DEPARTMENT OF HEALTH

Rule Outs – Referring Specimens to MDH

Sentinel Lab Training

May 6th, 2026

MDH declares no conflict of interest regarding the topic of this presentation

Course Objectives

- Understand the MDH list of reportable diseases
  - Identify which diseases must be reported **immediately by phone**
  - Identify which diseases must be reported **within one business day**
  - Locate the list of reportable diseases on the MDH website
- Understand the role of the Federal Select Agent Program
  - Identify Tier 1 select agents
- Know the characteristics of organisms that may need to be sent to MDH for rule out

  - *Bacillus anthracis*
  - Brucellosis-causing *Brucella* spp.
  - *Francisella tularensis*
  - *Burkholderia mallei* & *B. pseudomallei*
  - *Yersinia pestis*
  - Botulinum toxin-producing clostridial organisms *(Clostridium botulinum, etc.)*
  - Viral hemorrhagic fevers

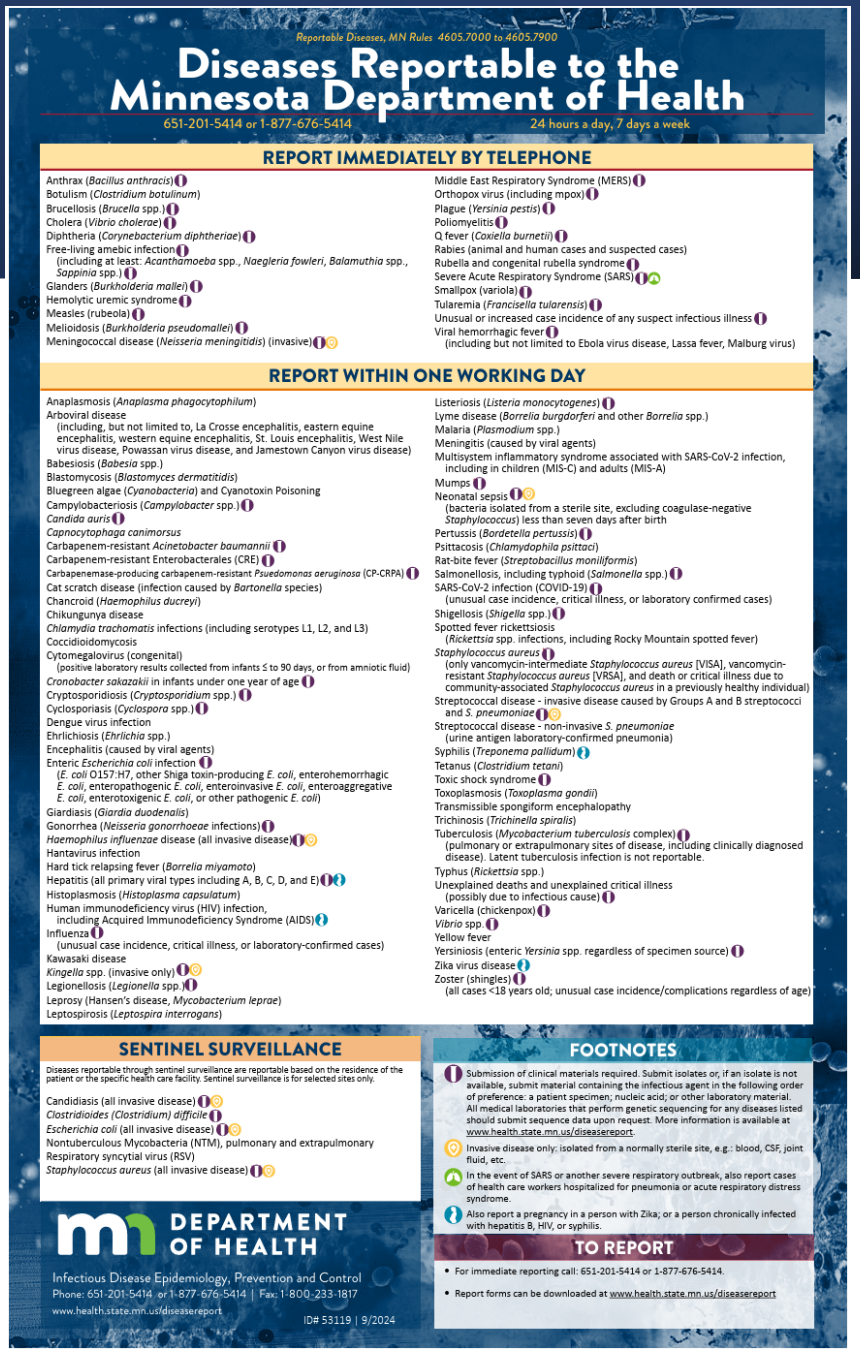
Reportable Diseases

- Information about reportable diseases is available at www.health.state.mn.us/diseases/reportable/rule/index.html

- Two categories:
  - Report immediately by telephone
  - Report withing one working day

- For immediate reporting call:
  - 651-201-5414 or 1-877-676-5414

- Report forms can be downloaded at www.health.state.mn.us/diseasereport



Reportable Diseases, MN Rules 4605.7000 to 4605.7900

Diseases Reportable to the Minnesota Department of Health

651-201-5414 or 1-877-676-5414 — 24 hours a day, 7 days a week

REPORT IMMEDIATELY BY TELEPHONE

Anthrax (*Bacillus anthracis*)
Botulism (*Clostridium botulinum*)
Brucellosis (*Brucella* spp.)
Cholera (*Vibrio cholerae*)
Diphtheria (*Corynebacterium diphtheriae*)
Free-living amebic infection
(including at least: *Acanthamoeba* spp., *Naegleria fowleri*, *Balamuthia* spp., *Sappinia* spp.)
Glanders (*Burkholderia mallei*)
Hemolytic uremic syndrome
Measles (rubeola)
Melioidosis (*Burkholderia pseudomallei*)
Meningococcal disease (*Neisseria meningitidis*) (invasive)
Middle East Respiratory Syndrome (MERS)
Orthopox virus (including mpox)
Plague (*Yersinia pestis*)
Poliomyelitis
Q fever (*Coxiella burnetii*)
Rabies (animal and human cases and suspected cases)
Rubella and congenital rubella syndrome
Severe Acute Respiratory Syndrome (SARS)
Smallpox (variola)
Tularemia (*Francisella tularensis*)
Unusual or increased case incidence of any suspect infectious illness
Viral hemorrhagic fever
(including but not limited to Ebola virus disease, Lassa fever, Malburg virus)

REPORT WITHIN ONE WORKING DAY

Anaplasmosis (*Anaplasma phagocytophilum*)
Arboviral disease
(including, but not limited to, La Crosse encephalitis, eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, West Nile virus disease, Powassan virus disease, and Jamestown Canyon virus disease)
Babesiosis (*Babesia* spp.)
Blastomycosis (*Blastomyces dermatitidis*)
Bluegreen algae (*Cyanobacteria*) and Cyanotoxin Poisoning
Campylobacteriosis (*Campylobacter* spp.)
Candida auris
Capnocytophaga canimorsus
Carbapenem-resistant *Acinetobacter baumannii*
Carbapenem-resistant Enterobacterales (CRE)
Carbapenemase-producing carbapenem-resistant *Psuedomonas aeruginosa* (CP-CRPA)
Cat scratch disease (infection caused by *Bartonella* species)
Chancroid (*Haemophilus ducreyi*)
Chikungunya disease
Chlamydia trachomatis infections (including serotypes L1, L2, and L3)
Coccidioidomycosis
Cytomegalovirus (congenital)
(positive laboratory results collected from infants ≤ to 90 days, or from amniotic fluid)
Cronobacter sakazakii in infants under one year of age
Cryptosporidiosis (*Cryptosporidium* spp.)
Cyclosporiasis (*Cyclospora* spp.)
Dengue virus infection
Ehrlichiosis (*Ehrlichia* spp.)
Encephalitis (caused by viral agents)
Enteric *Escherichia coli* infection
(*E. coli* O157:H7, other Shiga toxin-producing *E. coli*, enterohemorrhagic *E. coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli*, enterotoxigenic *E. coli*, or other pathogenic *E. coli*)
Giardiasis (*Giardia duodenalis*)
Gonorrhea (*Neisseria gonorrhoeae* infections)
Haemophilus influenzae disease (all invasive disease)
Hantavirus infection
Hard tick relapsing fever (*Borrelia miyamotoi*)
Hepatitis (all primary viral types including A, B, C, D, and E)
Histoplasmosis (*Histoplasma capsulatum*)
Human immunodeficiency virus (HIV) infection,
including Acquired Immunodeficiency Syndrome (AIDS)
Influenza
(unusual case incidence, critical illness, or laboratory-confirmed cases)
Kawasaki disease
Kingella spp. (invasive only)
Legionellosis (*Legionella* spp.)
Leprosy (Hansen's disease, *Mycobacterium leprae*)
Leptospirosis (*Leptospira interrogans*)
Listeriosis (*Listeria monocytogenes*)
Lyme disease (*Borrelia burgdorferi* and other *Borrelia* spp.)
Malaria (*Plasmodium* spp.)
Meningitis (caused by viral agents)
Multisystem inflammatory syndrome associated with SARS-CoV-2 infection, including in children (MIS-C) and adults (MIS-A)
Mumps
Neonatal sepsis
(bacteria isolated from a sterile site, excluding coagulase-negative *Staphylococcus*) less than seven days after birth
Pertussis (*Bordetella pertussis*)
Psittacosis (*Chlamydophila psittaci*)
Rat-bite fever (*Streptobacillus moniliformis*)
Salmonellosis, including typhoid (*Salmonella* spp.)
SARS-CoV-2 infection (COVID-19)
(unusual case incidence, critical illness, or laboratory confirmed cases)
Shigellosis (*Shigella* spp.)
Spotted fever rickettsiosis
(*Rickettsia* spp. infections, including Rocky Mountain spotted fever)
Staphylococcus aureus
(only vancomycin-intermediate *Staphylococcus aureus* [VISA], vancomycin-resistant *Staphylococcus aureus* [VRSA], and death or critical illness due to community-associated *Staphylococcus aureus* in a previously healthy individual)
Streptococcal disease - invasive disease caused by Groups A and B streptococci and *S. pneumoniae*
Streptococcal disease - non-invasive *S. pneumoniae*
(urine antigen laboratory-confirmed pneumonia)
Syphilis (*Treponema pallidum*)
Tetanus (*Clostridium tetani*)
Toxic shock syndrome
Toxoplasmosis (*Toxoplasma gondii*)
Transmissible spongiform encephalopathy
Trichinosis (*Trichinella spiralis*)
Tuberculosis (*Mycobacterium tuberculosis* complex)
(pulmonary or extrapulmonary sites of disease, including clinically diagnosed disease). Latent tuberculosis infection is not reportable.
Typhus (*Rickettsia* spp.)
Unexplained deaths and unexplained critical illness
(possibly due to infectious cause)
Varicella (chickenpox)
Vibrio spp.
Yellow fever
Yersiniosis (enteric *Yersinia* spp. regardless of specimen source)
Zika virus disease
Zoster (shingles)
(all cases <18 years old; unusual case incidence/complications regardless of age)

SENTINEL SURVEILLANCE

Diseases reportable through sentinel surveillance are reportable based on the residence of the patient or the specific health care facility. Sentinel surveillance is for selected sites only.

Candidiasis (all invasive disease)
Clostridioides (Clostridium) difficile
Escherichia coli (all invasive disease)
Nontuberculous Mycobacteria (NTM), pulmonary and extrapulmonary
Respiratory syncytial virus (RSV)
Staphylococcus aureus (all invasive disease)

FOOTNOTES

Submission of clinical materials required. Submit isolates or, if an isolate is not available, submit material containing the infectious agent in the following order of preference: a patient specimen; nucleic acid; or other laboratory material. All medical laboratories that perform genetic sequencing for any diseases listed should submit sequence data upon request. More information is available at www.health.state.mn.us/diseasereport.

Invasive disease only: isolated from a normally sterile site, e.g.: blood, CSF, joint fluid, etc.

In the event of SARS or another severe respiratory outbreak, also report cases of health care workers hospitalized for pneumonia or acute respiratory distress syndrome.

Also report a pregnancy in a person with Zika; or a person chronically infected with hepatitis B, HIV, or syphilis.

TO REPORT

- For immediate reporting call: 651-201-5414 or 1-877-676-5414.
- Report forms can be downloaded at www.health.state.mn.us/diseasereport

DEPARTMENT OF HEALTH

Infectious Disease Epidemiology, Prevention and Control
Phone: 651-201-5414 or 1-877-676-5414 | Fax: 1-800-233-1817
www.health.state.mn.us/diseasereport

ID# 53119 | 9/2024

Reportable Diseases

Arboviruses

CREs

Dengue

Giardia

Legionella

REPORT WITHIN ONE WORKING DAY

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  (all cases <18 years old; unusual case incidence/complications regardless of age)

Plasmodium spp

Treponema pallidum

Rickettsia spp

Enteric *Yersinia* spp

Reportable Diseases

REPORT IMMEDIATELY BY TELEPHONE

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- Botulism (*Clostridium botulinum*)
- Brucellosis (*Brucella* spp.)
- Cholera (*Vibrio cholerae*)
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- Free-living amebic infection (including at least: *Acanthamoeba* spp., *Naegleria fowleri*, *Balamuthia* spp., *Sappinia* spp.)
- Glanders (*Burkholderia mallei*)
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- Q fever (*Coxiella burnetii*)
- Rabies (animal and human cases and suspected cases)
- Rubella and congenital rubella syndrome
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- Smallpox (variola)
- Tularemia (*Francisella tularensis*)
- Unusual or increased case incidence of any suspect infectious illness
- Viral hemorrhagic fever (including but not limited to Ebola virus disease, Lassa fever, Malburg virus)

Federal Select Agent Program
(HHS and Overlap)

- The Federal Select Agent Program oversees the possession, use, and transfer of biological select agents and toxins which have the potential to pose a severe threat to public, animal or plant health or to animal or plant products.

- Complete list of select agents can be found at www.SelectAgents.gov

1. Abrin
2. *Bacillus cereus* Biovar *anthracis*
3. Botulinum neurotoxins
4. Botulinum neurotoxin producing species of Clostridium
5. Conotoxins
6. *Coxiella burnetii*
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol
9. Eastern equine encephalitis virus
10. Ebolavirus
11. *Francisella tularensis*
12. Lassa fever virus
13. Lujo virus
14. Marburg virus
15. Monkeypox virus
16. Reconstructed 1918 Influenza virus
17. Ricin
18. *Rickettsia prowazekii*
19. Severe acute respiratory syndrome coronavirus (SARS-CoV)
20. SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation to incorporate nucleic acids coding for SARS-CoV virulence factors
21. Saxitoxin
22. Chapare
23. Guanarito
24. Junín
25. Machupo
26. Sabia
27. *Staphylococcal* enterotoxins (subtypes A,B,C,D,E)
28. T-2 toxin
29. Tetrodotoxin

Tick-borne encephalitis complex (flavi) viruses:

30. Far Eastern subtype
31. Siberian subtype
32. Kyasanur Forest disease virus
33. Omsk hemorrhagic fever virus
34. Variola major virus (Smallpox virus)
35. Variola minor virus (Alastrim)
36. *Yersinia pestis*

Overlap Select Agents and Toxins

37. *Bacillus anthracis*
38. *Bacillus anthracis* Pasteur strain
39. *Burkholderia mallei*
40. *Burkholderia pseudomallei*
41. Hendra virus
42. Nipah virus
43. Rift Valley fever virus
44. Venezuelan equine encephalitis virus

FEDERAL SELECT AGENT PROGRAM • 2024 ANNUAL REPORT | KEY STATISTICS

INTRODUCTION

The *2024 Annual Report of the Federal Select Agent Program* (FSAP) provides insight into the program's regulatory activities and includes a look at compliance with the select agent and toxin regulations at laboratories across the nation. The report also highlights FSAP's efforts to engage with the regulated community throughout the year to enhance regulatory compliance.

The content underscores that overall, laboratories registered with the program are compliant with the regulations, and none of the small number of reported incidents during the year resulted in a significant risk to public health or agriculture. The publication, now in its tenth year, reflects the program's ongoing commitment to transparency.

BACKGROUND

FSAP is managed jointly by the Centers for Disease Control and Prevention and the Animal and Plant Health Inspection Service. The program regulates the possession, use, and transfer of biological select agents and toxins – which are materials that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products – so that important work with dangerous and deadly pathogens can be conducted as safely and securely as possible.

230 ENTITIES WERE REGISTERED WITH FSAP

- **36%** ACADEMIC
- **29%** NON-FEDERAL GOVERNMENT
- **17%** COMMERCIAL
- **13%** FEDERAL GOVERNMENT
- **5%** PRIVATE

52% of entities were registered for Tier 1* select agents and toxins

TOP REGISTERED AGENTS BY AGENCY

CENTERS FOR DISEASE CONTROL AND PREVENTION

1. *Brucella melitensis*
2. *Brucella suis*
3. *Brucella abortus*
4. *Bacillus anthracis* (Pasteur strain)
5. *Francisella tularensis**

ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. Avian influenza virus
2. Newcastle disease virus
3. *Ralstonia solanacearum*
4. *Xanthomonas oryzae*
5. African swine fever virus *Bacillus anthracis**

Some positions have multiple agents tied.

LAB BIOSAFETY LEVELS (BSL)

ENTITIES MAY HAVE MULTIPLE TYPES

- **28%** had **BSL-2** and/or **ABSL-2** labs
- **77%** had **BSL-3** and/or **ABSL-3** labs
- **3%** had **BSL-4** and/or **ABSL-4** labs

OUTREACH

- **4** events held for the regulated community (**3** webinars/**1** multi-day workshop) on in-depth select agent and toxin topics **Estimated 1,093 total attendees**
- **26** entities contacted as a result of emergency management activities during which FSAP offered assistance due to events such as severe weather, natural disasters, or other situations with potential impacts to safety or security (**8** events, **0** entities required assistance)

196 TOTAL INSPECTIONS CONDUCTED BY FSAP

- **119** by CDC
- **24** by APHIS
- **53** joint CDC & APHIS

8,902 ACTIVE INDIVIDUAL SECURITY RISK ASSESSMENTS

Denied access for **18** individuals

COMPLIANCE & ENFORCEMENT

- **2** entities participated in a corrective action plan (with **1** entity newly participating in 2024)
- **0** entities had their registrations revoked
- **0** entities had suspended registrations
- **14** security-related matters were shared with the Federal Bureau of Investigation for potential investigation
- **1** entity was referred to APHIS Investigative and Enforcement Services (with **0** entities referred to the Department of Health and Human Services Office of Inspector General)

THEFT, LOSS, OR RELEASE

- **0** thefts reported
- **9** reports of losses; **1** loss was recovered by the entity and the other **8** were determined to be related to record keeping issues and not the actual loss of material
- **0** releases resulted in illness, death, or transmission among workers or to the outside of a laboratory into the surrounding environment or community (out of **273** reports)

KEY ABBREVIATIONS:

FSAP: Federal Select Agent Program
CDC: Centers for Disease Control and Prevention
ABSL: Animal Biosafety Level
APHIS: Animal and Plant Health Inspection Service

***Tier 1 agent:** those that pose the greatest risk through misuse

Source: *2024 Annual Report of the Federal Select Agent Program*, September 2025 | www.selectagents.gov/annualreport2024.html

Tier 1 Select Agents

- A subset of select agents and toxins that present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence.
- Pose a **severe** threat to public health and safety
- **If a Tier 1 Select Agent cannot be ruled out, it must be referred to MDH**

HHS Tier 1 Select Agents

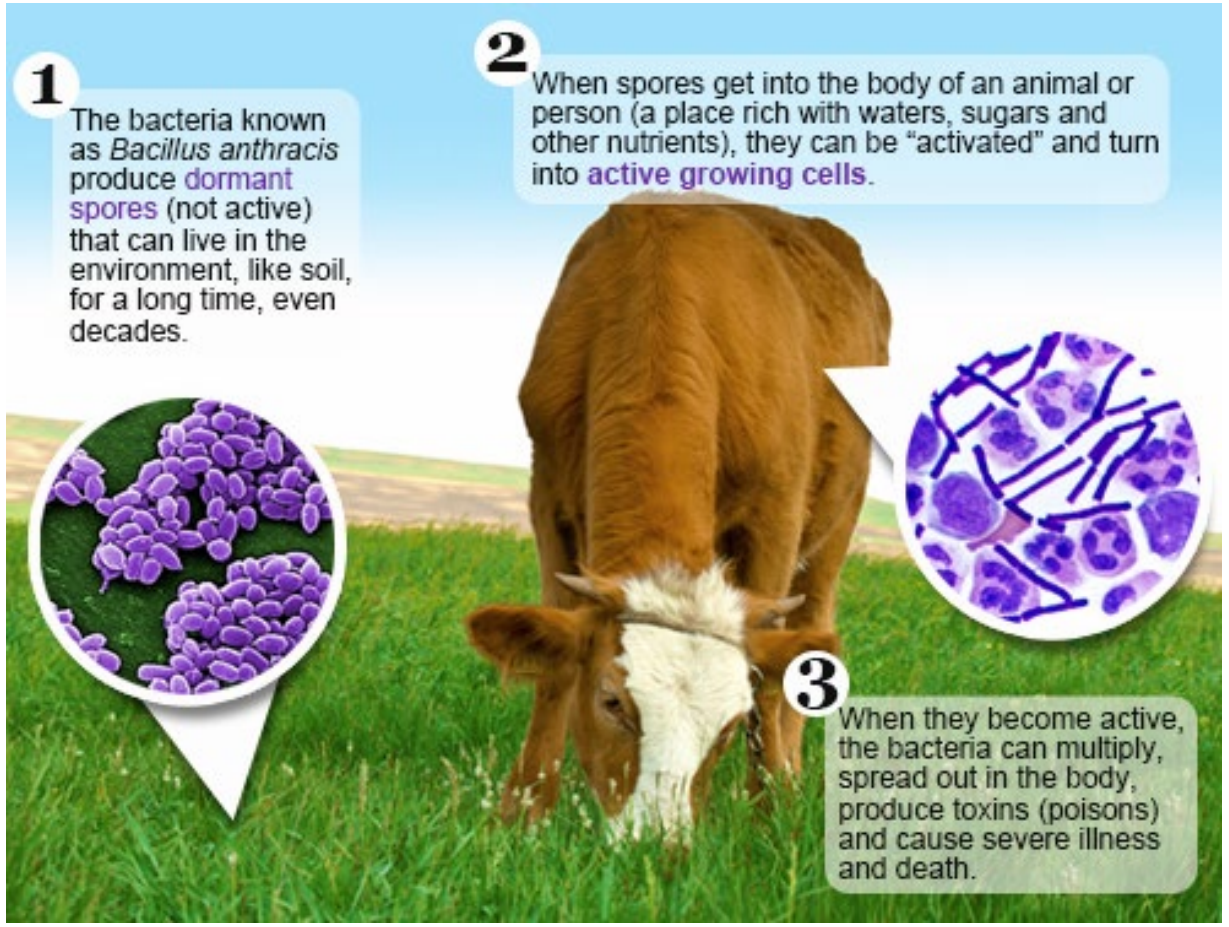
- *Bacillus anthracis*
- *Bacillus cereus* Biovar *anthracis*
- Botulinum neurotoxins
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- Ebola virus
- *Francisella tularensis*
- Marburg virus
- Variola major virus
- Variola minor virus
- *Yersinia pestis*

USDA Tier 1 Select Agents

- Foot-And-Mouth Disease virus
- Rinderpest virus

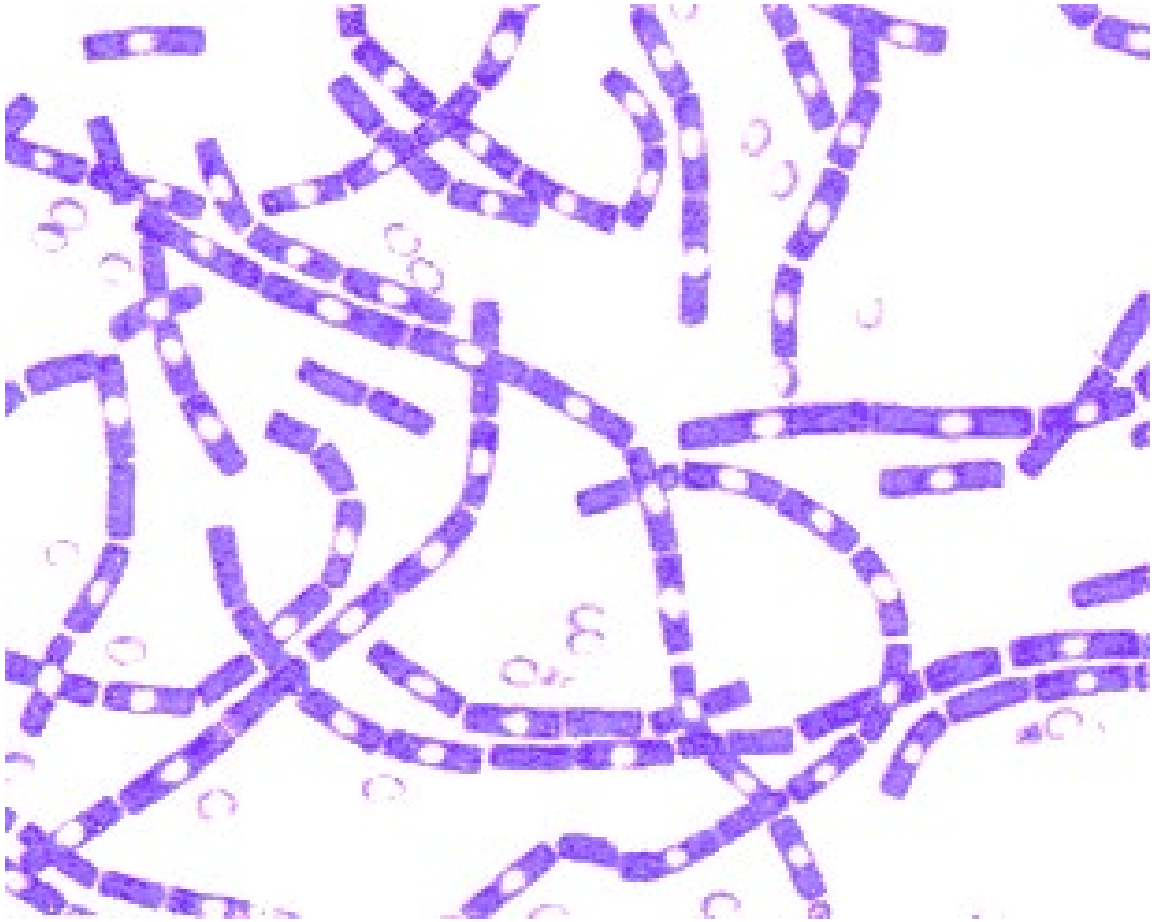
Bacillus anthracis – Anthrax

- Primarily a disease of grazing herbivores
- Spores are highly infectious and cause severe illness
  - Not contagious
- Four types of anthrax
  - Cutaneous (most common)
  - Inhalational
  - Gastrointestinal
  - Injection
- Bioterrorist threat
  - Tier 1 select agent
  - Powdered anthrax spores mailed in 2001 resulting in 22 infections and 5 deaths
  - Inhalation case in MN - 2011



Bacillus anthracis – Cell morphology

- Aerobic, Gram-positive rod
- 1–1.5 × 3–10 µm
- Non-motile, non-hemolytic
- Chain formation is common
- Spore forming



Bacillus anthracis – Culture

- Rough “ground glass” colonies
- Irregular edges with comma-like projections (“comet-tails”)
- Sticky and adheres to the agar (“medusa heads”)
- Non-hemolytic

Ground glass appearance

Sticky, adherent colonies

Comma-like “comet tails”

Bacillus anthracis – Biochemical Tests

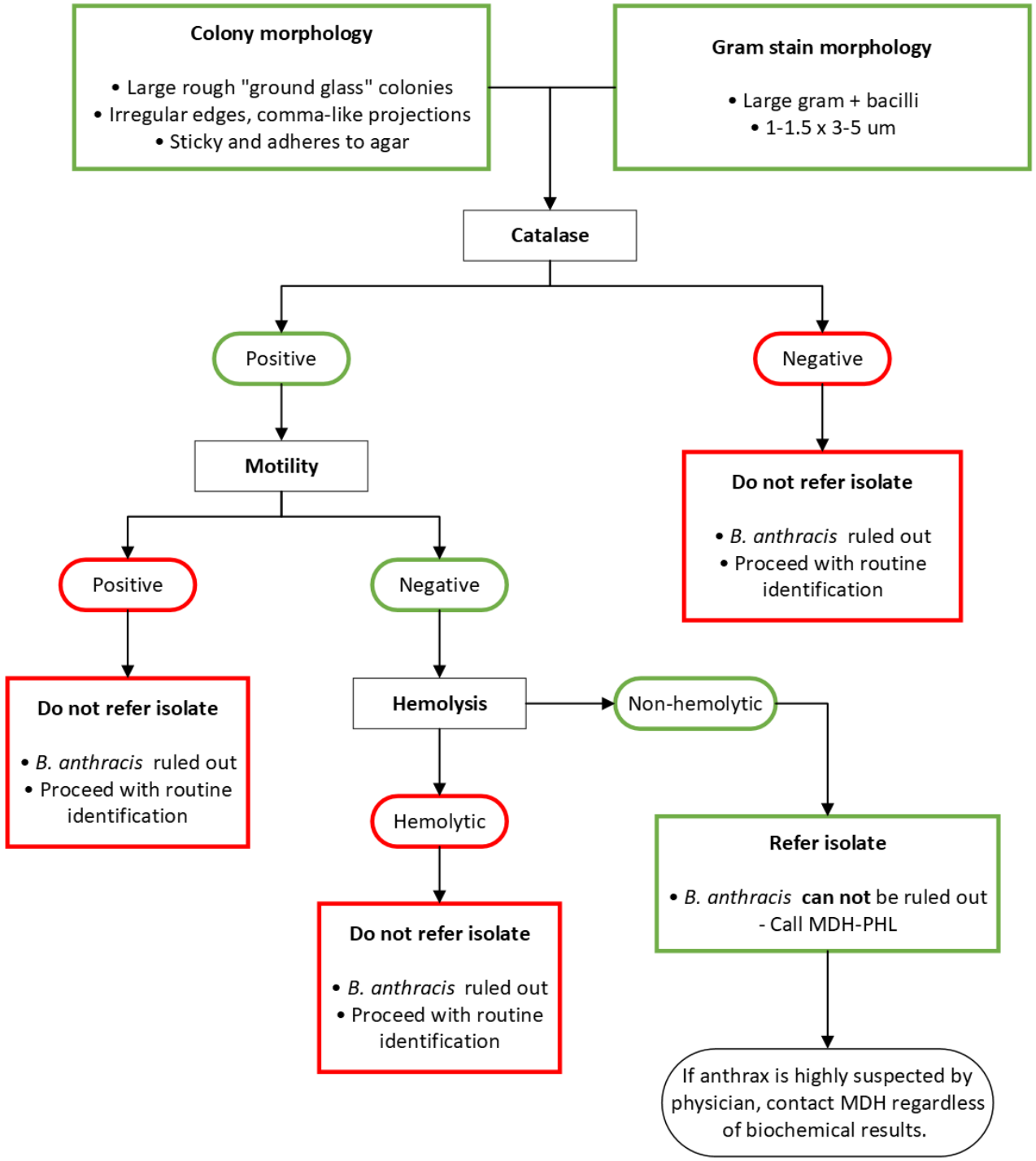
	B. anthracis	*B. cereus*	*B. cereus* biovar *anthracis* CI Primary[1]	*B. cereus* biovar *anthracis* CI Sub[2]	*B. cereus* biovar *anthracis* CA Primary[3]	*B. cereus* biovar *anthracis* CA Sub[4]
Hemolysis	-	+	-	+/- [5]	-	+/-
Motility	-	+	+	+	+	+
Gamma Phage Susceptibility	+	-	-	+/-	-	+/-
Penicillin G	S	R	S	S/R	R	R
Capsule	+	Absent in vitro	+	+/-	+	+/-

1: CI = Côte d'Ivoire strains, primary culture
2: Côte d'Ivoire, subculture
3: CA = Cameroon strains, subculture
4: Cameroon strains, subculture
5: +/- = some subclones positive, others negative
6: S = susceptible, R = resistant

Bacillus anthracis

When to refer an isolate to MDH for rule-out:

- **Biochemical tests:**
  - Non-hemolytic
  - Catalase positive
  - Non-motile
- **Gram stain:**
  - Large gram positive bacilli
- **Colony morphology:**
  - Large irregular shaped colonies
  - Sticky

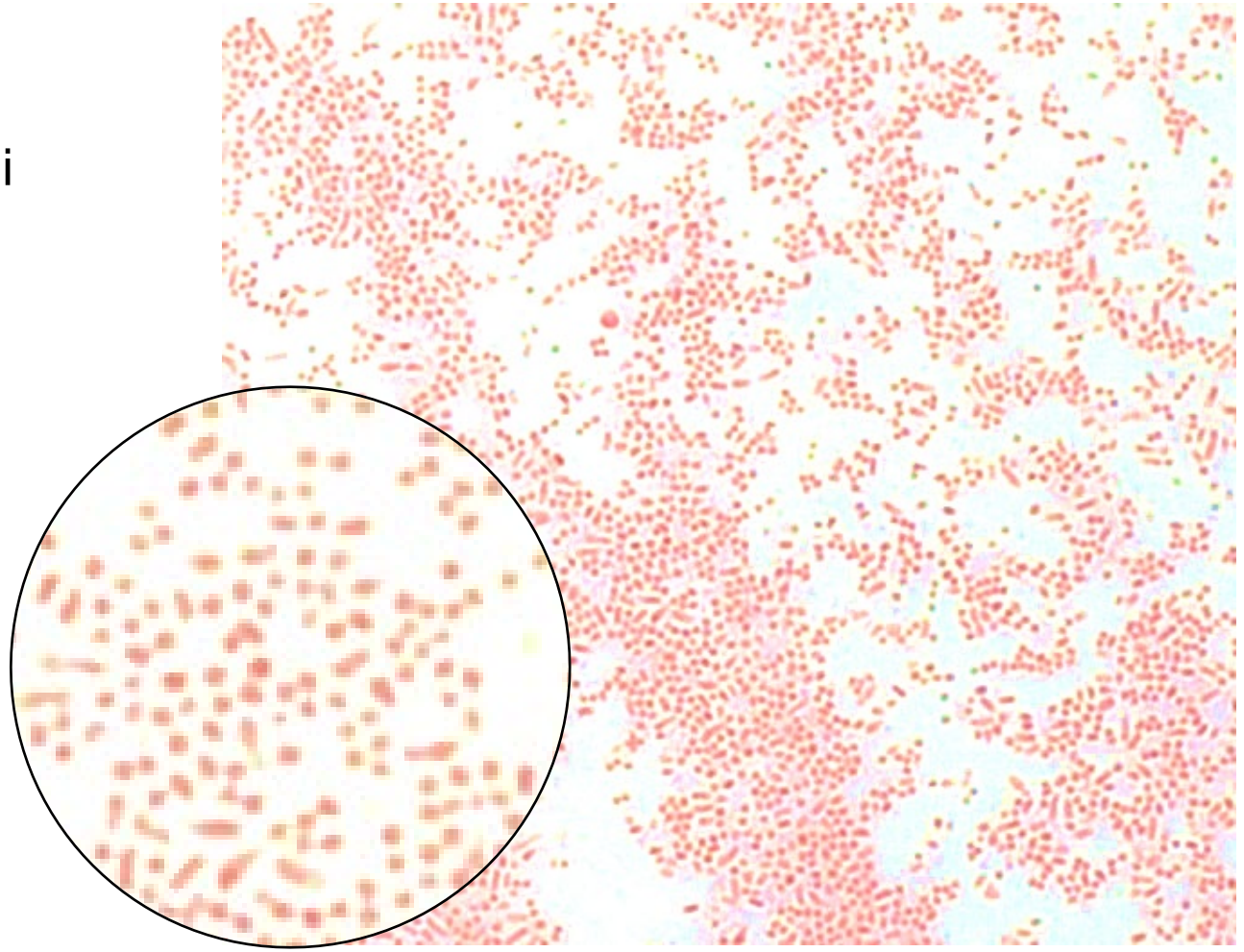


Brucella spp - Brucellosis

- Zoonotic disease that can cause human infection
  - *B. melitensis* (sheep and goats)
  - *B. suis* (pigs)
  - *B. abortus* (cows)
  - *B. canis* (dogs)
- Highly infectious
  - Low infective dose (10-100 organisms)
- Exposures common in veterinarians, slaughterhouse workers, and hunters
- Non-specific systemic symptoms; rarely fatal but can be extremely debilitating
- Removed from SA registry in Jan 2025….but still significant risk for laboratorians

Brucella spp – Cell Morphology

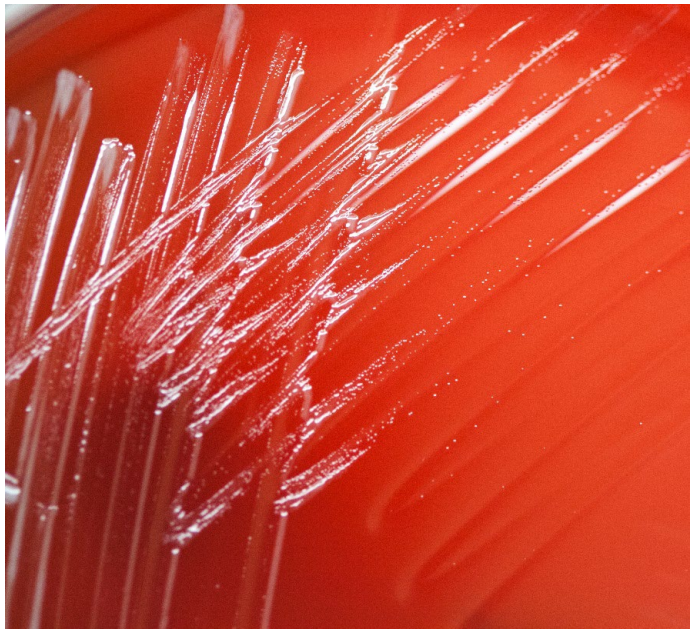
- Faintly-staining, Gram negative coccobacilli
- 0.6 to 1.5 µm x 0.5-0.7 µm
- Non-spore forming



Brucella spp - Culture

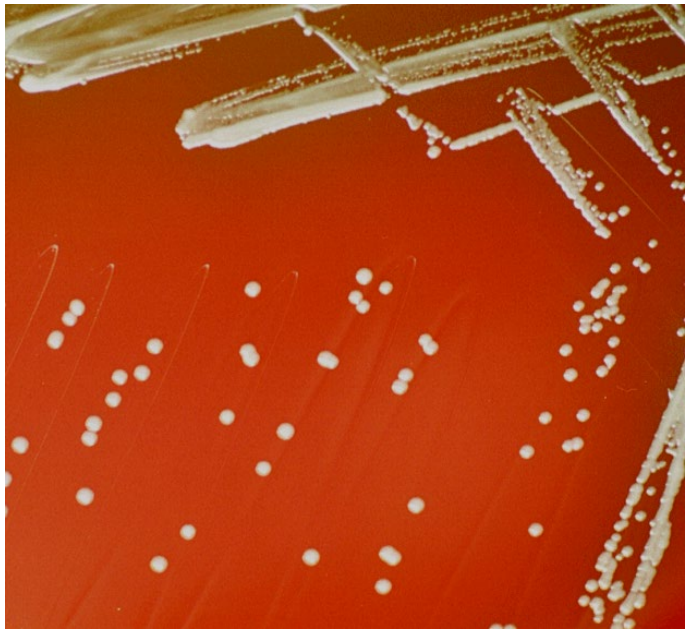
- Slow growing – may not be visible at 24 hours
- Small, raised, glistening white/cream colonies
- Incubate up to 7 days

- Grows on SBA, CHOC, Martin Lewis, and TM
- Does not grow well on MAC
- Non-hemolytic



B. melitensis
24-hour growth on SBA

B. melitensis
6 days growth on SBA



B. abortus
72-hour growth on SBA

Brucella spp – Biochemical Tests

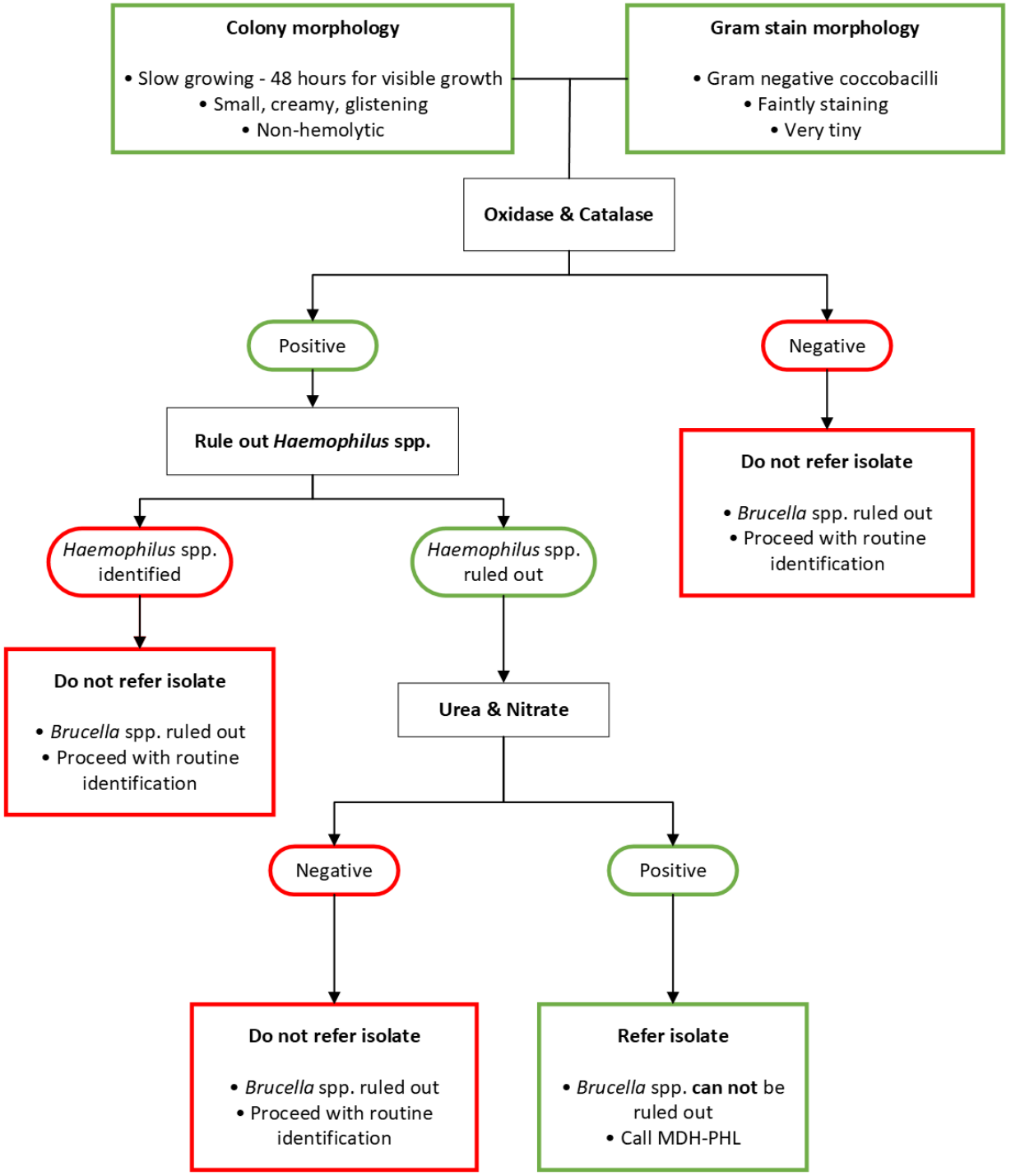
- Most automated identification systems will **not** identify brucellosis-causing *Brucella* spp.
    - **Additional complications:** *Ochrobactrum* species reclassified to *Brucella* genus in 2020, which may lead to *Brucella* misidentifications and brucellosis misdiagnosis
- If *Brucella* is suspected, **do not** perform MALDI-TOF testing.
- *Brucella* spp do not exhibit enhanced growth (satellite) around *S. aureus,* indicating no requirement for X and V factors.

Test	Result
Oxidase	Positive
Catalase	Positive
Urea	Positive (sometimes rapid)
Nitrate	Positive
Motility	Negative

Brucella spp

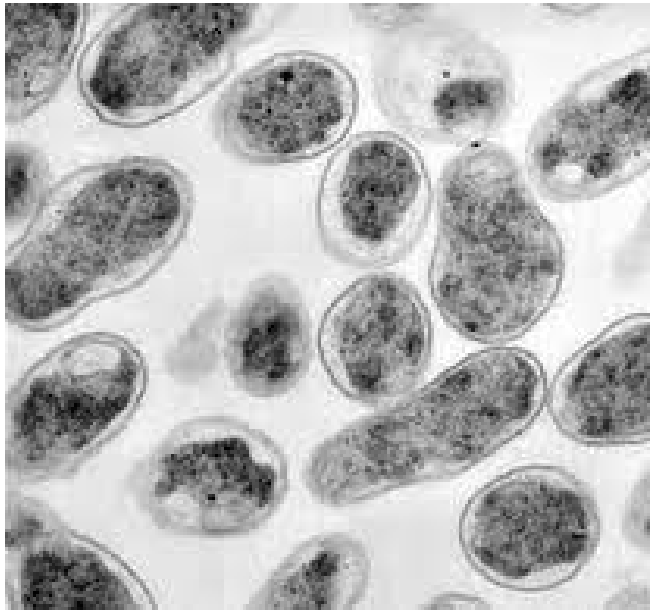
When to refer an isolate to MDH for rule-out:

- **Biochemical tests:**
  - Non-hemolytic
  - Oxidase, catalase, urease, nitrate positive
- **Gram stain:**
  - Gram negative coccobacilli
  - Faintly staining
  - Very tiny
- **Colony morphology:**
  - Small, creamy, glistening colonies
  - Slow growing (48 hours for visible growth)



Brucella spp – FSAP Updates

- *Brucella* spp are no longer Select Agents as of January 16th, 2025
  - Presumptive identification of *Brucella* spp **does not require a Form 4** within 7 days
  - **Mandatory destruction of clinical and diagnostic material within 7 days is not required**



- *Brucella* spp must be reported immediately by telephone and submission of clinical materials is required.
  - (651) 201-5414
  - Standard submission timelines are acceptable—specimens do not need to be transferred immediately for biothreat testing

- **Despite the regulatory change, *Brucella* spp are still a significant risk to laboratorians.**
  - Over 50% of all reported laboratory acquired infections were caused by *Brucella* spp during routine clinical work.
  - Proper PPE and engineering controls are critical for protecting laboratory staff.

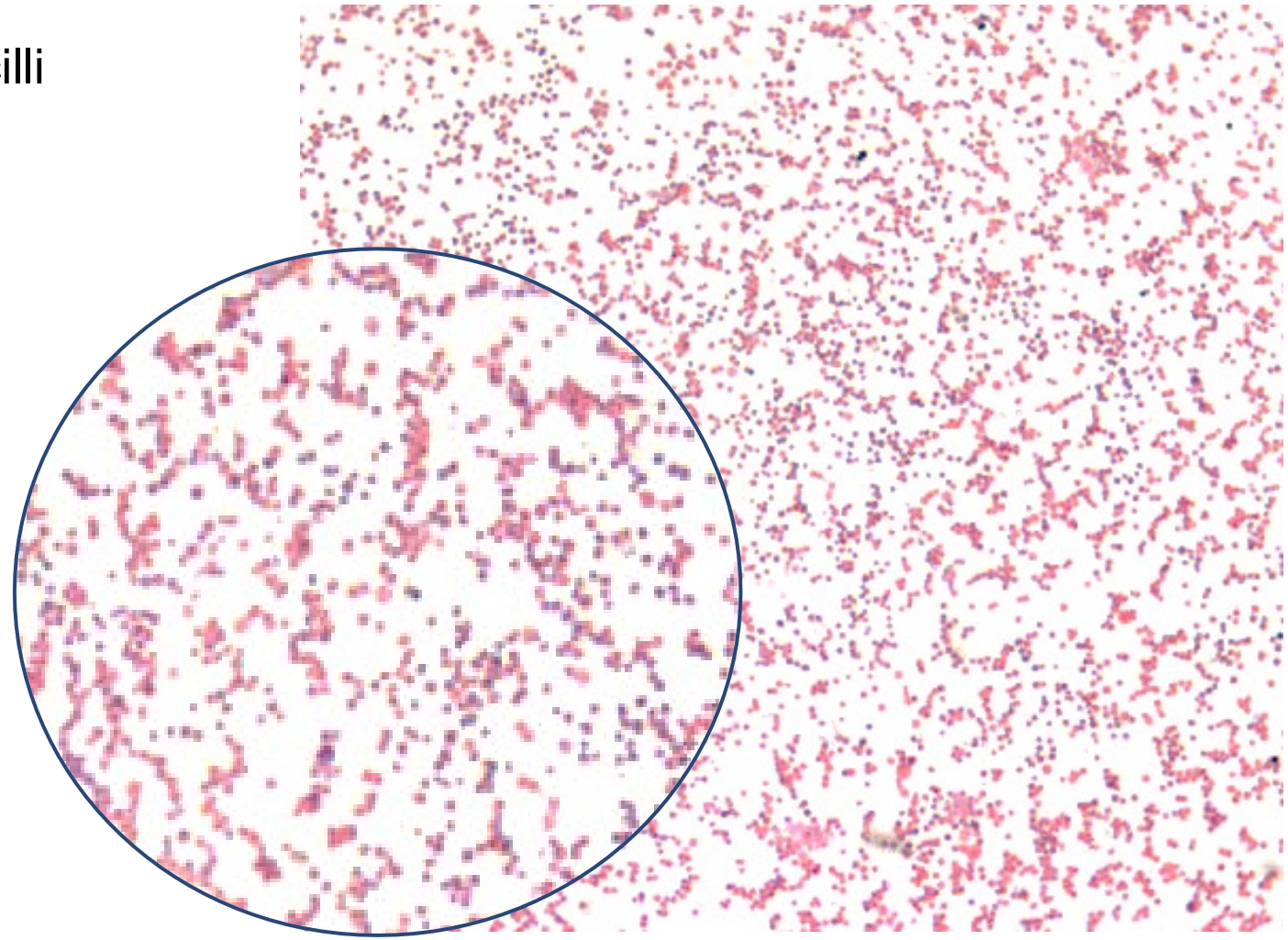
- For additional information, see the MDH-PHL MLS laboratory update from January 9th, 2025
  - https://www.health.state.mn.us/diseases/idlab/mls/labalerts/250109brucellafsapchanges.pdf

Francisella tularensis - Tularemia

- Highly infectious
  - As few as 10-50 bacteria can cause disease
  - Can be fatal if not treated with antibiotics
  - Tier 1 select agent
- Most common in South-central and Western states
  - Typically found in rabbits, hares, and rodents
  - Can survive for weeks in water and soil
- Routes of transmission:
  - Bite of infected insects (ticks, deerflies)
  - Handling sick or dead animals
  - Inhaling airborne bacteria
  - Eating or drinking contaminated food or water

Francisella tularensis – Cell morphology

- Pleomorphic, Gram negative coccobacilli
- Extremely tiny
- 0.2 to 0.5 µm x 0.5-0.7 µm
- Poorly staining
- Can look like *Brucella* spp.



Francisella tularensis – Culture

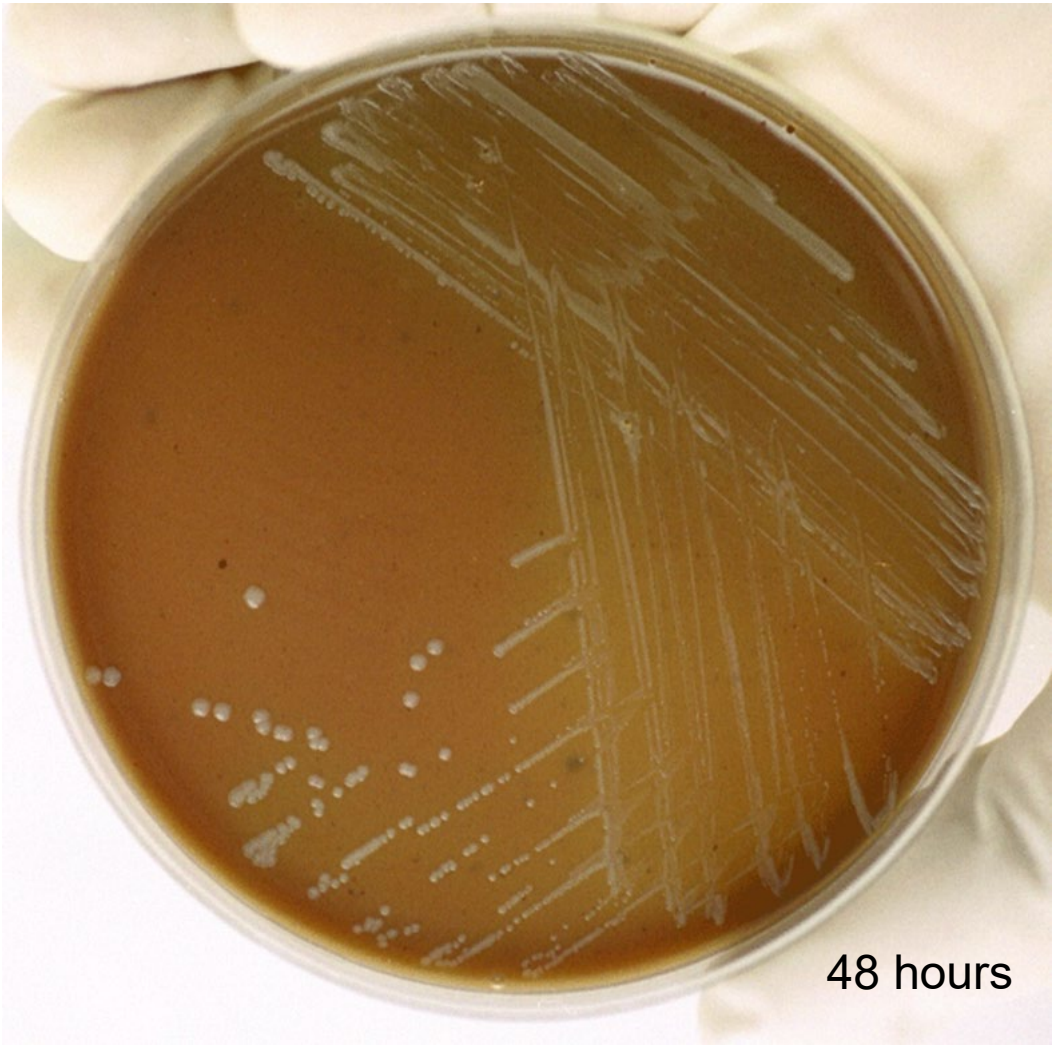
F. tularensis requires cysteine supplementation

24 hour growth:

- Little to no growth
- Tiny, pinpoint colonies

48 hour growth:

- White to grey / bluish grey
- Opaque
- Flat colonies with smooth edges
- 1-2 mm diameter



Francisella tularensis – Biochemical Tests

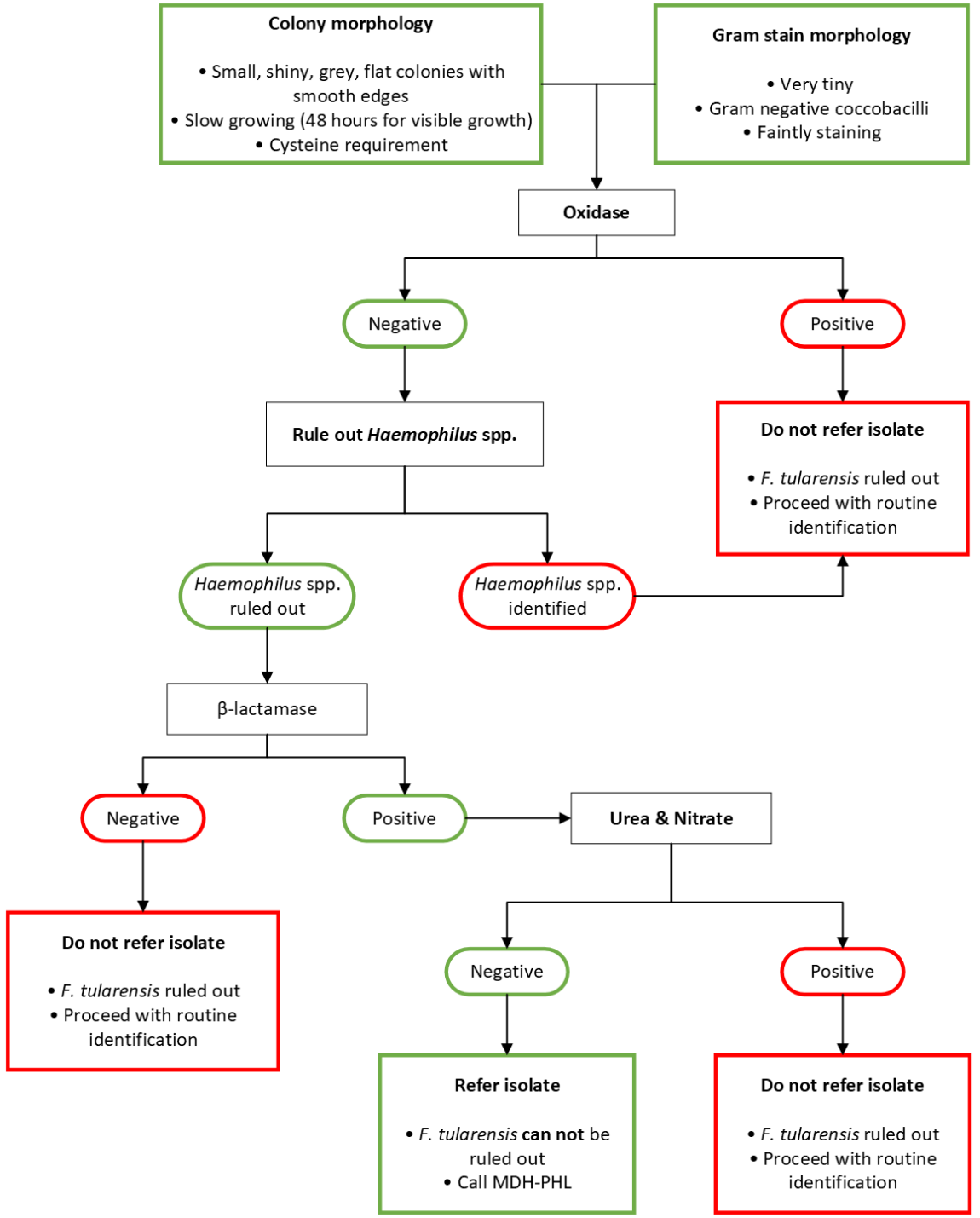
- Misidentification using automated detection systems is common so **classic biochemical testing is preferred**.
  - Vitek NHI panel may identify as:
    - *Aggregatibacter* spp.
    - *Haemophilus influenza*
  - Bruker MALDI
    - 100% gave no ID
- If *F. tularensis* is suspected, **do not** perform testing using automated systems
  - Aerosol generating potential

Test	Result
Oxidase	Negative
Catalase	Weak positive
Motility	Negative
Nitrate	Negative
Urea	Negative
β-lactamase	Positive

Francisella tularensis

When to refer an isolate to MDH for rule-out:

- **Biochemical tests:**
  - Oxidase, urease, nitrate negative
  - β-lactamase positive
- **Gram stain:**
  - Gram negative coccobacilli
  - Faintly staining
  - Very tiny
- **Colony morphology (Chocolate agar):**
  - Small, shiny, gray colonies
  - Flat colonies with smooth edges
  - Slow growing (48 hours for visible growth)



Burkholderia spp – Glanders & Melioidosis

Glanders – *B. mallei*

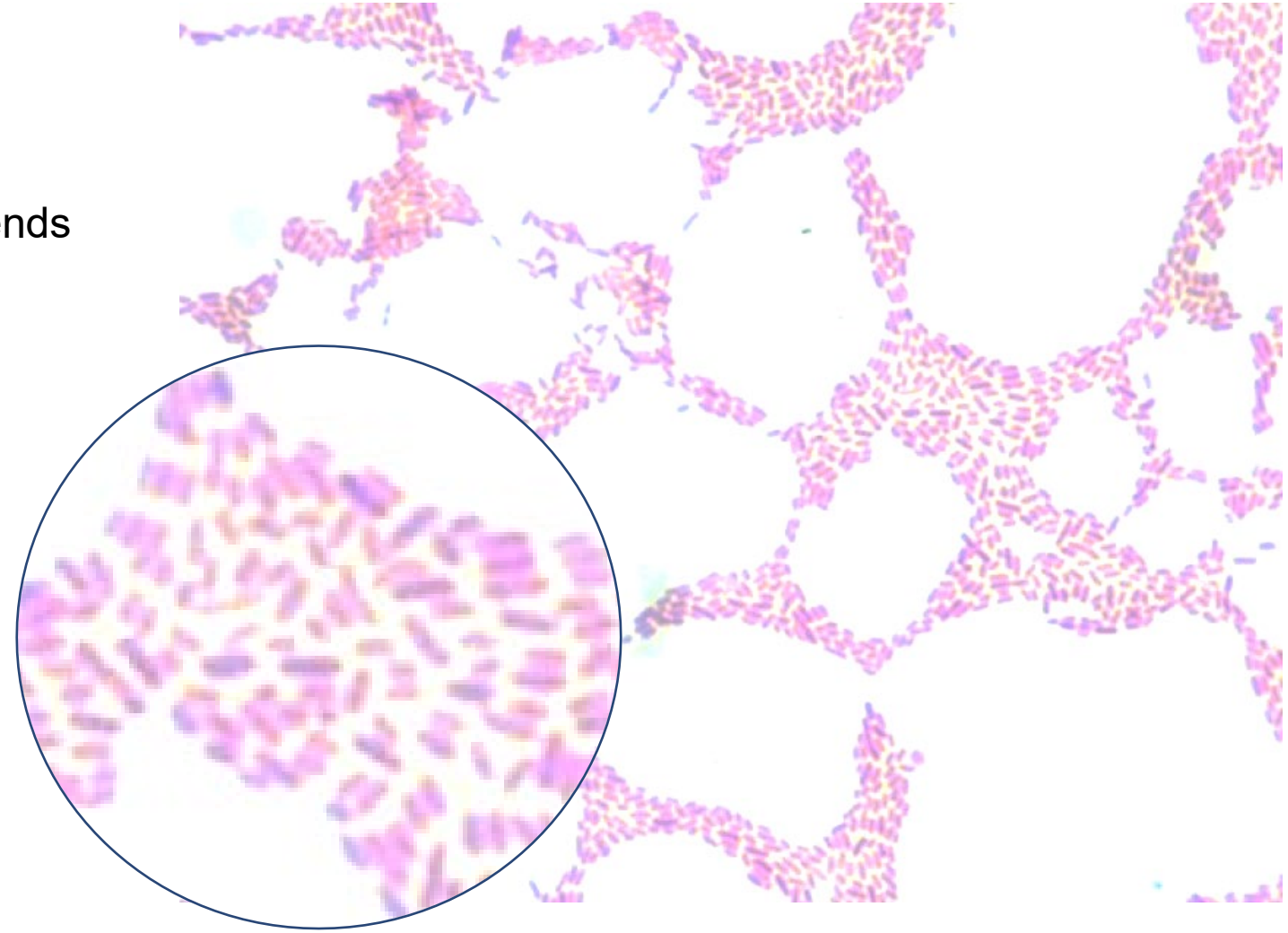
- Primary reservoir is horses
- Eradicated from the US in the 1940s
  - Infections still occur sporadically in SE Asia, Europe, and the Middle East
- Occupational disease of veterinarians, slaughterhouse workers, and laboratorians
- Infection caused by ingestion, inhalation, and cutaneous inoculation
- Tier 1 select agent

Melioidosis – *B. pseudomallei*

- Found in soil and water
- Endemic in SE Asia and Northern Australia
  - Possibly U.S. Gulf Coast (as of 2023)
- Spread through cutaneous inoculation and inhalation or ingestion of contaminated dust or water
- Can be mistaken for tuberculosis
- Tier 1 select agent

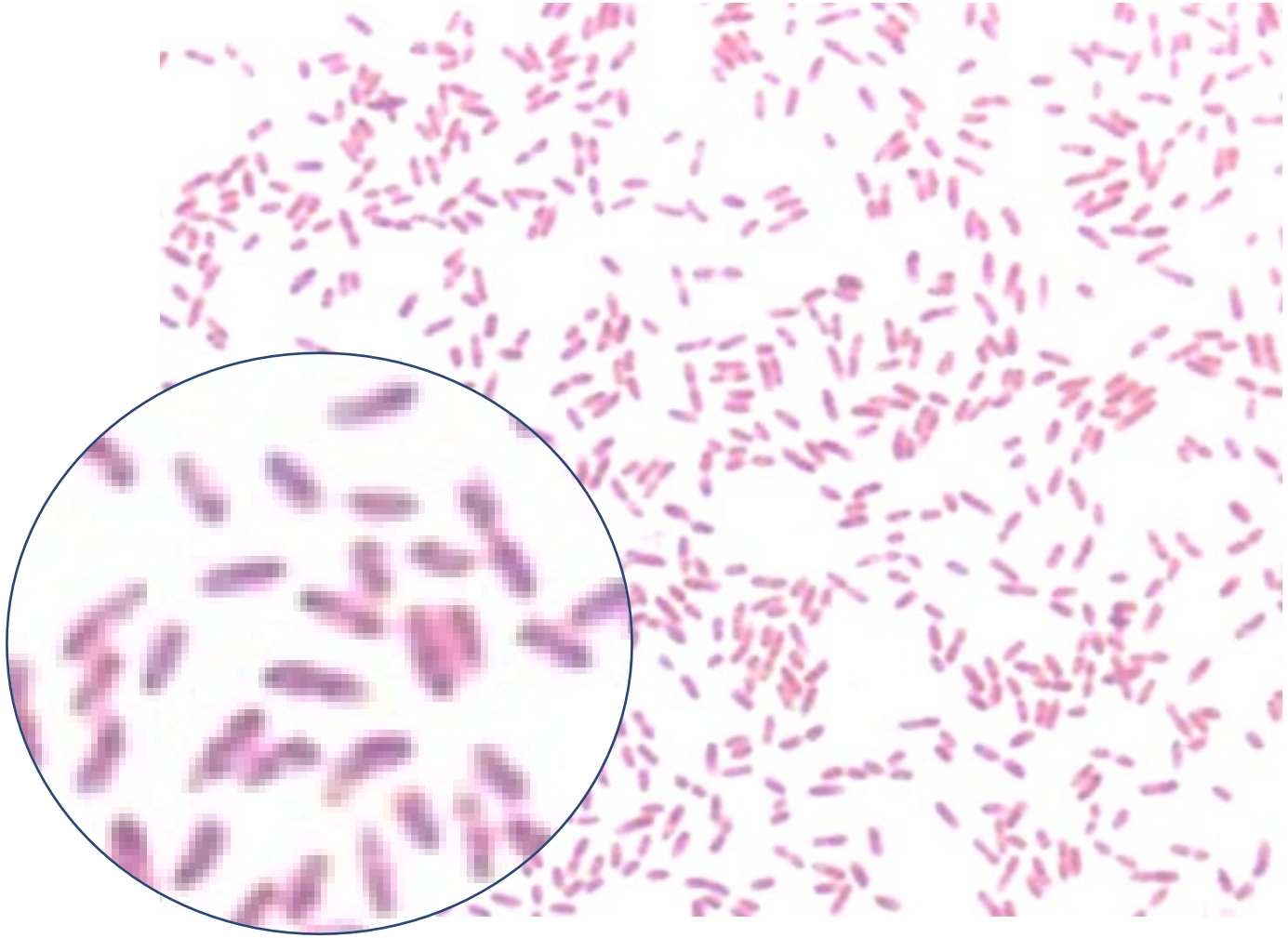
Burkholderia mallei – Cell morphology

- Gram-negative coccobacilli
- Straight or slightly curved with rounded ends
- Irregularly parallel or wavy sides
- 1-3 µm x 0.3-0.5µm
- Arranged in pairs end-to-end, in parallel bundles, or in “Chinese letters” form

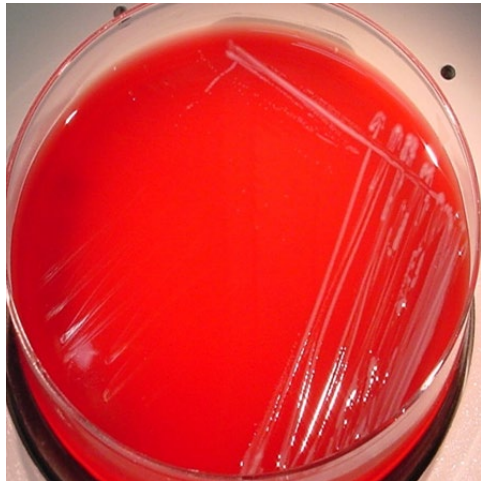
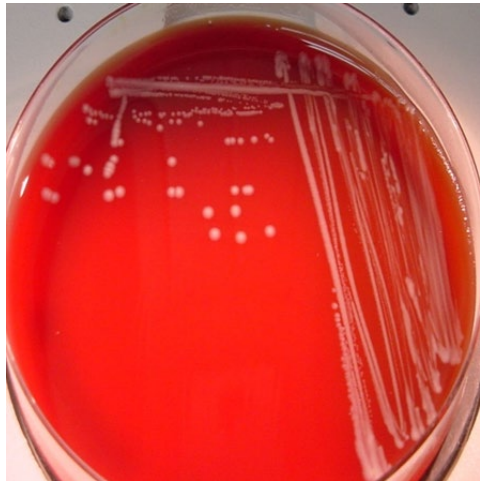
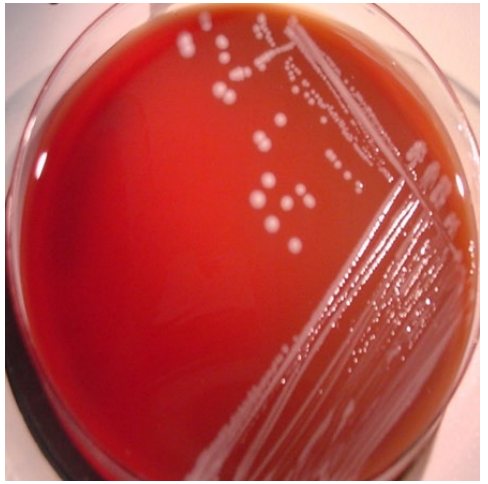
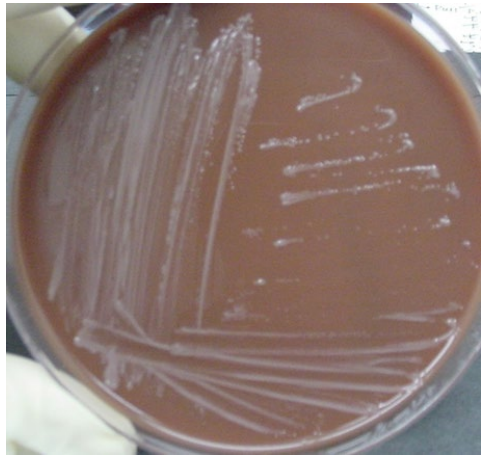
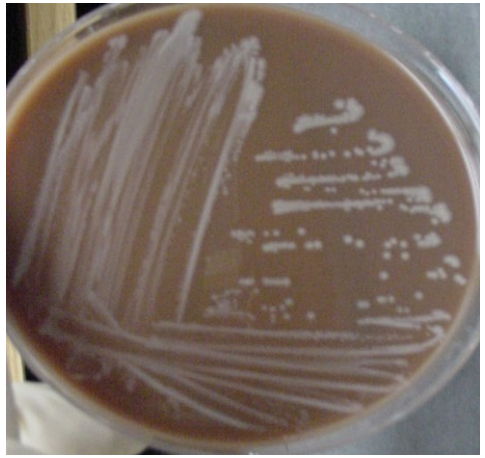


Burkholderia pseudomallei – Cell morphology

- Gram-negative bacilli
- Bipolar / Safety pin staining
- 2-5 µm length x < 2µm width
- Smooth form and rough form
  - Smooth form: long parallel bundles resembling filaments
  - Rough form: irregularly arranged

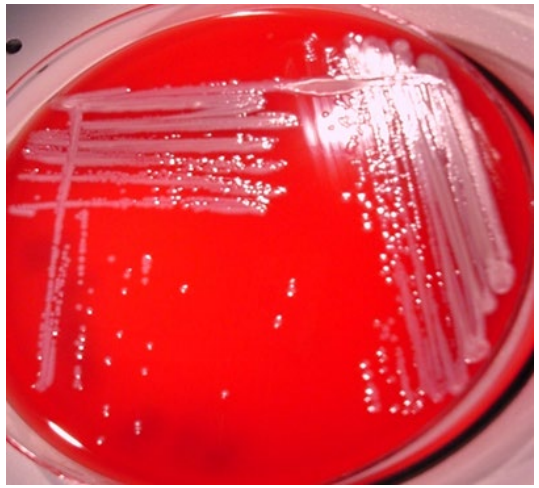
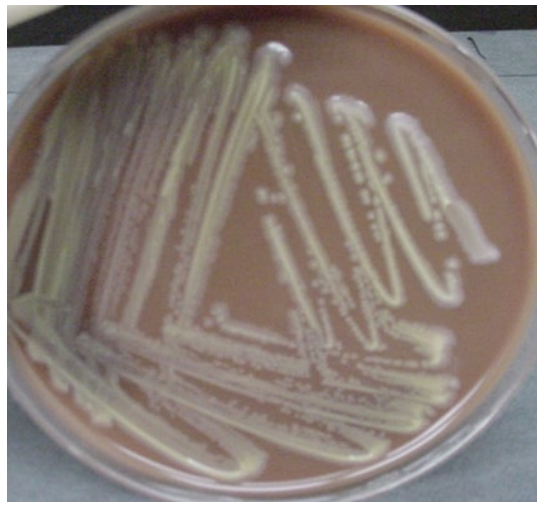
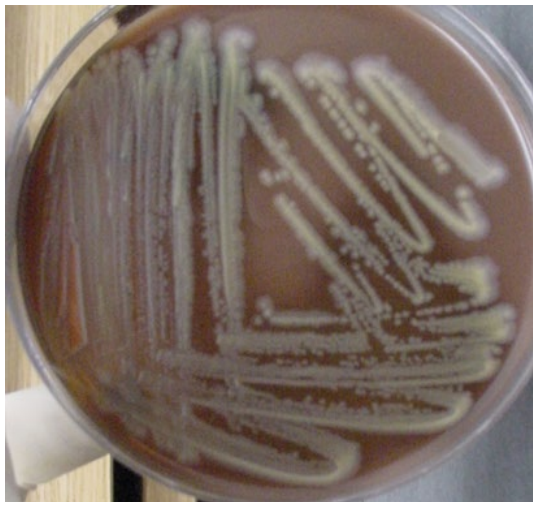


Burkholderia mallei – Culture

	24 hours	48 hours	72 hours
SBA			
Chocolate			

- Smooth colonies with uniform edges
- Grey, white, or translucent colonies

Burkholderia pseudomallei – Culture

	24 hours	48 hours	72 hours
SBA			
Chocolate			

- Smooth colonies with uniform edges
- White / cream colonies with a metallic sheen
- Some colonies dry out and become wrinkled after 48-72 hours
- Characteristic musty, earthy odor after 48 hours.

Burkholderia spp – Biochemical Tests

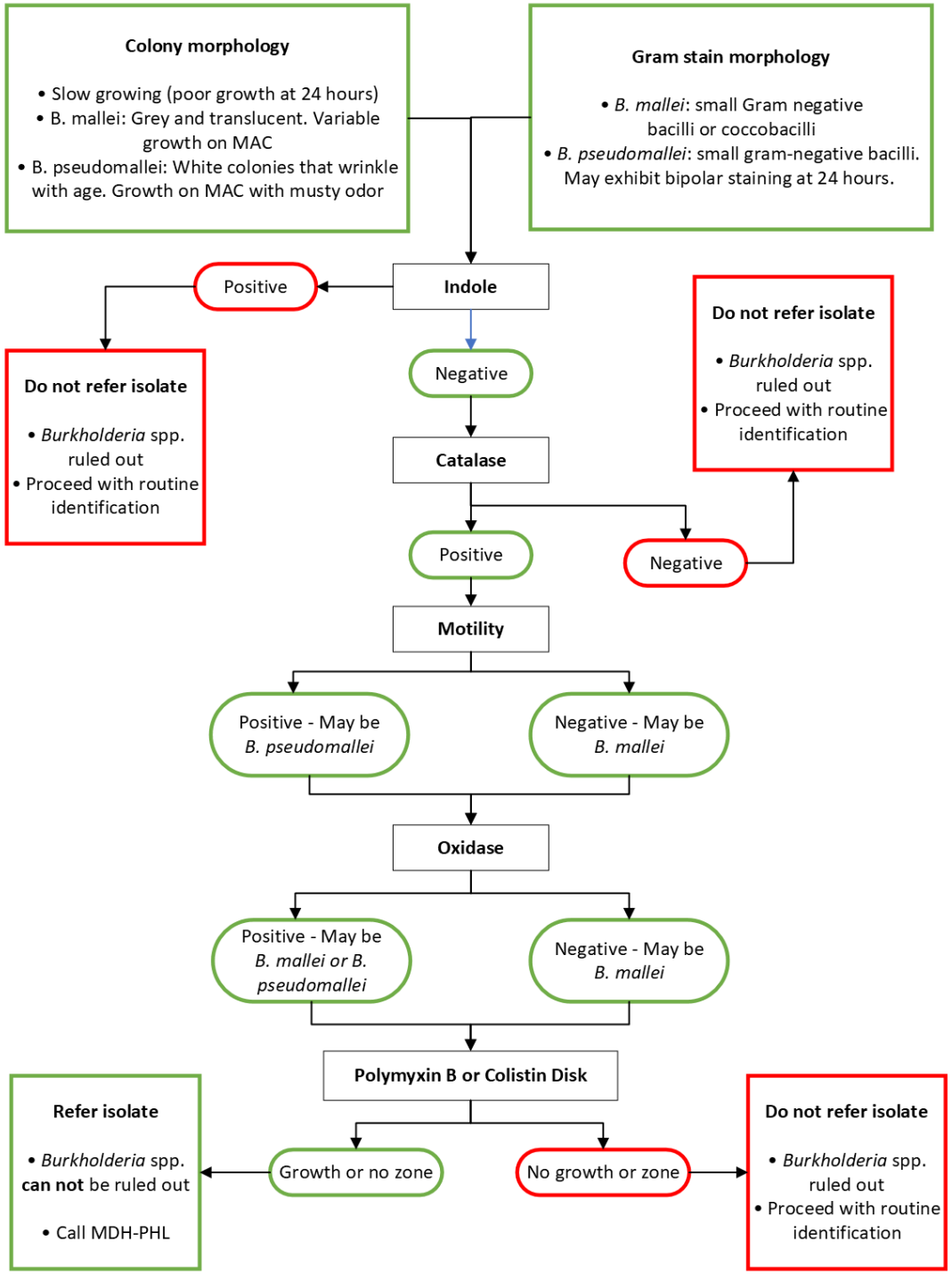
- Misidentification using automated detection systems is common so **classic biochemical testing is preferred**.
- Most automated identification systems can not identify *Burkholderia mallei* & *B. pseudomallei* reliably
  - Most systems only have a small number of *Burkholderia* species included in their libraries.

Test	***B. mallei***	***B. pseudomallei***
Motility	**Negative**	**Positive**
Catalase	Positive	Positive
Oxidase	Variable	Positive
Indole	Negative	Negative
B. cepacia selective agar	No growth	No growth
Polymyxin B / Colistin disk	No zone	No zone

Burkholderia mallei

When to refer an isolate to MDH for rule-out:

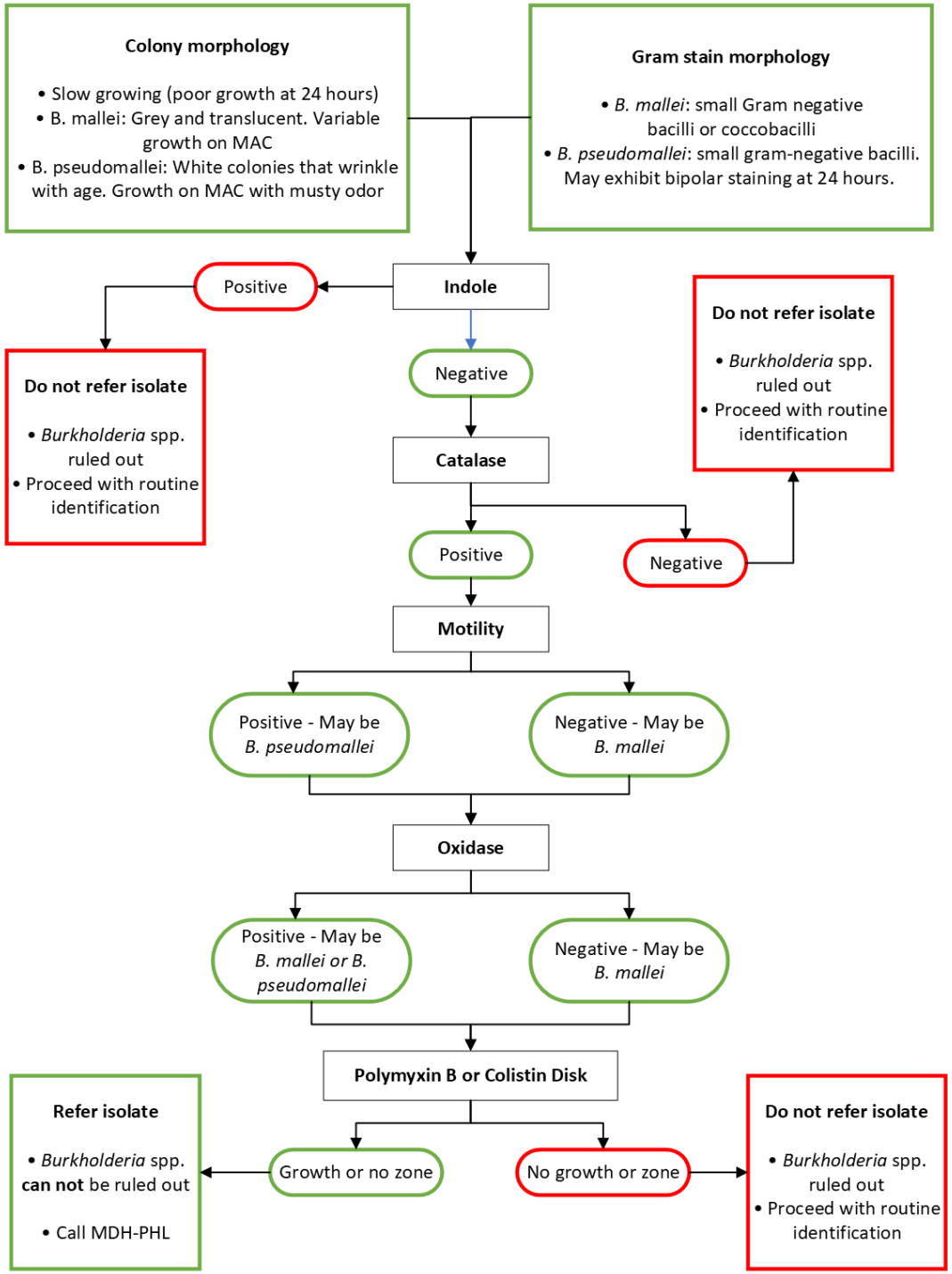
- **Biochemical tests:**
  - Indole negative
  - Catalase positive
  - Oxidase variable
  - **Non-motile**
  - No zone on Polymyxin B/Colistin disk
- **Gram stain:**
  - Small, Gram negative bacilli or coccobacilli
- **Colony morphology:**
  - Gray, translucent
  - Variable growth on MAC



Burkholderia pseudomallei

When to refer an isolate to MDH for rule-out:

- **Biochemical tests:**
  - Indole negative
  - Catalase and Oxidase positive
  - **Motile**
  - No zone on Polymyxin B/Colistin disk
- **Gram stain:**
  - Small, Gram negative bacilli
  - Straight or slightly curved
  - May exhibit bipolar staining
- **Colony morphology:**
  - White colonies that wrinkle with age
  - Growth on MAC

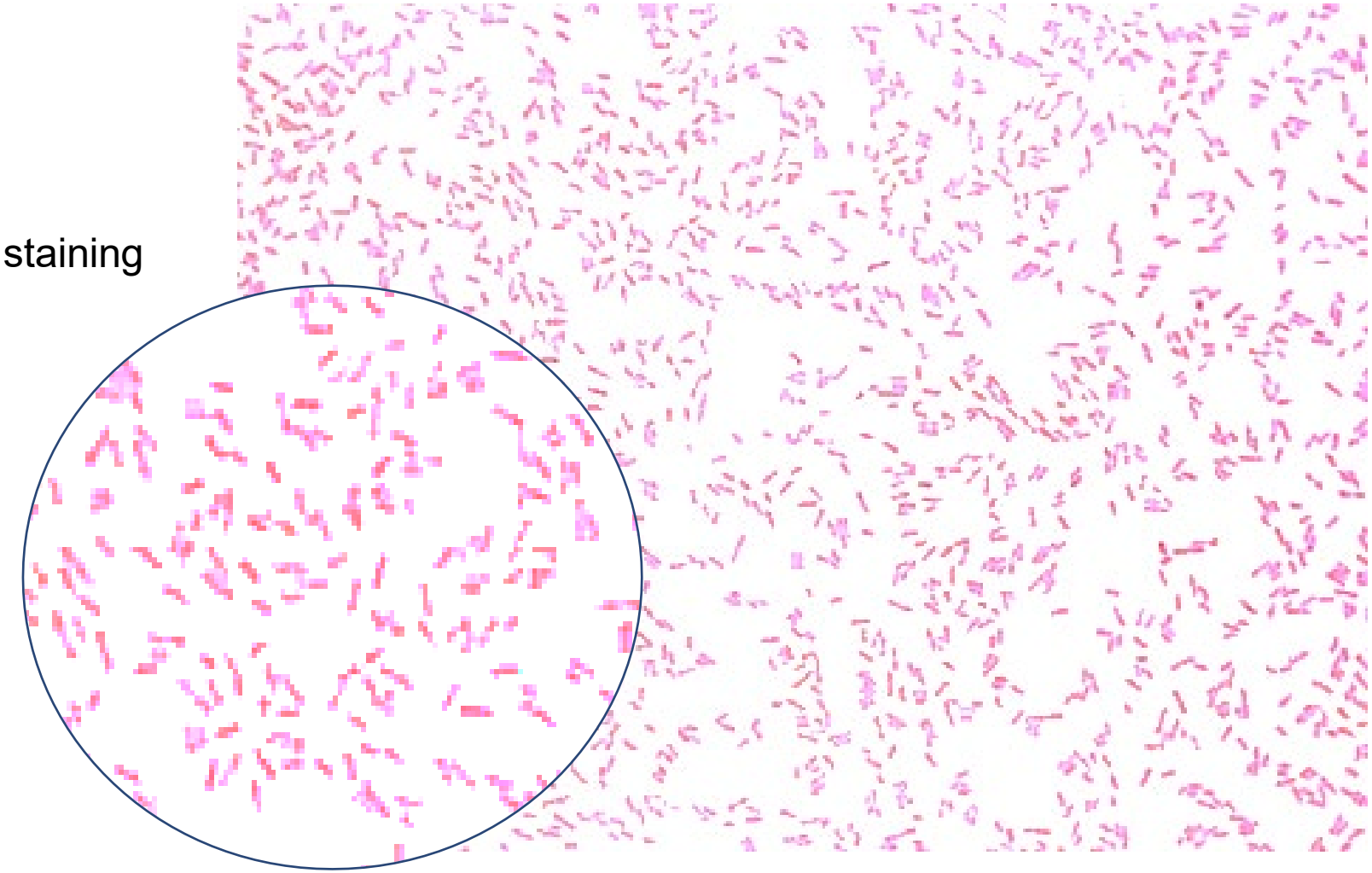


Yersinia pestis - Plague

- Affects humans and other mammals
  - Common vectors: rats, squirrels, prairie dogs
- Transmission:
  - Bites from rodent fleas
  - Infectious droplets
  - Contact with contaminated fluid or tissue
  - Wild carnivores can become infected by eating other infected animals
- Infections occur in rural areas of the Western US, Africa, and Asia
- 3 forms of plague: bubonic, septicemic, pneumonic

Yersinia pestis – Cell morphology

- Gram-negative bacilli
- Bipolar staining (variable)
  - Giemsa staining >> Gram staining
- 1-2 µm x 0.5µm



Yersinia pestis - Culture

24 hour growth:

- Tiny, pinpoint colonies
- Shiny and translucent
- Grey-white

48 hour growth:

- Raised, irregular colonies
- “Fried egg” or “hammered copper” appearance

48-hour growth on SBA

Yersinia pestis – Biochemical Tests

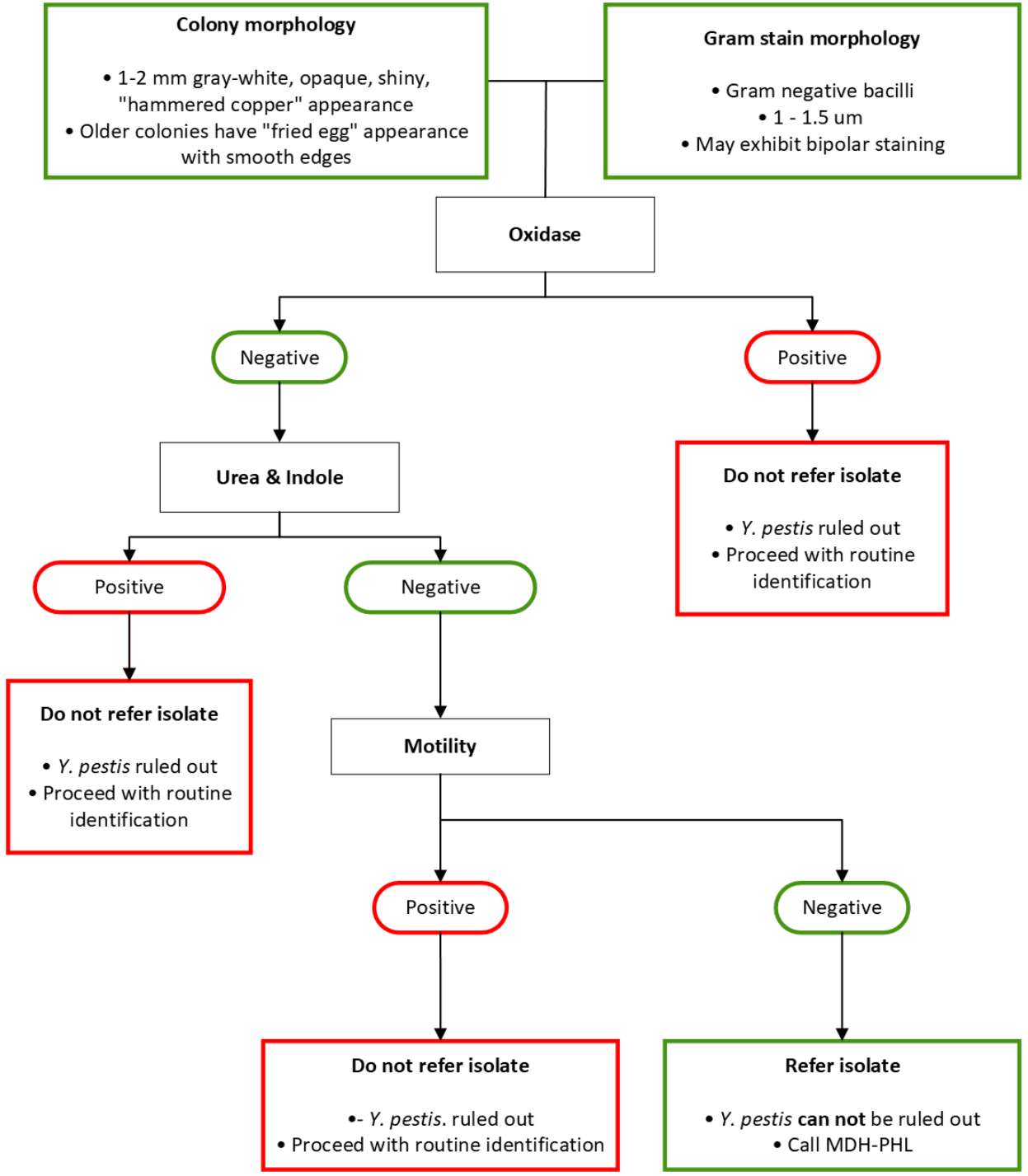
- Misidentification using automated detection systems is common so **classic biochemical testing is preferred**.
- Most automated identification systems cannot identify *Y. pestis* reliably
  - Often misidentified as *Y. pseudotuberculosis. intermedia,* or *Shigella* spp.
- Any positive ID from an automated system should be confirmed with biochemical testing.

Test	Result
Oxidase	Negative
Catalase	Positive
Urea	Negative
Motility	Negative
Indole	Negative
Glucose fermentation	Positive
Mannitol fermentation	Positive

Yersinia pestis

When to refer an isolate to MDH for rule-out:

- **Biochemical tests:**
  - Indole, urease, oxidase negative
  - Non-motile
- **Gram stain:**
  - Gram negative bacilli
  - May exhibit bipolar staining
    - Hard to see with Gram stain; Giemsa better
- **Colony morphology:**
  - Gray-white opaque colonies
  - 1-2 mm diameter
  - Shiny “hammered copper” appearance
  - Older colonies have “fried egg” appearance

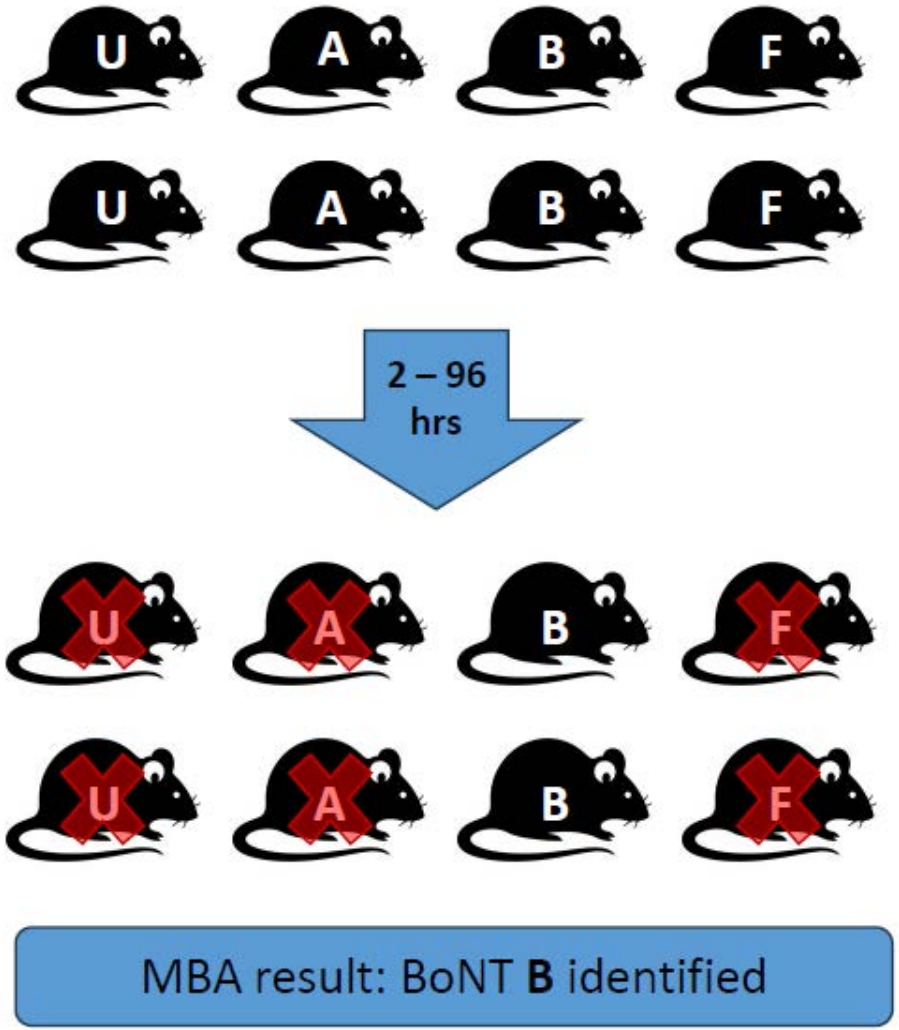


Clostridium botulinum - Botulism

- Spore-forming anaerobic organism commonly found in soil
- Toxin-forming spores are commonly found in improperly canned, preserved, or fermented foods
- 3 types of botulism
  - Foodborne
  - Wound
  - Infant
- One of the most toxic compounds in the world
  - 1 gram could kill 1 million people
  - Group of 7 related neurotoxins (BoNT A-G)
- Botulism diagnosis is made clinically based on patient case history and physical symptoms
  - **Suspected *C. botulinum* toxin must be referred to MDH**
  - Organism ***and*** toxin are on Tier 1 federal Select Agent registry

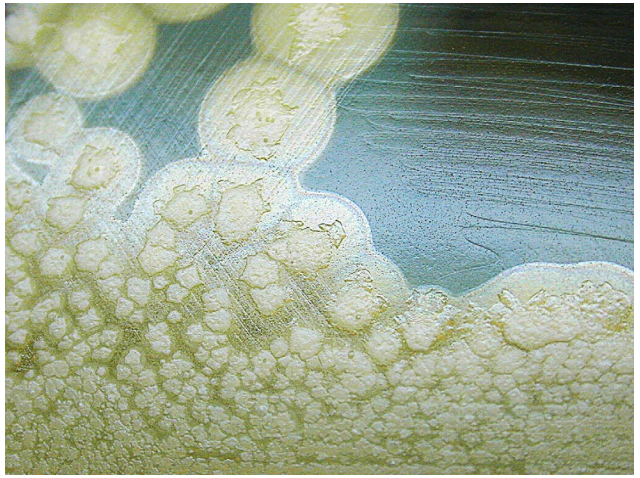
Clostridium botulinum – Identification

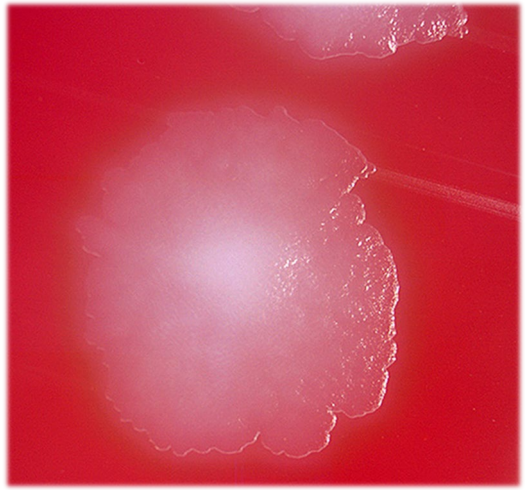
- **Mouse bioassay (MBA)**
  - Typical setup: IP injection of 8 mice (4 conditions, in duplicate)
    - **U**: Patient extract, no anti-toxin
    - **A**: Patient extract, BoNT A anti-toxin
    - **B**: Patient extract, BoNT B anti-toxin
    - **F**: Patient extract, BoNT F anti-toxin
  - Expected outcome: 2 mice (1 pair) survive, corresponding to the anti-toxin against the BoNT present in the specimen.
- **RT-PCR**
  - Seven singleplex RT-PCR targets (BoNT A-G genes)
    - **A**: mostly food/wound/infant
    - **B**: mostly food/wound/infant
    - **C & D**: non-human mammals
    - **E**: Salt-water associated
    - **F & G**: “Environmental”
    - **H**: B/F(5)
    - **Mixed BoNT**: Ab, Ba, etc
- **MALDI-TOF** (EndoPEP-MS) coming to MDH in 2026

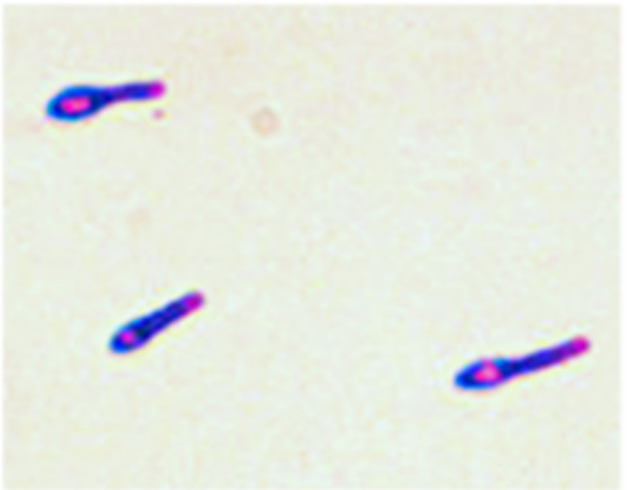


Clostridium botulinum – Culture / Staining

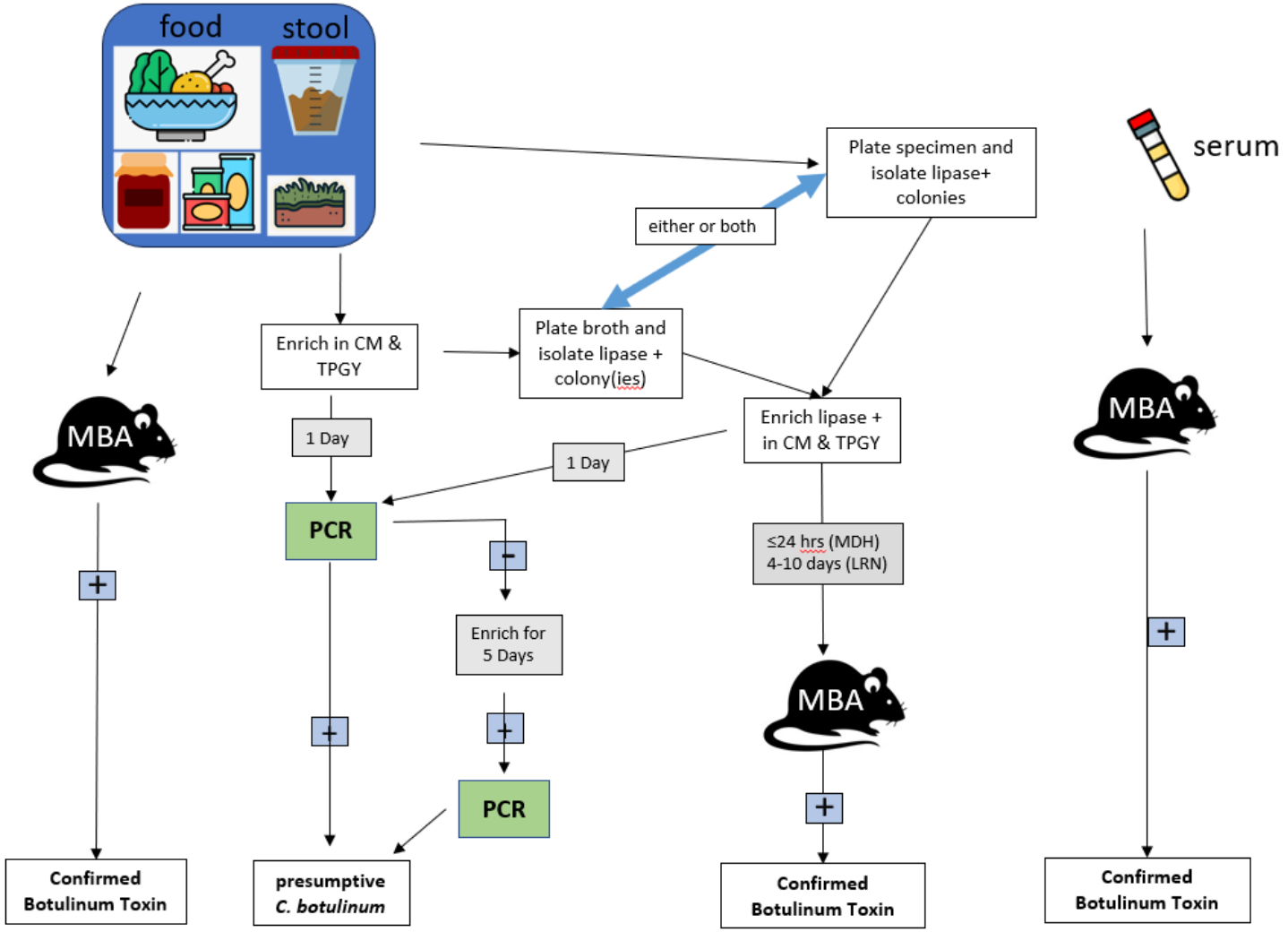
- Large, gram-positive rods
  - Oval, sub-terminal spores
- Yellow to yellow-green colonies
- Large colonies on 5% SBA
  - >2 mm, somewhat variable
  - Colony edges are often wavy
- β-hemolytic after 24 hrs
- Lipase positive
  - Oily sheen on egg yolk agar
- “Horse stable” odor





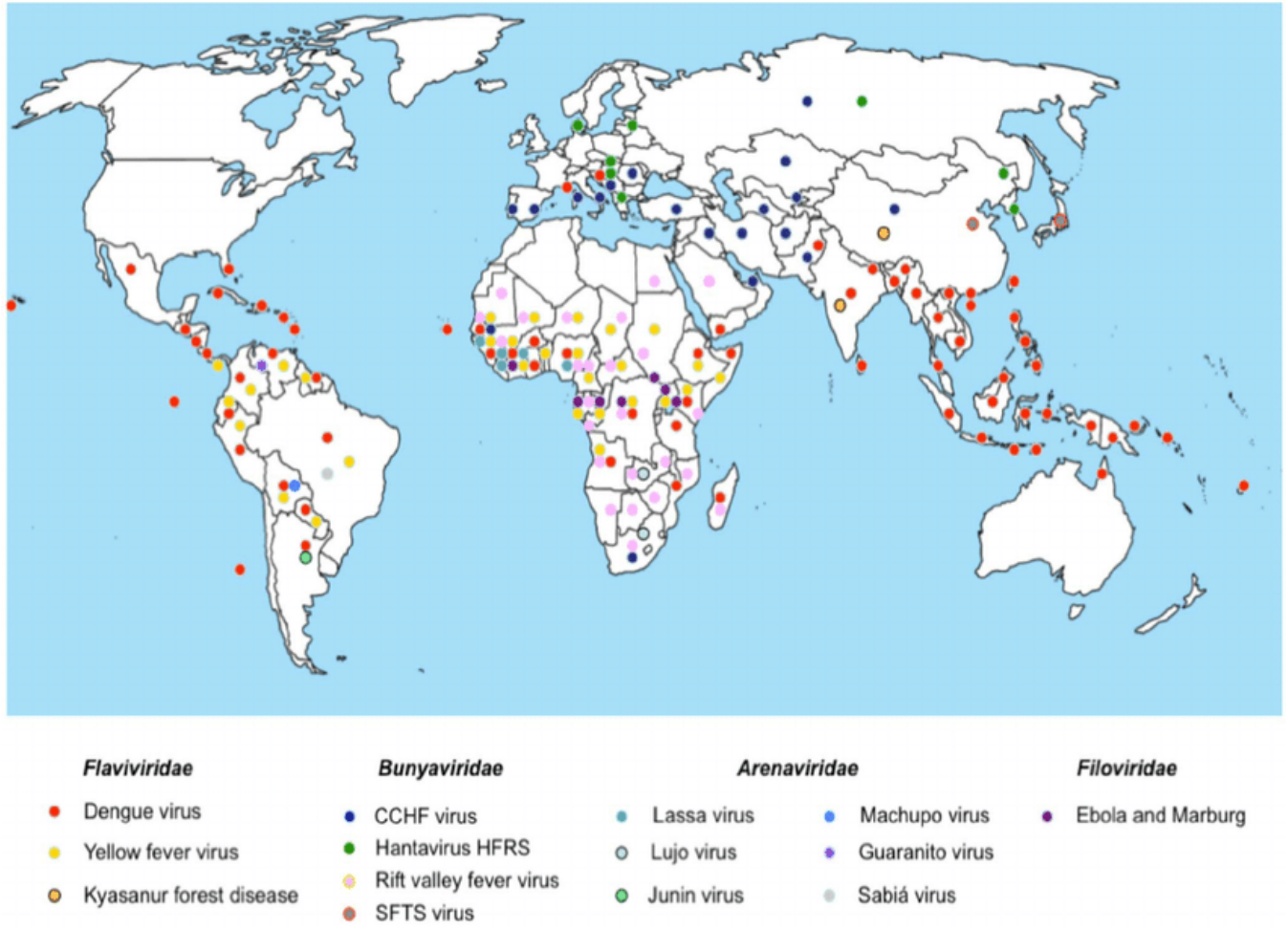


Clostridium botulinum – Testing algorithm



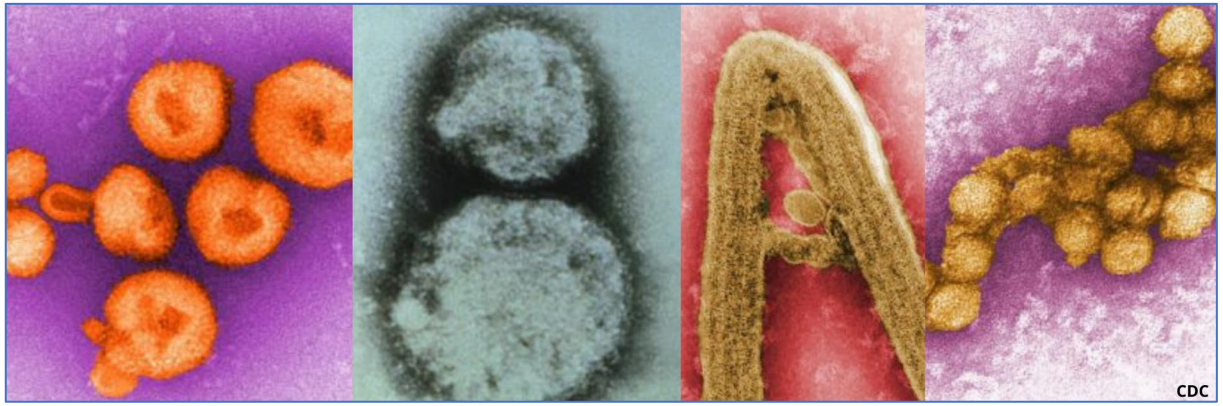
Viral hemorrhagic fevers (VHFs)

- Viral diseases that affect multiple organ systems
  - Caused by several families of viruses
  - Disease can range from mild to severe
- Common symptoms include:
  - Uncontrolled bleeding
  - Fever
  - Damage to the cardiovascular system
  - Nausea, vomiting, and diarrhea
  - Delirium
  - Respiratory, liver, and kidney failure
- Worldwide distribution, but most common in Africa, Asia, and South America



Viral hemorrhagic fevers

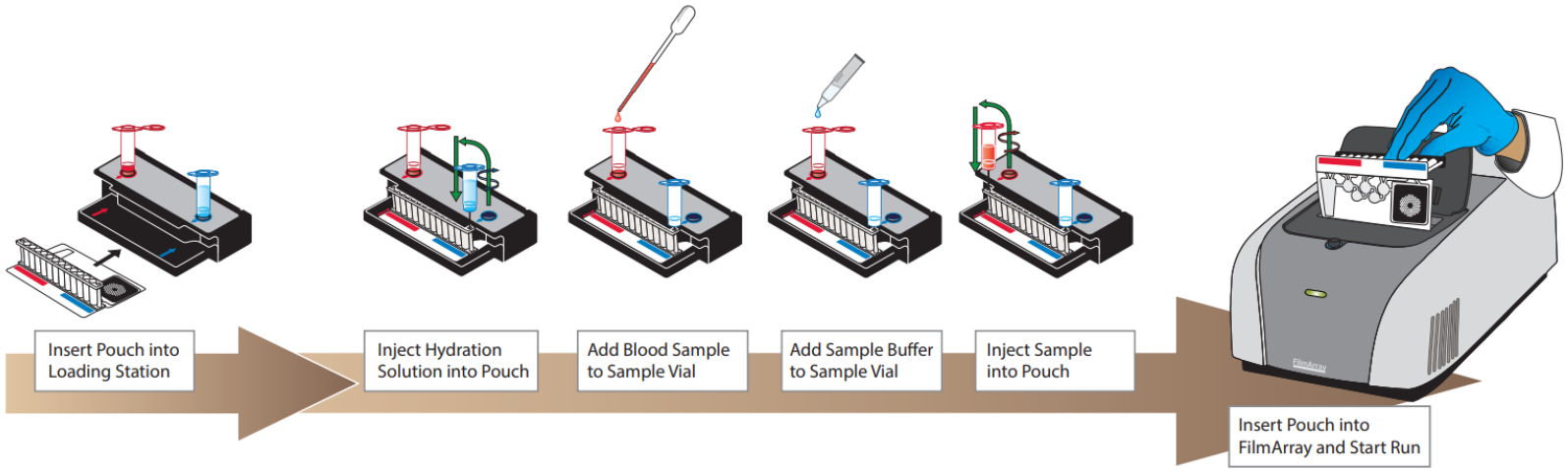
- No culturing or diagnostic testing currently available for Sentinel labs
  - Many VHFs are SA pathogens; require CDC testing approval
- Suspect VHF pathogens must be referred to MDH. Call either of the following to start the testing approval process:
  - MDH-PHL BioThreat phone: (612) 282-3723 [24x7]
  - MDH IDEPC Epi line: (651) 201-5414 [24x7]



VHF Pathogen	Common vector
Select Agents	
★ *Ebolavirus*	Bats, primates
★ Marburg virus	Bats, primates
Crimean-Congo fever virus	Ticks
Lassa fever virus	Rats
Hendra virus	Bats
★ Nipah virus	Bats
Non-Select Agents (SA adjacent)	
Yellow fever virus	Mosquitoes
Dengue fever virus	Mosquitoes
★ Denotes Tier 1 Select Agent	Testing available at MDH-PHL

VHFs – Testing at MDH-PHL

- **Global Fever Special Pathogens Panel**
  - Qualitative, multiplexed, nucleic acid-based test for the BioFire Film Array 2.0 or BioFire Film Array Torch systems
  - Allows rapid detection of VHFs and other diseases
    - 16 total targets
  - Testing performed on EDTA-Whole Blood (purple top tubes)
    - 2 x 5 mL tubes required
  - Results typically available within 6 hours (most VHFs)
    - May be preliminary results only; confirmation at CDC



Global Fever Special Pathogens Panel

Bacterial

Bacillus anthracis
Francisella tularensis
Leptospira spp.
Yersinia pestis

Viral

Chikungunya virus
Crimean-Congo hemorrhagic fever virus
Dengue virus (serotypes 1, 2, 3 and 4)
Ebolavirus spp. (Bundibugyo, Reston, Sudan, Taï Forest, Zaire)
Lassa virus
Marburgvirus
West Nile virus
Yellow fever virus

Protozoan

Leishmania spp.
Plasmodium spp.
Plasmodium falciparum
Plasmodium vivax/ovale

Contact Information

Consults and On-call resources

MDH Epi on call (24x7x365)	**Biothreat on call (24x7x365)**
• Clinical questions, case reporting, infection prevention, etc. • **651-201-5414** or **1-877-676-5414**	• Lab questions, rule out submissions, biosafety • **612-282-3723**