Agents of Bioterrorism Competency Exam

Instructions:

- Review this PPT presentation
- Answer exam questions associated with this PPT presentation in MTS
- Review Reference Guides (both are by the mail boxes):
  1. "Bioterrorism Guide for Clinical Labs" from the CDC
  2. Microbiology: Bioterrorism Procedure Manual (gray binder)

- DUE DATE: October 31, 2014

Laboratory Response Network (LRN) Classification of Laboratories

Level A: Clinical labs that operate under BSL2 levels, some may have a BSL3 lab that is used for Cat A infectious substances and also for possible bioterrorism agents.

Rule out or forward isolates

Level B: State Public Health Labs, BSL2 with BSL3 practice. Definitive confirmation of isolates

Level C: State Public Health Labs, BSL3 only practice. Rapid detection assays, molecular characterization

Level D: Federal Labs (ex CDC). BSL4 practice. Highest level of organism characterization and containment

(taken from the CDC-Bioterrorism Response Guide for Clinical Labs)

Brucella spp.

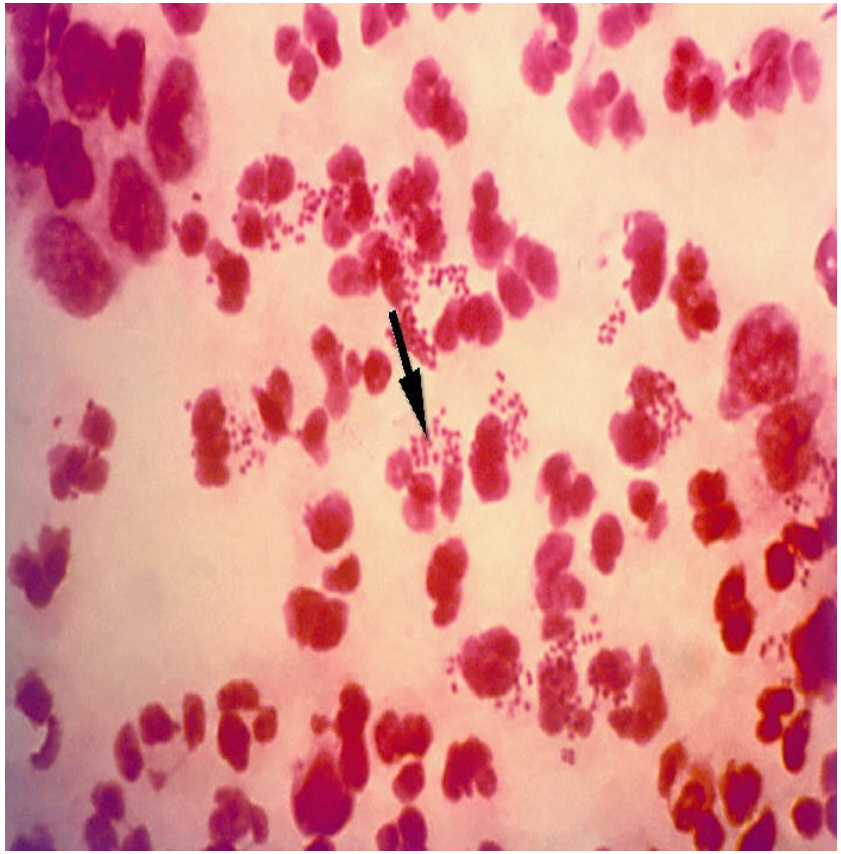
- Endemic to Mediterranean basin, Middle East, western Asia, Africa and Latin America
- Bioterrorism CDC list
- Best Sources:
  - Blood
  - Bone marrow
  - Tissue (lymph node)
- Symptoms:
  - profound weakness
  - diurnal fevers
  - generalized muscle aches
- Disease:
  - Brucellosis
  - Undulant fever
  - Malta fever

Brucella sp.

- Gram stain: tiny gram neg coccobacilli (GNCB) that stain faintly
- Growth: BAP,Choc and MTM, no growth on MAC (specialized media)
- Some species require 5-10% CO_2
- Slow grower: 48-72 hours, hold culture 7-10 days
- Colonies appear pinpoint and are not hemolytic
- Suspected: work up must be done in **BSL3 lab** with appropriate PPE (gloves, lab coat, N95 mask)

Brucella sp

Tiny Gram negative coccobacilli



Small gamma, gray colonies at 48-72 hrs

Key Characteristics of Brucella spp. (Rule in/out process)

If organism is:

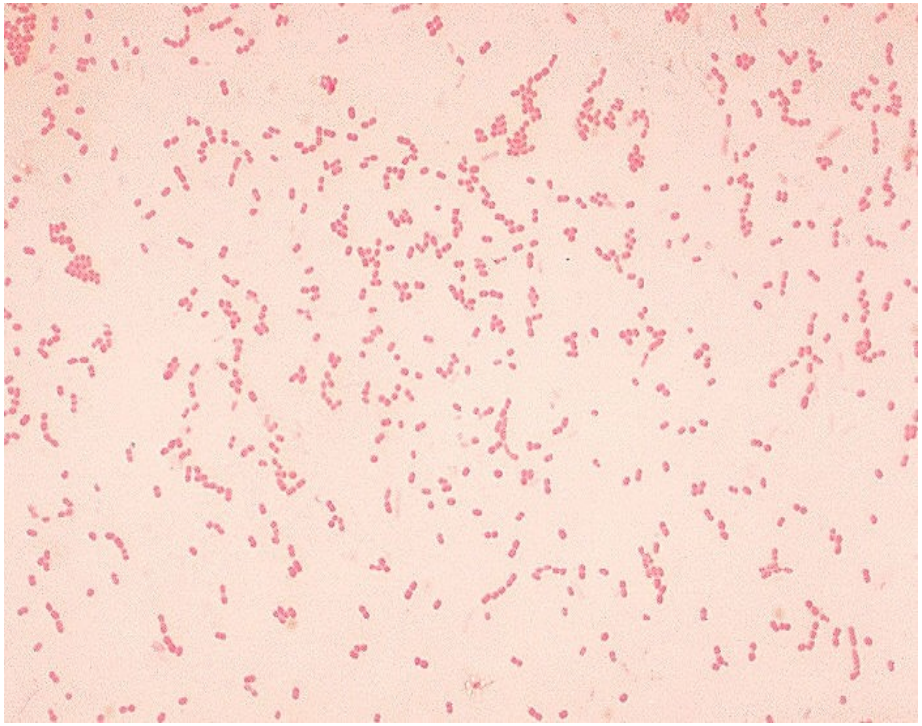
- Slow Grower: 48-72 hrs or longer in CO_2
- Small gray, non-hemolytic
- Gram negative coccobacillus, stains faintly
- Catalase: Pos
- Oxidase: Pos
- Urea: Pos (strong < 2 hrs)
- Non-motile
- Presumptive ID-→Send to MDCH for confirmation

Francisella tularensis

- Isolated from 100's of animals:
  - mammals (wild rabbits, sheep, beaver, muskrat)
  - avian spp.
  - arthropods (ticks, deerflies, mosquitos)
  - domestic animals

Franciscella tularensis

Faint staining tiny GNCB



Glucose Cystine Blood agar

Key Characteristics of Francisella tularensis (Rule in/out process)

If organism is:

- Growth on: BAP with cystine, CHOC, MTM, BCYE, no growth on MAC
- 5% sheep blood: no growth or weak
- 35-37C , no CO_2 needed
- Slow Grower: 48-72 hrs or longer, hold 7 days
- Small gray, alpha-hemolytic
- Very tiny Gram negative coccobaccilli, stains faintly
- Catalase: Neg or weak pos
- Oxidase: Neg
- Urea: Neg
- Non-motile
- Work in **BSL3 lab**
- Presumptive ID-→Send to MDCH for confirmation

Yersinia pestis

- Gram stain : Gram negative rods (bipolar/safety pin)
- Growth: best at 4C or room temperature(21-25C)
- Selective media = CIN(cefsulodin-irgasan-novobiocin)
- Mac and Hek: slow-lactose fermenter
- CIN morphology: small, red center, transparent edge,(bulls eye)
- Suspect--→ **work in BSL3 lab**

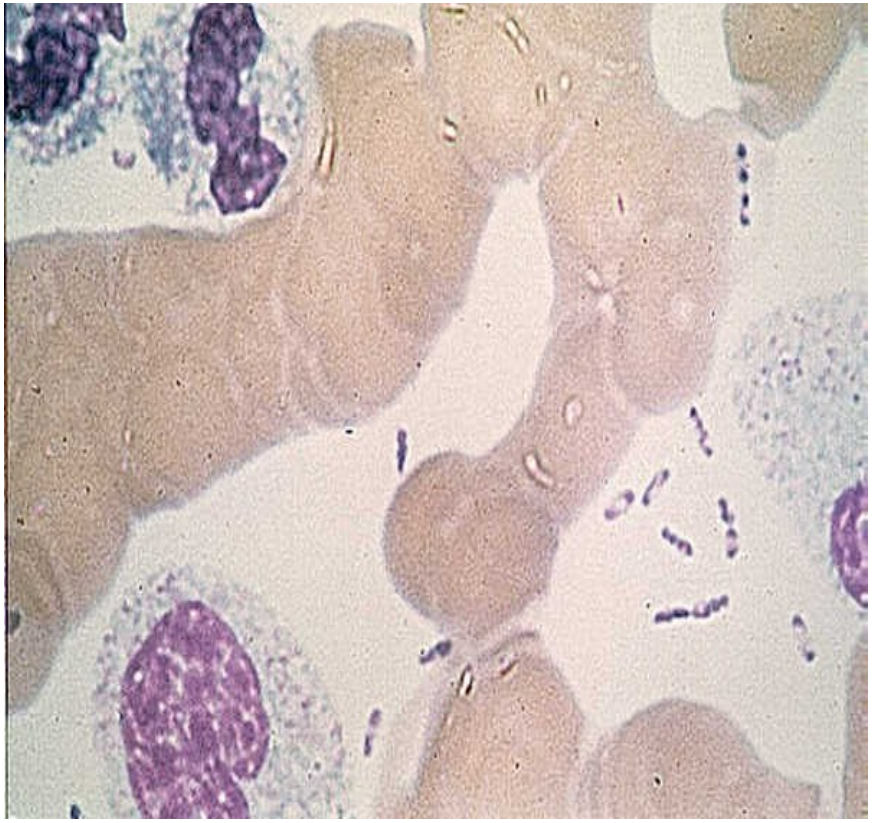
Yersinia pestis

Gram stain

(may or may not see safety pin)

Wright- Giemsa stain

(safety pin)



Yersinia pestis

MAC at 24-48hours

BAP at 48-72 hrs

Yersinia pestis

- CIN agar at RT

Key Characteristics of Yersinia pestis (Rule in/out process)

If organism is:

- Gram negative bacillus(may resemble “safety pin”)
- Grows at 35-37C, faster at room temperature
- Catalase : positive
- Non-motile at 37C and RT (other species of Yersinia are motile at RT)
- Oxidase: negative
- Identification by enteric biochemicals

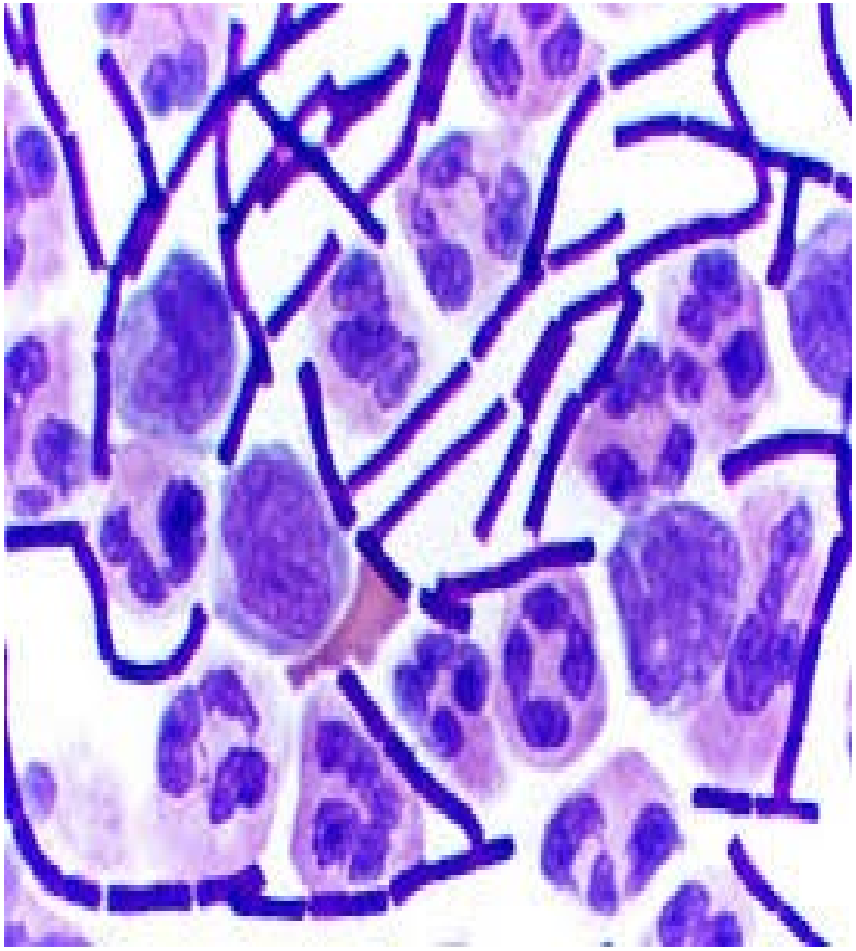
Presumptive ID-→ send to MDCH

Bacillus anthracis

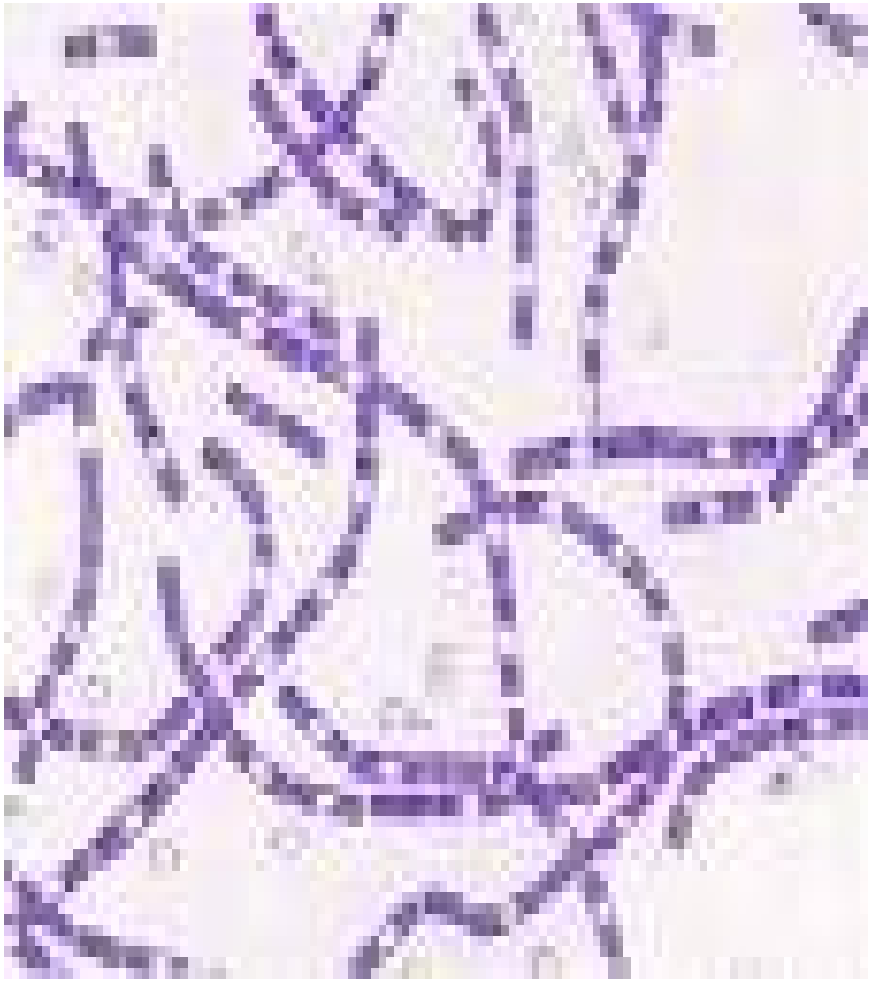
- **Non-motile**
- **Non-hemolytic**
- Have irregular edges to colonies
- Tenacious
- "Medusa head"
- Samples:
  - ✓ Blood
  - ✓ Vescular fluid
- Suspect on culture**:BSL3 lab**
- Send to MDCH to rule out anthracis

Gram stain of Bacillus species

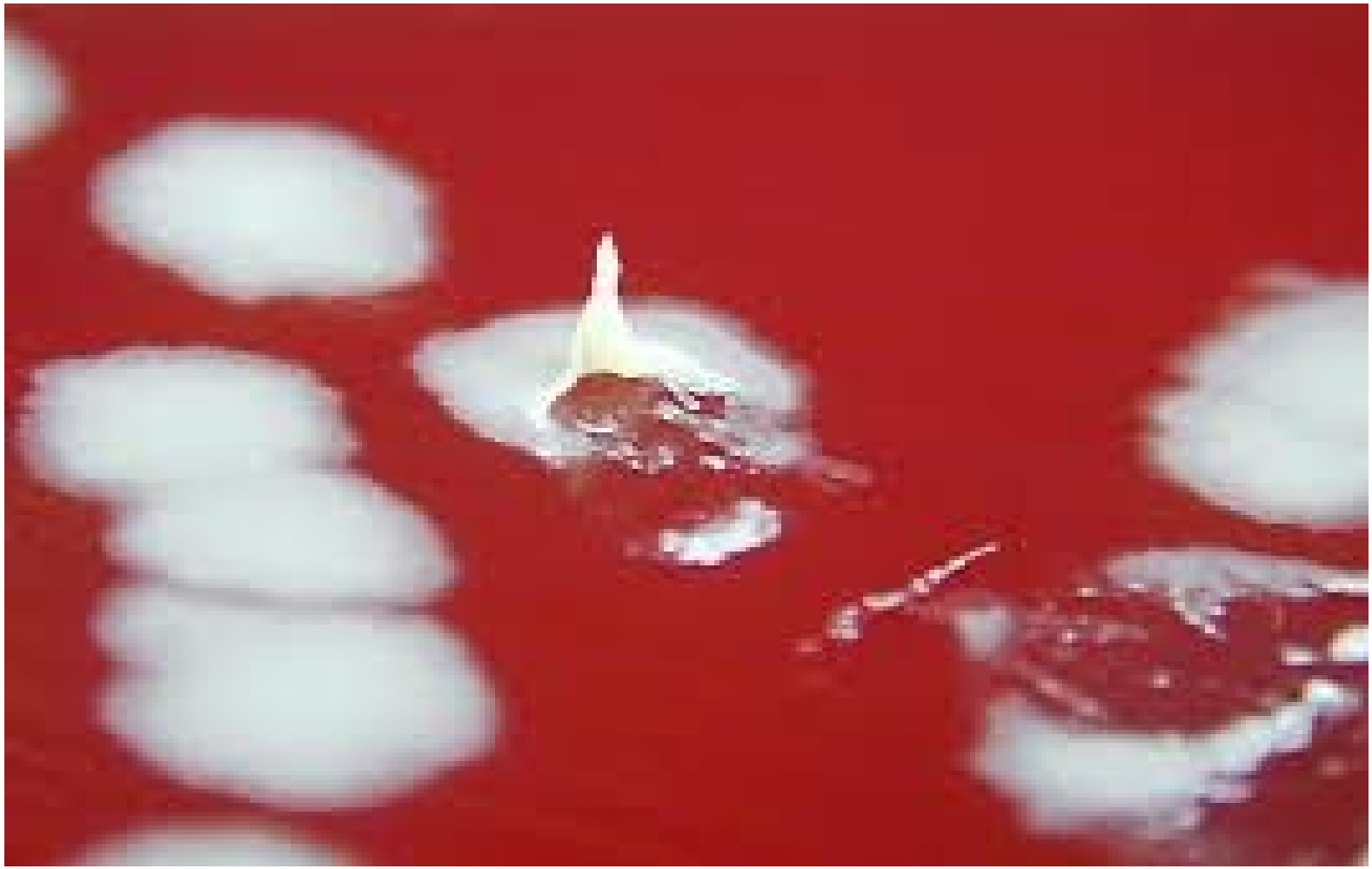
No spores seen



Spores seen (stressed)



Bacillus anthracis on BAP---tenacious

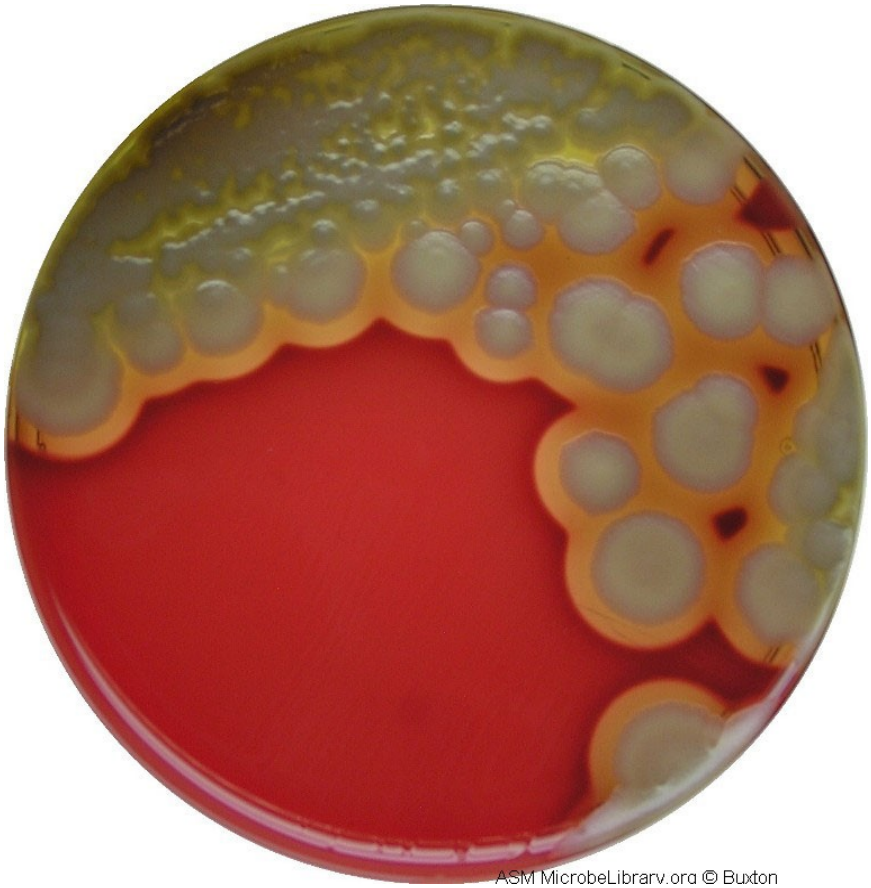


B. anthracis vs. B. species on BAP

Bacillus anthracis (medusa head)

Courtesy of Larry Stauffer, Oregon State Public Health Laboratory

Bacillus sp. not anthracis



Botulinum Toxin

- Lab must be notified if suspected
- MDCH must be notified immediately
- **Responsibility of the Level A** lab is limited to advising medical staff on specimen selection, packing, shipping and notification of recipient lab about suspected case.
- Specimens: Enema fluid, Food sample, Nasal swab, Serum, Stool, Enviromental **(if intentionally released)**
- **Level A labs should not attempt to culture this organism!!**

Clostrifium botulinum

ANA BAP/spready colony

Gram stain/terminal or subterminal spores

Agents of Bioterrorism
Competency Exam

Remember to complete the exam linked with this PPT presentation in MTS.

DUE DATE: 10/31/2014