindications to doxycycline or rifampin: TMPSMZ**, in addition to another appropriate antimicrobial, should be considered. Two antimicrobials effective against *Brucella* should be given.
- Pregnant women should consult their obstetrician.

Follow-up/Monitoring

- Regular symptom watch (eg, weekly) and daily self-fever checks through 24 weeks postexposure, after last known exposure.
- Sequential serological monitoring at 0 (baseline), 6, 12, 18, and 24 weeks postexposure, after last known exposure. Note: no serological monitoring currently available for RB51 and B. canis exposures in humans.

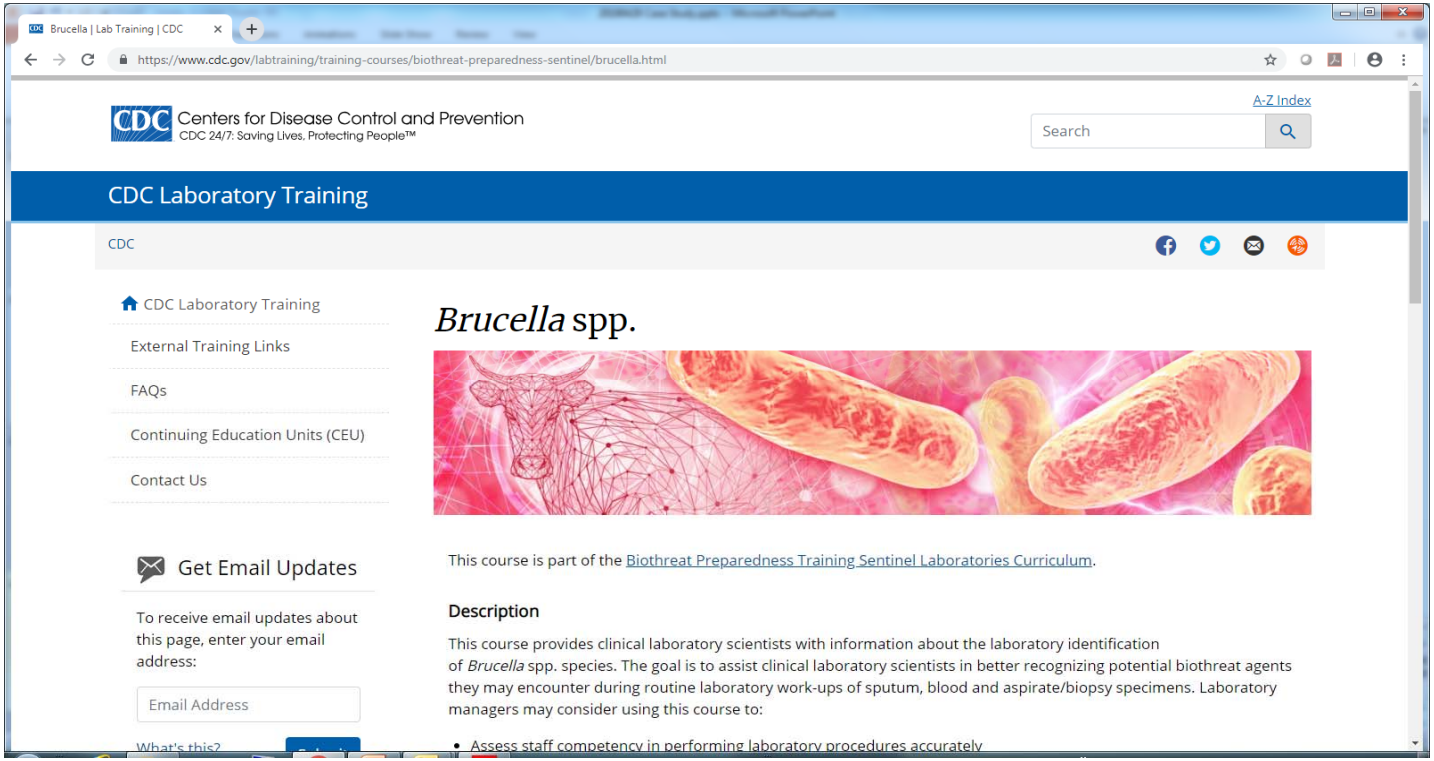
However, Who Were ≥ 5 Feet Away?

- Other lab technologists, technicians, aides
- In-House Personnel: Facilities, House Keeping, Planning, Materials Management, IT, Customer Solutions,
- Outside: Vendors, Field Service Reps
- There were >100

NJ Public Health and Environmental Laboratories Letter of Recommendations

- An opportunity to improve practices through increased education, programs, and training.

An Outstanding Resource



https//www.cdc.gov/labtraining/training-courses/biothreat-preparedness-sentinel/brucella.html

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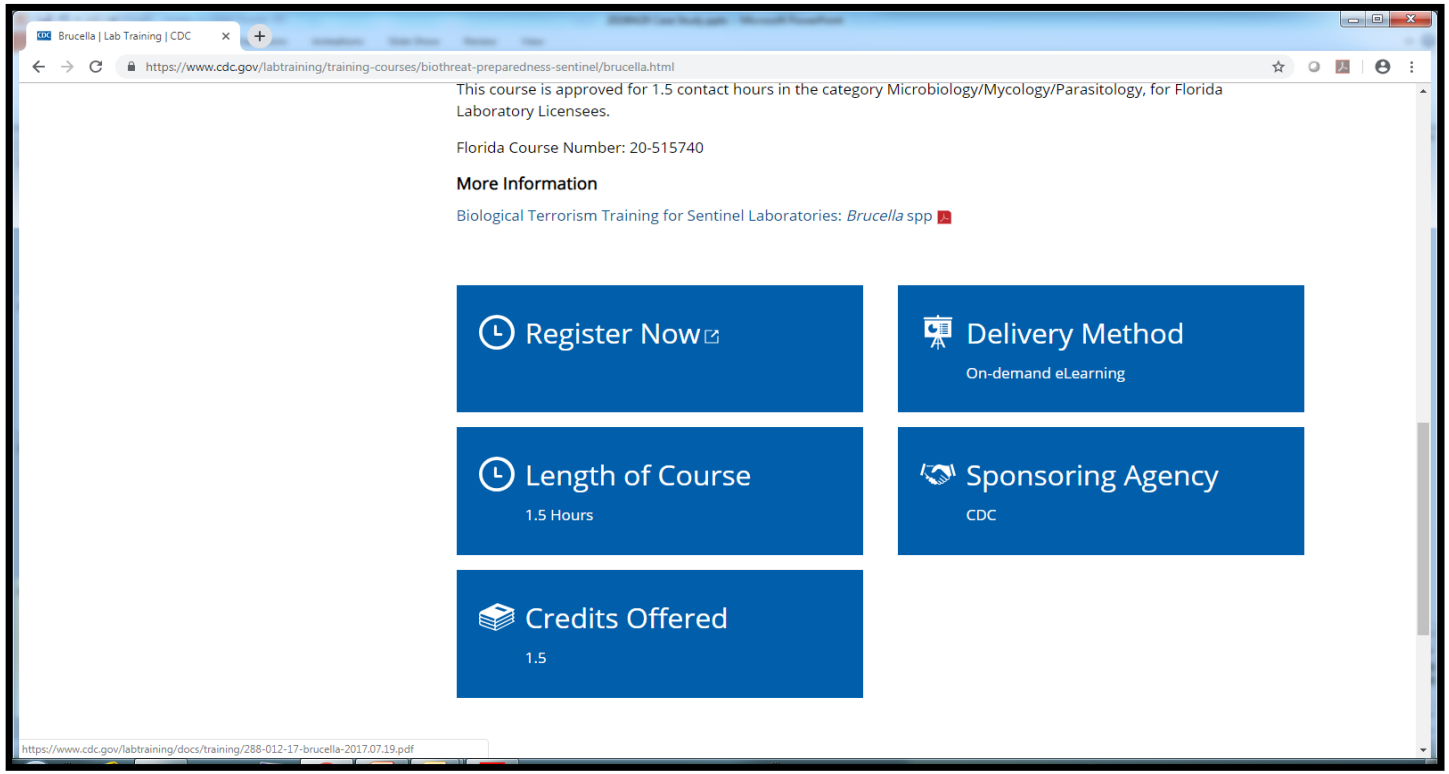
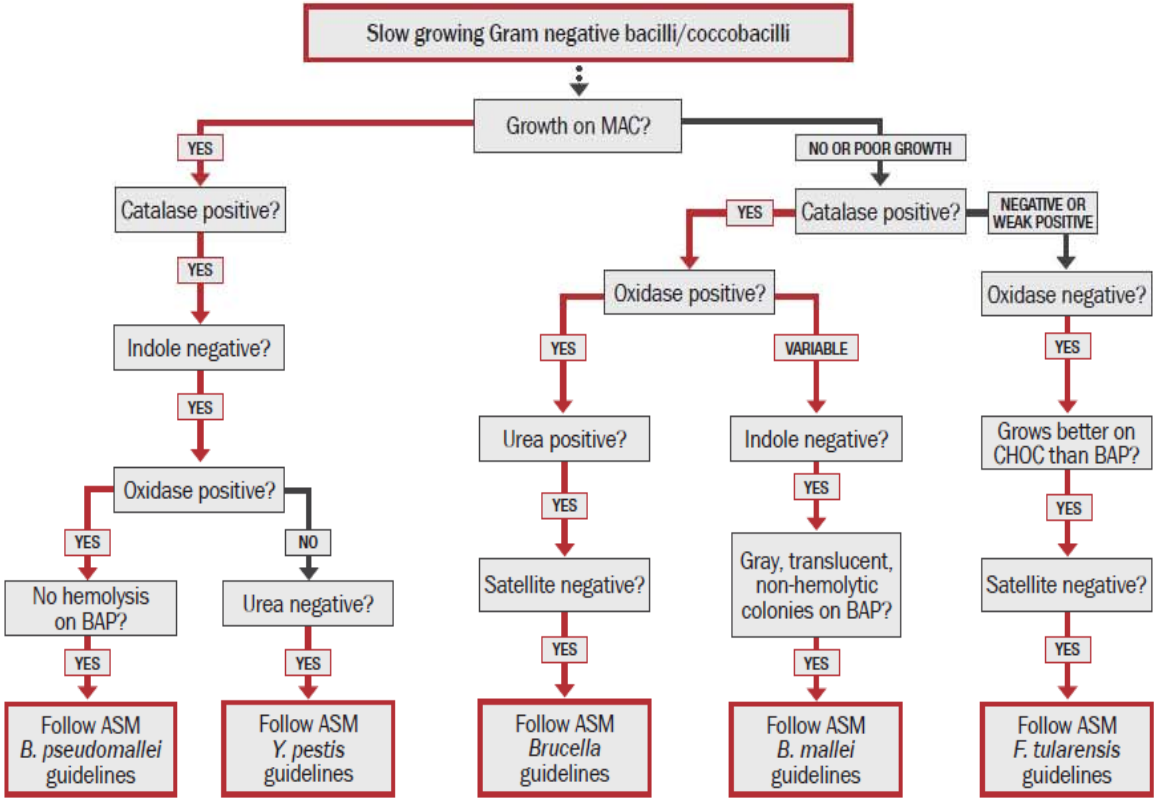


https//www.cdc.gov/labtraining/training-courses/biothreat-preparedness-sentinel/brucella.html

BIOTHREAT AGENT IDENTIFICATION

Gram Negative Bacilli/Coccobacilli Rule-Out Algorithm



https://www.aphl.org/aboutAPHL/publications/Documents/2018_BiothreatAgents_SentinelLab_BenchCards_WEB.pdf

	BAP	MAC	CHOC (or TM)
B. anthracis	Rapid growth. Flat, non-hemolytic, 'ground-glass' colonies. Medussa-head, comma-like projections. 'Stand-up' consistency	No growth.	Rapid growth. Flat, 'ground-glass' colonies. Medussa-head, comma-like projections. 'Stand-up' consistency
Y. pestis	Slow growth. Gray-white, transluscent at 24 h. No hemolysis. 'Fried-egg' at 48 h.	Slow growth. Small, opaque, colorless by 48 h.	Slow growth. Gray-white, transluscent colonies at 24 hours.
Burkholderia pseudomallei	Slow growth. Small, smooth creamy colonies by 24-48 hr, becoming dry and wrinkled. Musty odor apparent **without** sniffing.	Slow growth.	B. pseudomallei and B. mallei grow on TM media. Both are RESISTANT to Colistin and polymixin B found in TM.
Burkholderia mallei	Slow growth. Smooth, gray, transluscent colonies by 48 h without pigment or odor.	No growth, or pinpoint by 48 h.	B. pseudomallei and B. mallei grow on TM media. Both are RESISTANT to Colistin and polymixin B found in TM.
F. tularensis	Slow to No growth by 48 h. May fail to grow on subsequent passage.	No growth.	Slow growth by 48 h. Blue-white gray colony, flat, dense. Grows better than BAP.
Brucella spp.	Pinpoint at 24 h. Slow growth by 48 h. Small, dimpled, raised, white to cream, non-hemolytic, gllistening.	No growth.	Pinpoint at 24 h. Slow growth by 48 h. Small, dimpled, raised, white to cream, non-hemolytic, gllistening.

Elliot L. Rank. Table.

	Initial Rapid Tests to Perform				Follow-Up Tests Based on the Rapid Test Results					
	Gram stain	Catalase	Oxidase	Indole	Urease	22-25°C	42°C	Col/PolyB	β-lactamase	Motility
B. anthracis	GPR	+								-
Y. pestis	GNR	+	-	-	-	+				-
B. pseudomallei	GNR	+	+	-			+	+		+
B. mallei	GNR	+	v	-			-	+		
F. tularensis	Weak, GNcb	±	-		-				+	
Brucella spp.	GNcb	+	+		+*					

* Brucella spp. can turn the Urea slant positive within a few minutes.

Elliot L. Rank. Table.

What Is A Slow-Grower?

- 24 hrs
- 48 hrs
- 72 hrs
- More….?
- At what point do you become alarmed?
- Is it slow growth or no growth?
- Is it haze or is it really a microorganism?

IF	Then
Minimal or No Growth at 24 h for BAP, Mac and CHOC	Reincubate for 24 h. (Does not apply to Urines or anaerobes)
Similar observations after 48 h for BAP, Mac and CHOC (but slight growth or haze on CHOC)	Notify Supervisor. Confirm the possibility of a Slow Grower.* ASM/CDC/APHL algorithm takes precedence. (Reference1). ALERT notifications in place. Tape plates and place in sealable baggie for re-incubation. Seal the plates with laboratory film, tape, or in a CO2 permeable plastic bag prior to placing in incubator and place a biohazard sticker on plates/ slants and worksheets. (Reference 2 Sec. 8.1.2).All bench work effort transfers to BSC.
Gram Stain	GNB, or GN cocco-bacilli: faint stain, shorter rods Definitive POSSIBILITY of BT agent. ALERT Supervisor. ALERT notifications.
Catalase = **Positive**. Oxidase = **Positive**. Urease = **Positive**.	Slow Grower is a distinct possibility. Reactions to follow are shown in the ASM algorithm. Reaction results for *Brucella* are shown on the left. If all are positive, *Brucella* is a possibility. **STOP** Work on the open bench. Absolutely **NO** automated instrumentation identification systems including, MALDI, Vitek-MS, MicroScan, or Rapid NH systems. Alert the State PHL. Microorganism to be isolated in the locked incubator until approval for transfer to the State PHL. Package and send to PHL.

Approaching the Slow Grower

IF	Then
Minimal or No Growth at 24 h for BAP, Mac and CHOC	Reincubate for 24 h. Does not apply to urines or anaerobes.

Approaching the Slow Grower

IF	Then
Similar observations after 48 h for BAP. Mac and CHOC (slight haze on CHOC).	Contact Supervisor. Confirm the possibility of Slow Grower. ASM/CDC/APHL algorithm takes precedence. ALERT notifications in place. Tape plates and place in a sealable baggie for reincubation. Seal the plates with laboratory film, tape or place into a CO2 permeable plastic bag prior to placing in incubator and place a biohazard sticker on the plates/slants and worksheets. All bench work transfers to the BSC.

Approaching the Slow Grower

IF	Then
Gram Stain	GNdc, GNR, or GN cb: faint stain, shorter rods, Definitive possibility of a BT agent. ALERT Supervisor. Put up your alert notifications.

Approaching the Slow Grower

IF	Then
Catalase is positive; Oxidase is positive; Urease is positive.	Brucella is possible. STOP all bench work. Absolutely no further automated instrumentation identification systems including Maldi, Vitek-MS, or Rapid NH systems. Alert State PHL. The microorganism should be isolated in the locked incubator until approval transfer to the State PHL is granted. Package and send to PHL.

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

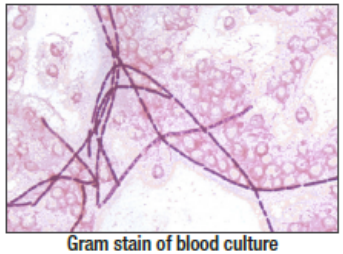
Biochemical/Test Reactions

- 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

APHL Best Risk Assessment Practices
https://www.aphl.org/programs/preparedness/Documents/APHL%20Risk%20Assessment%20Best%20Practices%20and%20Examples.pdf

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

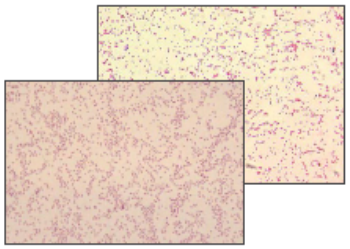
  Note: Oxidase may be variable and test should be performed on fresh cultures (18-24h)
- *S. aureus* streak negative (X & V Factor satellite test)

Colony Morphology

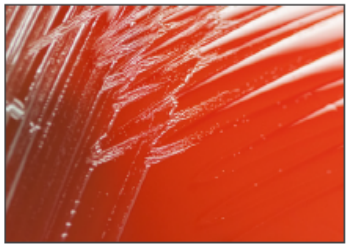
- Aerobic, slow growth
- Slow growth seen on BAP and CHOC (CO_2 may be required by some strains)
- Poor to variable growth on MAC. Pinpoint colonies may infrequently be observed with some strains after extended blood culture incubation (7 days)
- Non-mucoid
- Pinpoint colonies at 24h, and easily visible, 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

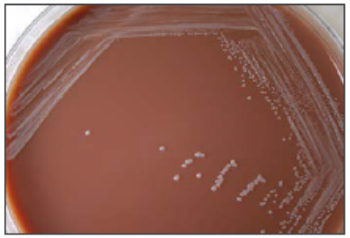
May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Moraxella* spp., *Micrococcus* spp., *Corynebacterium* spp., "slow growing" *Staphylococcus* spp., *Oligella ureolytica, Bordetella bronchiseptica, Haemophilus* spp., *Pasteurella*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



Gram Stain



48h growth on BAP



72h growth on CHOC

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

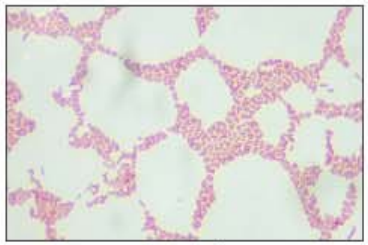
Biochemical/Test Reactions

- 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

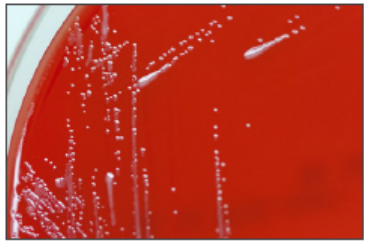
Common Misidentifications

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

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

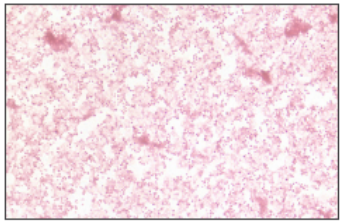
Biochemical/Test Reactions

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

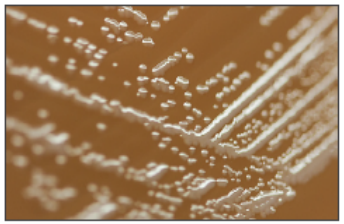
Common Misidentifications

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

APHL Best Risk Assessment Practices
https://www.aphl.org/programs/preparedness/Documents/APHL%20Risk%20Assessment%20Best%20Practices%20and%20Examples.pdf

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

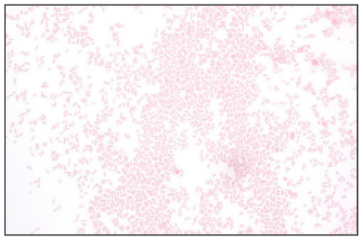
- Facultative anaerobe
- Slow growing at 35˚C, better growth at 25-28˚C
- Grey-white, translucent pinpoint colonies at 24h, usually too small to be seen
- On BAP:
  - After 48h: colonies approximately 1-2 mm in diameter, gray-white to slightly yellow and opaque
  - Older cultures (~96h): "Fried egg"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

Biochemical/Test Reactions

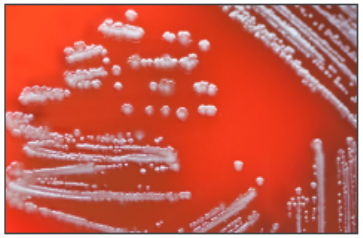
- Catalase positive
- Oxidase, urease (at 35˚C) and indole negative

Common Misidentifications

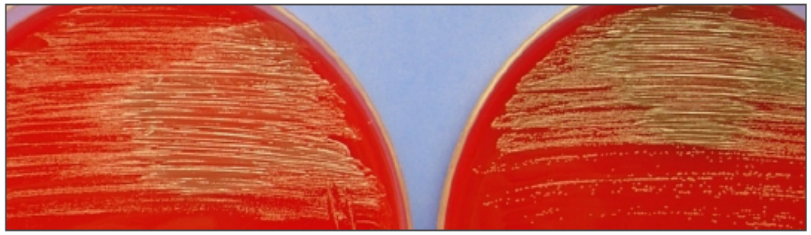
May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Shigella* spp., H_2S(-) *Salmonella* spp., *Acinetobacter* or *Pseudomonas* spp. and *Yersinia pseudotuberculosis*.



Gram Stain



48h growth on BAP



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

APHL Best Risk Assessment Practices
https://www.aphl.org/programs/preparedness/Documents/APHL%20Risk%20Assessment%20Best%20Practices%20and%20Examples.pdf

APPENDIX

Identification Tests

Arginine Dihydrolase (Decarboxylase)

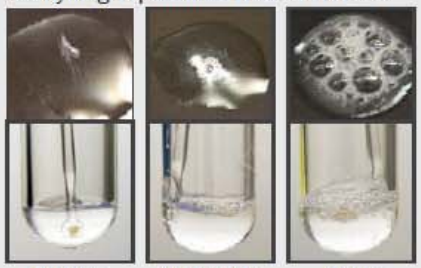
Look for pink/purple color change

Uninoculated Base | Positive Base | Negative | Positive | NF Base | Positive

Controls

Catalase

3% Hydrogen peroxide: look for bubbles

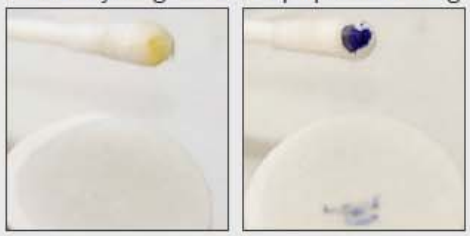


Negative | Weak Positive | Positive

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

Oxidase

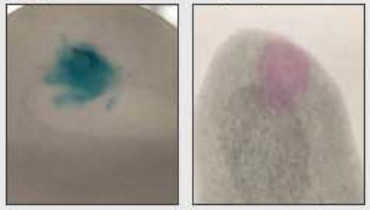
Tetramethyl reagent: look for purple color change



Negative | Positive

Spot Indole

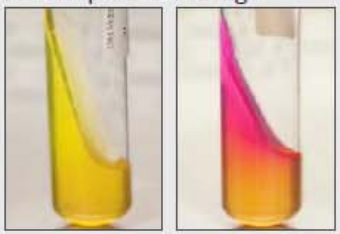
Look for color change, varies by reagent; Cinnamaldehyde preferred



Cinnamaldehyde: positive is blue | Benzaldehyde: positive is pink

Urea

Look for pink color change



Negative | Positive

APHL Best Risk Assessment Practices
https://www.aphl.org/programs/preparedness/Documents/APHL%20Risk%20Assessment%20Best%20Practices%20and%20Examples.pdf

Who Are Most Affected?

- Veterinarians
- Farmers
- Hunters
- Dairy workers
- Slaughter house personnel
- Folks who keep pet goats, sheep
- Travelers to endemic areas (eating local foods….)
- **Lab Workers**

"Which occupations are at greater risk for brucellosis?", MedScape, https://www.medscape.com/answers/213430-36565/which-occupations-are-at-greater-risk-for-brucellosis

Notes from the Field: Human Bru

https://www.cdc.gov/mmwr/volumes/68/wr/mm6807a6.htm

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Morbidity and Mortality Weekly Report (*MMWR*)

CDC

Notes from the Field: Human *Brucella abortus* RB51 Infections Caused by Consumption of Unpasteurized Domestic Dairy Products — United States, 2017–2019

Weekly / February 22, 2019 / 68(7);185

María E. Negrón, DVM, PhD[1]; Grishma A. Kharod, MPH[1]; William A. Bower, MD[1]; Henry Walke, MD[1] (View author affiliations)

View suggested citation

Since August 2017, CDC has confirmed three cases of brucellosis attributed to *Brucella abortus* cattle vaccine strain RB51 (RB51). Each case was associated with consumption of domestically acquired unpasteurized (raw) milk products (*1*). Patient symptoms varied and included fever, headache, overall malaise, and respiratory symptoms. In total, at least eight persons met the probable case definition of a clinically compatible illness epidemiologically linked to a shared contaminated source (*2*). In addition, hundreds of persons, from dozens of states, were potentially exposed to the contaminated raw milk products (*3*).

Consumption of raw milk products increases the risk for infection with pathogens such as *Escherichia coli*, *Campylobacter*, *Listeria*, and *Brucella* spp. Raw milk–related disease outbreaks occur more often in states with legalized raw milk sales (*4*). Approximately 75% of U.S. states have laws allowing various types of raw milk sales (*5*).

Brucellosis, caused by *Brucella* spp., is primarily an animal disease; however, exposure to infected animals or raw milk products

https://www.cdc.gov/mmwr/volumes/68/wr/mm6807a6.htm

Notes from the Field: Human Bru

https://www.cdc.gov/mmwr/volumes/68/wr/mm6807a6.htm

Brucellosis, caused by *Brucella* spp., is primarily an animal disease; however, exposure to infected animals or raw milk products can cause human disease. In humans, brucellosis is characterized by nonspecific symptoms, including fever, arthralgia, myalgia, and sweats; miscarriage and other sequelae can occur. Human brucellosis is rare in the United States, with 80–120 cases reported annually; most of these are associated with *Brucella* exposures abroad (CDC, unpublished data, 2019). The rarity of human brucellosis in the United States is mainly attributable to pasteurization and the successful U.S. State-Federal Cattle Brucellosis Eradication Program. As a result of the program's focus on disease surveillance and cattle vaccination, *B. abortus* in livestock has been eliminated, except in limited areas where disease reintroduction from infected wildlife occurs.

References

Related Materials

RB51 is a live, attenuated vaccine that has been used to vaccinate cattle against *B. abortus* in the United States since 1996. Although rare, it is possible for cattle to shed RB51 in their milk, even when vaccine label recommendations are followed (*6*). Consuming this raw milk can cause human infections, which, unlike infections caused by field *Brucella* strains, do not stimulate an antibody response detectable by commercially available serological assays and can be missed by tests normally used for diagnosis. In addition, RB51 is resistant to rifampin, a first-line antibiotic used to treat human brucellosis (*3*). When evaluating patients whose symptoms are consistent with brucellosis, clinicians should consider RB51 infection and inquire about raw milk consumption as part of the patient's exposure history (*3*).

Several actions could be considered to reduce the risk for raw milk–related RB51 human infections. CDC recommends that public health and regulatory authorities continue supporting pasteurization and consider further restricting the sale and distribution of raw milk and raw milk products in their jurisdictions.* States might explore options such as the United States Animal Health Association's recommendations that state animal health officials and cattle industry representatives evaluate the need for the RB51 vaccine in areas where *B. abortus* is not endemic in wildlife (*7*). Modifying current RB51 vaccine labels to include information about possible shedding in milk could also improve awareness. Finally, veterinarians and dairy farm owners need to be aware that RB51 vaccination might pose a risk when given to cows whose milk is intended to be consumed unpasteurized.

Top

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All authors have completed and submitted the ICMJE form for disclosure of potential conflicts of interest. No potential conflicts of interest were disclosed.

https://www.cdc.gov/mmwr/volumes/68/wr/mm6807a6.htm

Retrospective Patient Information

- Family lived an organic lifestyle upstate NY
- Organic cheese (unpasteurized) may have been responsible for the illness (PA farm)
- Family was reluctant to bring the child to the pediatrician
- Family was obstinate/opposed to lab testing
- Family refused empiric and follow-up antimicrobial therapy

The 6 Year Old Little Girl

- Recovered by herself
- Doing just fine
- Case was mentioned on the CDC site, but only in very broad terms.

CDC - Home - Brucellosis

https://www.cdc.gov/brucellosis/index.html

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Brucellosis

Brucellosis is an infectious disease caused by bacteria.

People can get the disease when they are in contact with infected animals or animal products contaminated with the bacteria. Animals that are most commonly infected include sheep, cattle, goats, pigs, and dogs, among others.

Exposures to Drug-Resistant Brucellosis Linked to Raw Milk

Food Safety Alert

February 8, 2019

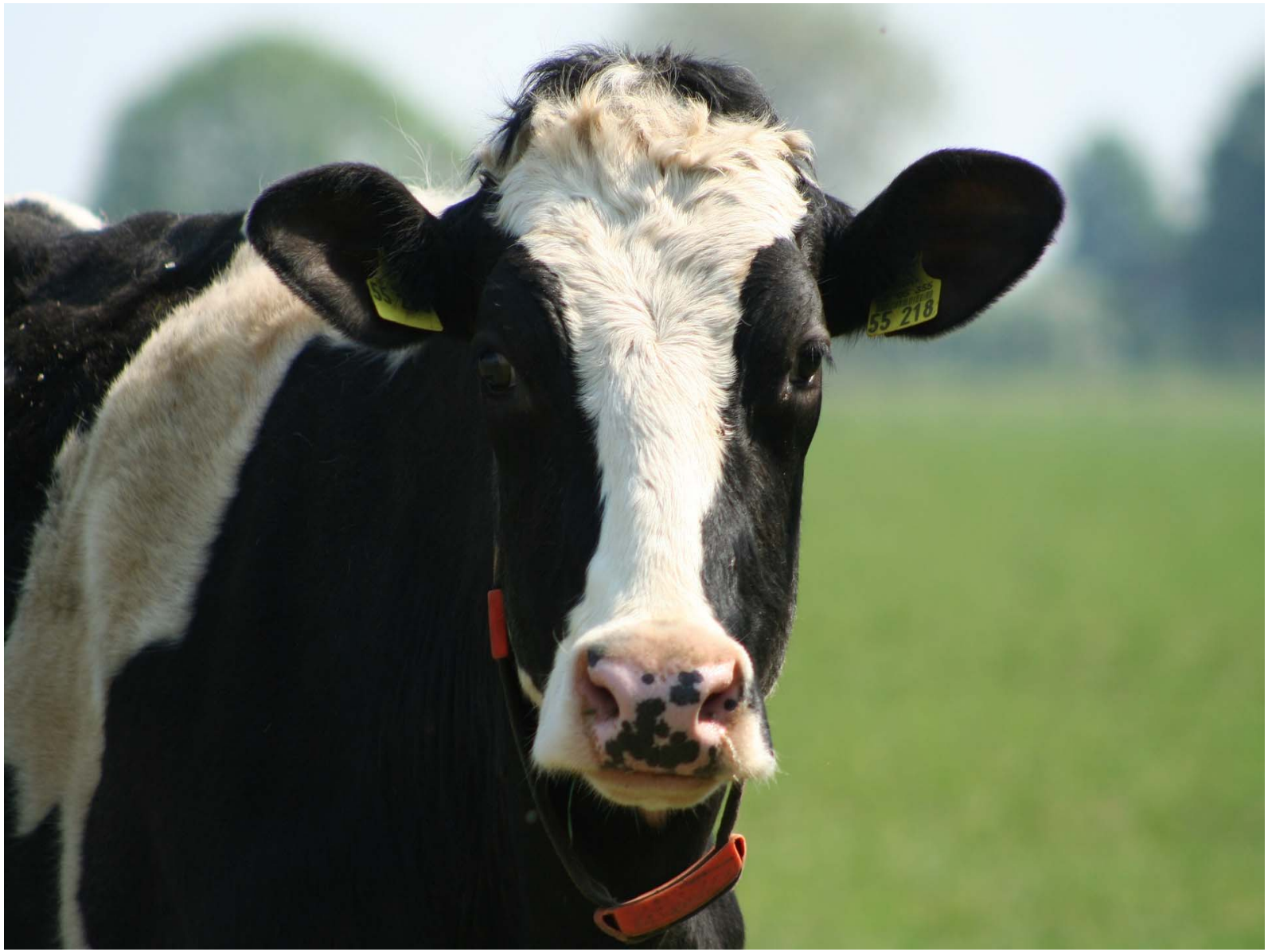
The CDC and state health officials are investigating potential exposures to *Brucella* RB51 in 19 states, connected to consuming raw (unpasteurized) milk from Miller's Biodiversity Farm in Quarryville, Pennsylvania.

Transmission

Prevention

Treatment

https://www.cdc.gov/brucellosis/index.html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Brucellosis: A Case Study and Discussion

Questions?

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