

## Using Exercises to Help Prevent Bioterrorism Agent Exposures

WCLN Webinar  
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Eric Beck, PhD, D(ABMM), ACL Laboratories  
Erin Bowles, BS, MT(ASCP), WSLH  
Amanda Weiss, BS, MT(ASCP), WSLH

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## Objectives

- 1) Discuss bioterrorism agents that have been recently found in WI, what indicators may be observed that suggest a possible bioterrorism agent and what mitigation steps can be taken to help prevent exposure.
- 2) Explain the basic thought process used in ruling out possible bioterrorism agents.
- 3) Identify the resources that are available to aid in performing rule-out testing of bioterrorism agents and where they are located.

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## Laboratory Exposure to *Brucella suis* from a Blood Culture

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## Outline

- Overview of the case
  - Patient history, presentation, lab results
- Isolation of a BT Agent
  - Identify Exposures, determine reporting
- Post-exposure follow up
  - Root cause analysis, risk assessment
- Lessons learned
  - Procedural updates, practice improvements
- Summary

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## Overview of the Case

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## Patient History

- 50 year old male
- No Hx of TB exposure
- Spends significant time outdoors
  - Forages for mushrooms
  - Hunts deer, squirrel, and waterfowl

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**Clinical Presentation**

- Fever, rigors, night sweats, and non-productive cough for 5 weeks
- Depressed appetite; lost 15 pounds
- Neck stiffness/headaches following rigors
- Denies confusion
- 6 mm pulmonary nodule in left lower lobe
- 4 mm subpleural nodule in right middle lobe
- Worked up for sepsis and other inf. diseases

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**Infectious Disease Testing Orders**

- Procalcitonin = 0.23 (sepsis not likely)
- Lactic acid = 0.9 (normal)
- C-Reactive protein = 2.5 (elevated)
- Erythrocyte Sed. Rate = 54 (elevated)
- Quantiferon = Negative
- HIV Ag/Ab = Nonreactive
- EBV and CMV IgM = Negative
- Blood cultures x 2 = Aerobic bottles positive with GNR at 60 and 90 hours

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**Blood Culture Processing**

- Aerobic bottle #1
  - Positive with GNR at 60 hours
  - Plates processed in BSC
  - Subcultured for MALDI-TOF analysis
    - Minimal growth, but MALDI-TOF still attempted
    - No identification
  - Culture reincubated for additional workup
  - Plates were not taped up

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**Blood Culture Processing Cont'd**

- Aerobic bottle #2
  - Positive with GNR at 90 hours
  - Processed and subcultured in BSC
  - Taped up due to risk of bioterrorism agent
    - No MALDI-TOF plate was prepared
  - Gram stain of subculture:
    - Gram negative rod
    - Not gram negative coccobacilli or small rod
    - Not plump gram negative rod
    - Assumed bioterrorism agent ruled out

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**Blood Culture Processing (cont.)**

- Proceeded to perform rapid tests on benchtop:
  - Catalase and oxidase = positive
  - Indole = negative
- Pattern suspicious for *Brucella*
  - Urease test in BSC = positive in about 5 min.
- *Brucella* sp. can not be ruled out
- Specimen sent to WSLH for confirmation
  - PCR confirmed *Brucella* sp.
  - CDC confirmed identity as *Brucella suis*

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**Patient Follow Up**

- Brucella antibody 1:640 (reference is <1:20)
- *Brucella suis* associated with wild hogs
- Where did it come from
  - Patient had been on a recent hunting trip for wild pigs (neglected to mention that)
  - Handled raw meat with open abrasions on arms
- Treated with doxycycline + rifampin
- Patient recovered after completing 6 week course of treatment

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## You Isolated a Bioterrorism Agent; Now What?

Were there any exposures?  
 What post-exposure follow up is required?  
 Who needs to be notified?  
 What do you do with the isolates?

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## What Constitutes an Exposure

- Described in CDC Brucellosis Reference Guide<sup>1</sup>



Specimen handling	Exposure scenario	PEP	Follow-up
Isolation (e.g., blood, serum, cerebrospinal fluid, ascitic fluid, urine, sputum, feces, nasal secretions, vaginal secretions, milk, or other body fluids) in a certified Class II biosafety cabinet, with an additional exposure control procedure implemented (e.g., gloves, gown, eye protection)	Person who manipulates a routine clinical specimen (e.g., blood, serum, cerebrospinal fluid, ascitic fluid, urine, sputum, feces, nasal secretions, vaginal secretions, milk, or other body fluids) in a certified Class II biosafety cabinet, with an additional exposure control procedure implemented (e.g., gloves, gown, eye protection)	Discontinue (DID) face shield, and change clothing and shoes for 30 minutes.	Regular contact with (e.g., sexual contact, kissing, etc.) daily until face shield through 28 days post-exposure, after last routine specimen.
Isolation (e.g., a blood, serum, cerebrospinal fluid, ascitic fluid, urine, sputum, feces, nasal secretions, vaginal secretions, milk, or other body fluids) in a certified Class II biosafety cabinet, with an additional exposure control procedure implemented (e.g., gloves, gown, eye protection)	Person who manipulates (e.g., a) a fluid from animal mammalian, non-ruminant animal (e.g., a blood, urine, genital tract fluid) or ruminant animal specimen (e.g., amniotic fluid, placental products, milk) in a certified Class II biosafety cabinet.	For poultry and ruminants: 14 days of oral antibiotic (e.g., tetracycline, trimethoprim-sulfamethoxazole, streptomycin) for 28 days post-exposure, after last routine specimen.	Regular contact with (e.g., sexual contact, kissing, etc.) daily until 28 days post-exposure, after last routine specimen.
Isolation (e.g., a blood, serum, cerebrospinal fluid, ascitic fluid, urine, sputum, feces, nasal secretions, vaginal secretions, milk, or other body fluids) in a certified Class II biosafety cabinet, with an additional exposure control procedure implemented (e.g., gloves, gown, eye protection)	Person who manipulates animal material (e.g., a blood, urine, genital tract fluid) or ruminant animal specimen (e.g., amniotic fluid, placental products, milk) in a certified Class II biosafety cabinet, without appropriate personal protective equipment (e.g., gloves, gown, eye protection).	Two intramuscular injections of rifampin, 600 mg, 12 hours apart, plus 14 days of oral antibiotic (e.g., tetracycline, trimethoprim-sulfamethoxazole, streptomycin).	Regular contact with (e.g., sexual contact, kissing, etc.) daily until 14 days post-exposure, after last routine specimen.
Isolation (e.g., a blood, serum, cerebrospinal fluid, ascitic fluid, urine, sputum, feces, nasal secretions, vaginal secretions, milk, or other body fluids) in a certified Class II biosafety cabinet, with an additional exposure control procedure implemented (e.g., gloves, gown, eye protection)	All persons present during the occurrence of animal generating events (e.g., centrifuging, animal handling, milking, lambing, parturition, necropsy) in a Class II biosafety cabinet, without appropriate personal protective equipment (e.g., gloves, gown, eye protection).	None (N0).	None (N0).

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## Laboratory Exposure

- Aerobic bottle #1
  - Positive with GNR at 60 hours
  - Positive bloods subcultured for workup and for MALDI-TOF analysis; not taped up
  - MALDI-TOF performed on 5 hour old subculture outside BSC
    - Tech performing MALDI not aware specimen was GNR > 48 hours
- This is an exposure

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**Laboratory Exposure (cont.)**

- Aerobic bottle #2
  - Positive with GNR at 90 hours
  - Processed and subcultured appropriately; taped up for bioterrorism rule out
  - Not submitted for MALDI testing
  - Performed the following on benchtop:
    - Catalase = positive
    - Oxidase = positive
    - Indole = negative
- This is an exposure

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**Laboratory Exposure (cont.)**

- Resulted in 9 high risk exposures
  - 4 technologists opened subcultures outside BSC
    - 2 looked closely at open plates and reincubated; no manipulation
    - 1 attempted MALDI from scant growth outside BSC
    - 1 performed rapid biochemical testing outside BSC
  - 5 technologists within 5 feet of manipulations being performed outside BSC

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**Post-Exposure Follow Up**

- All staff members
  - Offered consult by employee health
  - Serial *Brucella* serology tests at 0, 12, and 24 wks
  - Offered 3 week course of doxycycline + rifampin
    - 3 declined due to past issues with these drugs
    - 6 took antibiotics for at least two weeks
      - 2 discontinued one or both components due to side effects at 2.5 weeks
- No seroconversions or illnesses associated with exposure

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## What Notifications Must Be Made

- All bioterrorism agents in normal rule-out process are select agents
- Federal Select Agent Program
  - Agents which have the potential to pose a severe threat to human, animal, or plant health, or to animal and plant products



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## Federal Select Agent Program

- Isolation of select agents must be reported:
  - Complete APHIS/CDC Form 4 within 7 days
- Some require additional notification within 24 hours by phone, fax, email
- Additional paperwork when agent released:
  - Includes exposure, theft, or loss
  - APHIS/CDC Form 3
- Isolates/specimens destroyed or transferred
  - Transfer requires APHIS/CDC Form 2

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## What Went Wrong?

Deviations from current policies?  
Are there gaps in current policies?

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**Root Cause Analysis**

- Determine where the processes failed
- JCAHO has an RCA framework template<sup>3</sup>
  - Consists of 24 analysis questions
  - Investigation performed by unbiased party
- Includes:
  - Interviews of all parties involved
  - Review of procedure manuals
  - Walk through of operations

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**Root Cause Analysis (cont.)**

- Technologist experience
  - Processing technologist completed training 1 week earlier
    - Thought GNRs growing > 72 hours not >48 hours entered bioterrorism rule-out
- Lack of Communication
  - Some risk factors not relayed to provider
  - No risk factors relayed to the lab
  - Staff not all aware of the exposure event

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**Root Cause Analysis (cont.)**

- Rule out charts can be subjective
  - Blood bench ended up with untaped plates from bottle #1 and taped plates from bottle #2
  - Questioned why taped and untaped plates
  - Reviewed gram stains
  - Determined gram stains to be gram negative rods
    - Bioterrorism agents ruled out because
      - Not plump gram negative rods
      - Not small gram negative rods or coccobacilli

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**Additional Lessons Learned**

- Frequent risk assessment is important
- Work flow issues on blood bench
  - BT rule out charts can be subjective
  - Individual BT rule out charts can lead to missed rule out steps
  - Bench techs assume BT agents ruled out if plates not taped
- Well defined post exposure plan is critical

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**Risk Assessments**

- Do not skip or rush!!
- Perform when:
  - Implementing new testing
  - Significant workflow changes
  - After biosafety failures (e.g. exposures)
- Involve testing personnel
  - Practice may not always match procedure

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**Risk Assessments (cont.)**

- Share results with staff
  - Identify changes and explain why
  - Ensure staff understands biggest risks
- Share results with other department
  - Microbiologists often have the keenest eye for biosafety issues
- Share results with administration
  - Significant issues may require capital

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### Work Flow Issues

- Techs pulling bottles from instruments differ from those working up cultures
  - Requires trust that specimens processed correctly
- Some steps are subjective
  - Differences in interpretation of coccobacilli
  - Can colony morphology rule out organisms
- Issues with bioterrorism rule out key
  - Discrepancies between versions
  - Individual charts can lead to missed steps

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### Post Exposure Plan is Critical

- Exposure happened on a weekend
- Staff thought they had an exposure, but:
  - Didn't know what constituted an exposure
  - Didn't understand the risks to themselves or their family
  - Employee health not available
  - Limited supervisory staff in lab
  - Who should they call, what should they do

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### Procedural Updates and Preventive Measures

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**Workflow Adjustments**

- Changed timing of GNR rule out
  - Positive blood bottles > 36 hours worked up as possible BT agents
- Blood subculture plate receive sticker:
  - Gram stain result
  - Time to positivity
  - Allows subsequent technologists a chance to catch an untaped plated before they are exposed

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**Workflow Adjustments (cont.)**

- Develop simplified rule out charts
  - One chart for Gram positives (*B. anthracis*)
  - One chart for Gram negatives
  - Combines APHL charts into one step by step document
    - Eliminates missed steps while flipping through several charts
  - Allows rule outs to proceed more quickly

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**Detailed Post-Exposure Plan**

- Current procedures focus on prevention
- Detailed post-exposure plan is lacking
- Developed clear post-exposure plan including:
  - What constitutes an exposure
  - Who must be notified
  - Information relating to transmissibility
  - What situations require prophylaxis/testing
  - Staff communication and follow up plan

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### Summary

- BT agents can find their way to the micro lab
- Labs must remain vigilant
  - Perform risk assessment to develop processes
  - Utilize proficiency testing to test processes
    - Adjust processes if gaps exist
  - Take the time to create detailed post-exposure plans

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### So How Does Participation in a Bioterrorism Preparedness Exercise Program Help Prevent Laboratory Exposures?

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### BT Agents Received at WSLH (2013 – 2018)

BT Agent	2013	2014	2015	2016	2017	2018
<i>B. anthracis</i>						
<i>Brucella spp.</i>	2	4	2	2	2	1
<i>B. mallei</i>						
<i>B. pseudomallei</i>					1	
<i>F. tularensis</i>			3			1
<i>Y. pestis</i>						

NOTE: *B. cereus* biovar *anthracis* has not been detected in humans

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### What We Know

- Bioterrorism agents are isolated infrequently in WI
- It is unlikely that the ordering physician will communicate to the laboratory that a BT agent is suspected or that the lab will receive a travel history.
- Bioterrorism (BT) agents are most likely to be found in blood, lower respiratory and wound cultures.
- Although not a high incidence state, people may have traveled to a state where there is a larger naturally occurring reservoir of a specific BT agent and therefore a higher incidence of BT agent infection.

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### Where Are BT Agents Found?

Number of Reported Brucella Cases - 2010      Reported Cases of Human Plague 1970 - 2016

Reported Cases of Tularemia - 2016

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### What We Know (cont.)

**STOP** work and think "possible BT agent" when:

- An organism doesn't grow well, or at all on solid media or in a blood culture media until >48 hours
  - Move all work to a biosafety cabinet (BSC)
  - Perform Gram stain, catalase and oxidase in a BSC on these organisms before other testing.
- If Gram stain shows tiny Gram negative bacilli or coccobacilli, or large boxcar shaped Gram positive bacilli
- If blood culture instrument indicates bottle is positive but nothing is seen on Gram stain

*Brucella* sp.

*Francisella tularensis*

*Bacillus anthracis*

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## What We Know (cont.)

**Remember:**

- MALDI-TOF doesn't ID BT agents well and will actually misidentify BT agents because there is not enough BT isolate data in the standard database.
- Safely using the special MALDI-TOF BT database requires extra safety extraction and filtration steps before spotting to kill spores.
  - *"Safety and Accuracy of MALDI-TOF MS to Identify Highly Pathogenic Organisms"*
    - <https://jcm.asm.org/content/jcm/early/2017/10/06/JCM.01023-17.full.pdf>

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## What We Know (cont.)

**Your LRN Reference Laboratory is happy to answer any questions and help – contact them!**

- Rule-out resources have been harmonized
  - ASM: Guidelines for Suspected Agents of Bioterrorism
  - APHL: *"Clinical Laboratory Preparedness and Response Guide"*
  - APHL: Biothreat Agent Bench Cards for the Sentinel Laboratory
  - Always check on-line to make sure you are using the most up to date version
- Don't perform extra testing not listed in the flowcharts.
- Not recommended to develop your own testing algorithms
- Working on a tool for use to evaluate exposures

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## Definition of Sentinel Clinical Laboratories

<http://www.slh.wisc.edu/wp-content/uploads/2018/11/Sentinel-Clinical-Laboratories-Definition-Updated-April-2018-.pdf>

- *Responsibilities of the Sentinel Clinical Laboratory*
  - *Clinical core/central laboratories are responsible for providing their satellite facilities with written directions and training as needed for appropriate sample collection and handling. Core/central 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.*
  - *The laboratory maintains the capability to perform the testing outlined in the American Society of Microbiology (ASM) Sentinel Level Clinical Microbiology Laboratory Protocols and Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases and must demonstrate annual competency by participation in proficiency testing or exercises, such as the APHL, CDC and College of American Pathologists (CAP) Laboratory Preparedness Exercise (LPX), State-developed proficiency/challenge sets, or other equivalent assessment.*

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## Learning by Doing

- Styles of Learning
 

<b>Visual</b> • Visual learners prefer the use of images, maps, and graphic organizers to access and understand new information.	<b>Auditory</b> • Auditory learners best understand new content through listening and speaking in situations such as lectures and group discussions. Aural learners use repetition as a study technique and benefit from the use of mnemonic devices.
<b>Read &amp; Write</b> • Students with a strong reading/writing preference learn best through words. These students may present themselves as copious note takers or avid readers, and are able to translate abstract concepts into words and essays.	<b>Kinesthetic</b> • Students who are kinesthetic learners best understand information through tactile representations of information. These students are hands-on learners and learn best through figuring things out by hand (i.e., understanding how a clock works by putting one together).
- Frequency and repetition

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## Teach New Hires




- Use extra samples to teach - don't assign a new hire to perform a bioterrorism preparedness exercise by reading directions without practice.
 

Cross neck and pull	Cross legs over
Place L-shaped on the back	Make a bow, pull tightly
Push shoulder through hole to form a loop, repeat with the other side	

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## Best Use of Preparedness Exercise

- Assign one person to perform the actual preparedness exercise and instruct them to work up the specimen as if it is from a patient suspected of having exposure to a bioterrorism agent
  - The provided scenarios are meant to provide clues
- After results are received, use residual samples for competency assessment of all staff in recognizing and safely ruling out bioterrorism agents
- Hands on practice is essential for learning

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## Tips for Working With the WSLH BT Preparedness Exercise

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## WSLH BT Preparedness Exercise

- General procedure overview
- Example from 2018 challenge
- How to get the most out of the exercise (do's and don'ts)

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## WSLH BPE Worksheet

Procedure	Culture choices	Comments for Sample	Comments for Sample
Growth on Blood agar at 35°C	24 hours / 48 hours / >48 hours / No growth		
Growth on Chocolate agar at 35°C	24 hours / 48 hours / >48 hours / No growth		
Growth on MacConkey (540) at 35°C	24 hours / 48 hours / >48 hours / No growth		
Hemolysin description	Beta hemolysin / Not beta hemolysin / No growth on BAP		
Gram stain (from agar growth)	Green powder bacilli, large bacilli, cocci Green negative bacilli, small bacilli, coccobacilli, small coccobacilli, cocci		

**STOP!** Consider possible BT agents based on the agar growth, colony appearance, and Gram stain. Only perform further testing for suspected BT agents.

Procedure	Culture choices	Comments for Sample	Comments for Sample
Catalase	Positive / Negative / T20*		
Oxidase	Positive / Negative / T20*		
Indole	Positive / Negative / T20*		
Motility	Positive by tube / Positive by wet mount / Negative by tube / Negative by wet mount / T20*		
Urease	Positive / Negative / T20*		
Satellite test	Positive / Negative / T20*		
Susceptibility Disk <sup>a</sup>	Resistant / Susceptible / T20*		
Beta lactamase	Positive / Negative / T20*		
Growth at 42°C	Growth / No growth / T20*		

\*Only perform the procedures appropriate for the suspected bioterrorism agent(s) that have not been ruled out by growth and/or Gram stain.  
<sup>a</sup>Test not indicated  
<sup>c</sup>Colistin, polymyxin B, penicillin, and/or amoxicillin-clavulanate disks

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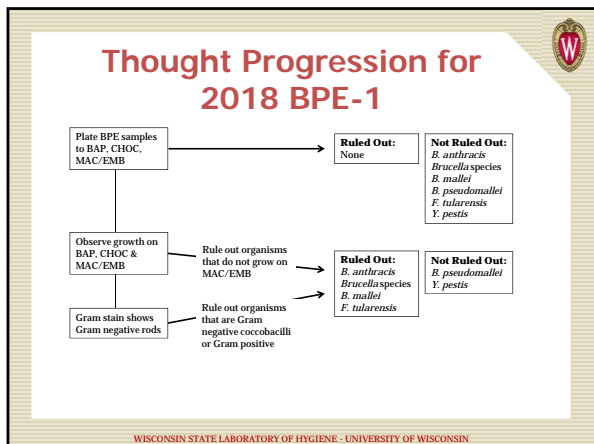
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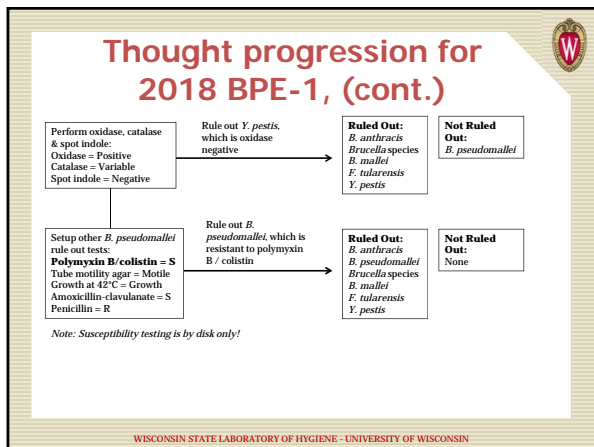
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- ### BPE Do's and Don't's
- Do**

  - Use agar growth and Gram stain to decide what BT agents need to be ruled out.
  - Perform rapid testing first, then additional testing appropriate only for BT's not ruled out.
  - Submit a response for every procedure.
  - Add Comments!
  - Contact us with questions.

**Don't**

  - Use growth rate or be too reliant on Gram stain results to rule out BT agents.
  - Perform every test possible and then decide what has been ruled out.
  - Leave any result entry areas blank.
  - Leave us guessing why procedures were performed or BT agents were or were not ruled out.
  - Assume you can't ask for help during the live event.
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### References (cont.)

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- The Joint Commission: "Framework for Conducting a Root Cause Analysis and Action Plan"  
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### Questions??

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