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INTRODUCTION

The UHN/SHS clinical microbiology laboratory is a Containment Level 2 facility licensed to safely possess and handle most Risk Group 2 organisms. However, as the laboratory processes unknown specimens, an inherent risk exists to isolate Risk Group 3 organisms.
Hence, the microbiology laboratory plays an essential role in identifying and limiting the spread of potentially dangerous infectious agents. The following procedures are in place to safely identify and handle organisms of high biosafety risk.

WHEN TO SUSPECT HIGH BIOSAFETY RISK AGENTS:

A. Presumptive diagnosis provided

SUSPECT CJD or other relevant agents listed below

B. Gram smear

Bacteria:

- **Small Gram-negative bacilli or cocco-bacilli from all sites**
  SUSPECT *Brucella, Francisella, Yersinia pestis, Burkholderia pseudomallei or Burkholderia mallei*
- **Gram-negative diplococci from sterile sites**
  SUSPECT *Neisseria meningitidis*
  Note: *N. meningitidis* is a Risk Group 2 organism, but given the potential for serious infection, culture should only be opened in a BSC.

C. Gram smear or fungal smear

Mould:

- **Large thick-walled broad-based budding yeast from all sites**
  - **SUSPECT *Blastomyces dermatitidis***

D. Culture

Bacteria:

- **Slow-growing Gram-negative bacilli/cocco-bacilli from all sites**
  SUSPECT *Brucella, Francisella, Yersinia pestis,* or *Burkholderia pseudomallei*
- **Rapid-growing non-hemolytic large spore-forming Gram-positive bacilli from all sites**
  SUSPECT *Bacillus anthracis*

Mould:

- **White mould growing on cycloheximide-containing agar after ≥ 3 days of incubation**
  SUSPECT *Histoplasma, Blastomyces, Coccidioides, or Paracoccidioides*
- **Black, olivaceous green or brown mould from brain tissue**
  SUSPECT *Cladophialophora bantiana* or *Rhinocladiella mackenziei*

IF YOU ENCOUNTER ANY OF THE ABOVE, NOTIFY SENIORS IMMEDIATELY AND FOLLOW THE BIOSAFETY MANUAL FOR FURTHER INSTRUCTIONS.

- *See "Profile of Relevant Risk Group 3 Organisms" in the section below for complete characteristic profile of common RG 3 pathogens.*
- For a complete list of Risk Group 3 and 4 organisms, see PHAC's ePATHogen – Risk Group Database available online. Refer to Isolate Notification and Freezing Table

WHAT TO DO IF A RISK GROUP 3/4 ORGANISM IS SUSPECTED

Small Gram-negative bacilli or cocco-bacilli from all sites (Brucella, Francisella, Yersinia, B. pseudomallei or Burkholderia mallei)
Should a suspect Risk Group 3 concern be provided from the clinical team or recognized from the Gram stain or culture, follow steps below:

1. **Notify Senior/Charge** of potential Risk Group 3 organism.

2. **Process samples offline** within a BSC (DO NOT load into WASP / WASPLAB)
   - **Incubate BC for 21 days; for cultures add *Staph* streak to BA plate**
   - **Immediately seal all plates with parafilm** circumferentially from all relevant specimens for the patient with the isolate in question. This will require expunging any already processed plates within WASPLAB.
   - **Label** plates and plate rack **with RG3 Alert labels**.

3. Continue **incubating all plates offline** until growth is observed.
   Any further handling of sealed plates must be done in a Class 2 Biological Safety Cabinet (BSC) with an N95 respirator and gloves.

4. **If suspicious growth is observed:**
   a. Work-up all organisms as per the Small Gram negative bacilli/cocco-bacilli Workup Flowchart. NOTE: **Perform oxidase and catalase only.** DO NOT SET UP MALDI OR MANIPULATE ANY FURTHER**.**
   b. Notify seniors, who shall:
      i. Ensure notification of Biological Safety Officer, Microbiologist (if concern for exposure, notify microbiologist-on-call in real-time; otherwise notify once report provided to Health Canada and isolate/plates discarded, and Infection Control (as appropriate).
      ii. Ensure notification Biological Safety Officer, Microbiologist, and Infection Control occurs.
      iii. Notify the Local Public Health Unit when a preliminary ID is available.
      iv. Send LIS email to all staff with patient demographics warnings.
      v. Add RG3 ESO flag.
      vi. Post patient demographics in specimen processing and bacteriology and incubators containing culture plates using the RG3 Alert signs.
   c. Send isolate to PHOL:
      i. Notify PHOL of incoming RG3 organism.
      ii. Package according to Transportation of Dangerous Goods regulations (Only certified staff are permitted to do the packaging)

5. **Store plates in the Seniors RG3 Basket** pending PHOL confirmation.

6. Ensure all plates with **confirmed RG3 organisms are disposed.** See Appendix III

Gram-negative diplococci from sterile sites (*N. meningitidis*) / C. diphtheriae isolated
Note: N. meningitides / C. diphtheriae are not Risk Group 3 organisms but given the potential for serious infection, culture should only be opened in a BSC.

Should a suspect *Neisseria meningitides / C. diphtheriae* concern be provided from the clinical team or recognized from the Gram stain or culture, follow steps below:

1. **Notify Senior/Charge** of potential Risk Group 3 organism.

2. **Process samples offline** within a BSC (DO NOT load into WASP / WASPLAB).
   Immediately seal all plates with parafilm circumferentially from all relevant specimens for the patient with the isolate in question. This will require expunging any already processed plates within WASPLAB.
   Label plates and plate rack **with RG3 Alert labels**.

3. **Incubate all plates offline** observing for growth at 24hrs.
   Any further handling of sealed plates must be done in a Class 2 Biological Safety Cabinet (BSC) with gloves.

4. **If suspicious growth is observed:**
   a. **Process culture in BSC with gloves for identification and susceptibilities.**
   b. **Notify Seniors, who shall:**
      i. Ensure notification of Biological Safety Officer, Microbiologist (if concern for exposure, notify microbiologist-on-call in real-time; otherwise notify once report provided to Health Canada and isolate/plates discarded, and Infection Control (as appropriate).
      ii. Notify the Local Public Health Unit when a preliminary ID is available.
      iii. Send LIS email to all staff with patient demographics warnings.
      iv. Add RG3 ESO flag.
      v. Post patient demographics in specimen processing and bacteriology and incubators containing culture plates using the RG3 Alert signs.
   c. **Send isolate to PHOL**:
      - Package according to Transportation of Dangerous goods regulations

      (Only certified staff are permitted to do the packaging)

5. **Store plates in the Seniors RG3 Basket** pending PHOL confirmation.

6. Ensure all plates with **confirmed RG3 organisms are disposed.** See Appendix III

Non-hemolytic large aerobic spore-forming Gram-positive bacilli from all sites (*B. anthracis)*

Should a suspect anthrax (*B. anthracis)* concern be provided from the clinical team or recognized from the Gram stain or culture, follow steps below:

1. **Notify Senior/Charge** in area of potential Risk Group 3 organism.

2. **Process further relevant samples offline** within a BSC (DO NOT load into WASP / WASPLAB).
   Immediately seal all plates with parafilm circumferentially from all relevant specimens for the patient with the isolate in question. This will require expunging any already processed plates within WASPLAB.
   Label plates and plate rack **with RG3 Alert labels**.

3. **Any further handling of sealed plates must be done in a Class 2 Biological Safety Cabinet (BSC) with gloves.**

4. **If suspicious growth is observed:**
   a. **Perform catalase and motility testing on all suspicious colonies.**
      i. **If catalase negative, continue processing culture routinely.**
      ii. **If catalase positive and motile, continue processing culture routinely.**
      iii. **If catalase positive and non-motile, STOP testing (possible anthrax).**
   b. **Notify Seniors, who shall:**
      i. Ensure notification of Biological Safety Officer, Microbiologist (if concern for exposure, notify microbiologist-on-call in real-time; otherwise notify once report provided to Health Canada and isolate/plates discarded, and Infection Control (as appropriate).
      ii. Notify the Local Public Health Unit when a preliminary ID is available.
      iii. Send LIS email to all staff with patient demographics warnings.
      iv. Add RG3 ESO flag.
      v. Post patient demographics in specimen processing and bacteriology and incubators containing culture plates using the RG3 Alert signs.
   c. **Send isolate to PHOL:**
      i. Notify PHOL of incoming RG3 organism.
      ii. Package according to Transportation of Dangerous goods regulations
      (Only certified staff are permitted to do the packaging)

5. **Store plates in the Mycology/Seniors RG3 Basket** pending PHOL confirmation.

6. Ensure all plates with **confirmed RG3 organisms are disposed.** See Appendix III

Suspect Risk Group 3 Moulds

A) <u>Suspect white mould growing on cycloheximide-containing agar after ≥ 3 days of incubation from all sites (*Histoplasma, Blastomyces, Coccidioides, Paracoccidioides*)</u>

B) <u>Black, olivaceous green/black mould from brain tissue (*Cladophialophora bantiana, Rhinocladiella mackenziei*)</u>

Should a suspect Risk Group 3 mould concern be provided from the clinical team or recognized from the Gram stain or culture, follow steps below:

1. **Notify Senior/Charge** of potential Risk Group 3 organism.
2. Smears suggestive of Blastomyces: Call ward

3. **Process samples offline within a BSC** (DO NOT load into WASP / WASPLAB)
   Immediately seal plates with parafilm circumferentially from all relevant specimens for the patient with the isolate in question. This will require expunging all plates within WASPLAB.
   Label plate**s** and plate rack **with RG3 Alert labels**.

4. Continue **incubating all plates offline** until growth is observed.
   Any further handling of sealed plates must be done in a Class 2 Biological Safety Cabinet (BSC) with gloves.

5. **If suspicious growth is observed:**
   - **a.** DO NOT PERFORM ANY SMEARS OR MANIPULATE ANY FURTHER**.**
   - **b.** Notify Seniors, who shall**:**
     i. Ensure notification of Biological Safety Officer, Microbiologist (if concern for exposure, notify microbiologist-on-call in real-time; otherwise notify once report provided to Health Canada and isolate/plates discarded, and Infection Control (as appropriate).
     ii. Notify the Local Public Health Unit when a preliminary ID is available.
     iii. Send LIS email to all staff with patient demographics warnings.
     iv. Add RG3 ESO flag.
     v. Post patient demographics in specimen processing and bacteriology and incubators containing culture plates using the RG3 Alert signs.
   - **c.** Send isolate to PHOL**:**
     i. Notify PHOL of incoming RG3 organism.
     ii. Package according to Transportation of Dangerous goods regulations

   (Only certified staff are permitted to do the packaging)

6. **Store plates in the Mycology** section pending PHOL confirmation area.

7. Ensure all plates with **confirmed RG3 organisms are disposed.** See Appendix III

Creutzfeldt-Jakob Disease (CJD)

While routine practices can be used to perform the lumbar puncture, additional precautions are in place for CJD processing in the clinical laboratory given the potential for aerosolization and contamination with how we process the CSF.

Should a suspect CJD concern be provided from the clinical team, Microbiology Special Queries team or any other staff follow steps below:

1. DO NOT PROCESS CSF for microbiology tests other than CJD.

2. Notify Senior Technologist/or designate in area of CJD request.

3. Senior Technologist/designate shall

   - Apply CJD ESO flag
   - Notify Microbiologist on call & Infection Control of request
   - Notify core lab of suspect CJD specimens
     i. For Sinai: MSH Hematology at ext.17- 4503
     ii. For UHN: UHN Hematology at 14-2525
   - Provide requestor/clinician with **CLINICIAN - CJD SPECIMEN COLLECTION, TRANSPORTATION AND ORDERING INSTRUCTIONS**
   - Notify specimen receiving area with all information

4. MLA should Accession and Process specimen according to **LAB - CJD CSF ORDER ENTRY AND SPECIMEN PROCESSING INSTRUCTIONS**

5. MLA should send-out / ship sample according to **SEND – OUT/ SHIPPING INSTRUCTIONS**

6. MLA should enter test comments and additional instructions in **CJD REPORTING INSTRUCTIONS**

7. MLA should follow **CLEAN UP AND PROPER DISPOSAL** instructions

CLINICIAN - CJD Specimen Collection, Transportation and Ordering Instructions

1. **Ordering**

i) CJD is orderable in EPIC (UHN) and Cerner (MSH) as "Creutzfeldt-Jakob Disease (CJD) CSF Protein Panel"

ii) Other microbiology tests:
Can be ordered but will be deferred until CJD results are received and confirmed as negative

iii) Non-microbiology tests:
Mitogen testing may be requested but only cell count will be done until CJD results are received and confirmed as negative, unless otherwise discussed and approved by the microbiologist-on-call. CSF protein and glucose will not be performed.

2. **Collection and Transportation**

i) Collection timing:

CSF specimens for CJD testing are accepted Monday to Friday between 8:00 and 13:00 hours

ii) Specimen collection and volume:

Routine collection containers can be used to collect CSF. A minimum volume of 2 ml non-xanthochromic CSF (no visible blood) is needed for CJD testing.

iii) Specimen transport:

CSF samples must be labelled and placed in a hard screw-top transport container prior to transportation, then placed in the usual biosafety transport bags

a) For UHN
The clinical team can call Specimen Management at the respective site in order to obtain 2 hard screw-top transport containers, one container for samples to be sent to MSH for CJD/microbiology testing and a separate container for samples to be sent to UHN core lab/cytology for their testing.

Extensions to specimen processing to request these containers are
TWH 13-5011
PMH 16-5683
TGH 14-8403

b) For MSH
The clinical team can call 17-2016 in order to obtain one container for all samples to be sent to microbiology for all testing ordered.

3. Notification of Toronto Public Health and the Canadian CJD Surveillance System

The ordering clinical team should contact local Public Health Unit to notify them of the suspect CJD case. Additionally, The Canadian CJD Surveillance System should be also contacted via the following phone # 1-888-489-2999 (https://www.canada.ca/en/public-health/services/surveillance/blood-safety-contribution-program/creutzfeldt-jakob-disease.html). This should be done for any suspect CJD cases, not just confirmed CJD cases.

LAB - CJD CSF Order Entry and Specimen Processing Instructions

Expected arrival of samples for CJD is Monday – Friday 8:00 – 13:00 only.

Outside of these hours, freeze aliquot as per specimen processing instructions and put CJD aliquot in freezer for sendout next shipping day (Monday –Thursday only)

- Sample should arrive in a hard screw-top transport container.
    - If received inside a Biohazard bag like a routine specimen, carefully place inside the hard screw-top transport container.
        - Decontaminate the area.
        - Notify the Senior technologists then continue processing for CJD
- **Do not open any containers in regular BSC – go to 2.5 level BSC**

1. ORDER ENTRY:

UHN Samples - CSF cell count is directly processed on their site

i) CSF will be submitted with EPIC labels provided.

ii) Accession CJD and other Microbiology Tests using EPIC labels provided, receive and print labels.

Sinai Health Samples

i) CSF will be submitted with MSH - Cerner paper requisition form.

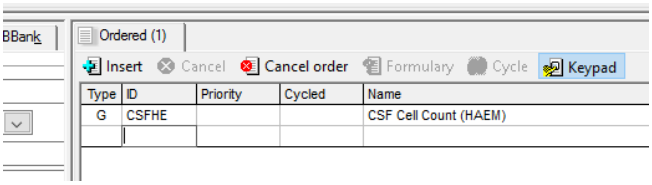
ii) Order CJD and other microbiology tests in LIS and print labels.

iii) If cell count is requested, select the last tube of CSF and follow steps below and then send to TGH Core Lab by KJV – STAT

a. Photocopy the form. Keep the original form for scanning in SOFTMEDIA

b. In SOFTLAB,

- Order Cell Count Test, Test code CSFHE, or test can be found under folder fluid/ CSFF



- Collect and Receive
- Add order comment "Sample(s) for CSF Cell count is(are) sent to UHN by Microbiology Lab."

c. Using the photocopied form, attach Large LIS Label and sticker "Copy results to MSH Core Lab, fax # 416 619 5533" found by Miscellaneous specimen processing bench.

d. Call both MSH Hematology at ext. 17-4503 and UHN Hematology at 14-2525 and notify them that you will be sending a suspect CJD CSF sample for cell count testing.

iv) Print out and complete CJD Requisition form

PRION DISEASE SECTION REQUISITION - CSF
https://cnphi.canada.ca/gts/laboratory/1025

2. SPECIMEN PROCESSING

Specimen processing of CSF must be performed with increased safety precautions within a biological safety cabinet due to the risk group and infectious nature of CJD
Process samples in BSC inside the "2.5" processing room.

- Prepare labels and supplies needed:
  - 2 White top CryoPure freezer tube 1.8 ml vial labeled with small LIS barcode
  - 4 biohazard bags
  - 4 clear Ziploc plastic bags
  - FEDEX Shipping box with Dry Ice
  - Hard screw-top transport container with absorbent (for MSH samples where CSF Cell count is requested)

- Small Life labs Courier Bag to TGH Core lab- PRIORITY (for MSH CSF Cell count if requested)
- Clear autoclave bag for in hood
- Daniels biohazard waste pail – round, red, found in washup

- Two MLAs required (1st MLA processes sample, 2nd MLA assists)

- 1st MLA – Don PPE (Single use gloves (double), disposable gown, mask and face shield)
  - 2nd MLA – Outside 2.5 Room to assist if more supplies are needed during processing.

i) FOR CJD TESTING

1. Check the sample for any visible blood or xanthochromic (yellow color). (If present, no need to aliquot NML will reject the sample. Return sample to container)
2. Aliquot 1.8 ml of CSF into one freezer vial (minimum 1ml required)
3. Put vial in biohazard bag and then directly into dry ice.

ii) FOR OTHER MICROBIOLOGY TESTS

Leave extra CSF in biohazard bag in a hard screw-capped container in white fridge in the planting area with LIS label on outside, to wait on CJD results and further testing. Place the hard screw-capped container inside a clear Ziploc plastic bag.

iii) FOR MSH CSF CELL COUNT

1. Select the last tube (tube number markings are etched on tubes)
2. Place the sample inside a biohazard bag, then to a hard -screw capped container, then to clear the Ziploc bag. Place the Photocopied requisition form (outside the hard container, in case of leakage, the form will not get contaminated)
3. Place the container in Lifelabs blue bag labeled TGH CORE LAB (Send out to TGH CORE LAB by KJV- STAT/PRIORITY)

iv) FOR OTHER NON- MICROBIOLOGY TESTS

1. Aliquot one extra tube of CSF into 2 ml freezer vials and label with patient's name, MRN if CSF remaining.
2. Store vial in freezer to wait on CJD results and further testing.

v) CLEAN UP AND PROPER DISPOSAL

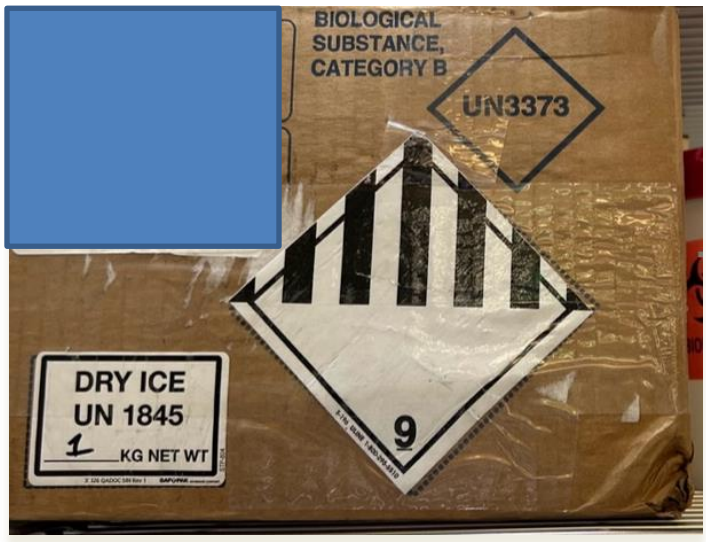
1. All disposable lab wear along with all extra items that were placed in hood along with 1st pair of gloves should be placed in autoclave bag that was kept in hood
2. All waste should be double bagged. Knot bag and place in a large, round red Daniel's pail.
3. Label container with LIS label and placed by the Bacteriology senior's bench to obtain proper disposal documentation. See
4. For hard surfaces (e.g., BSC): remove visible soil; flood with 2N NaOH or undiluted sodium hypochlorite; let stand for 1 hour; then mop up and rinse with water.

SEND – OUT/ Shipping Instructions

Follow Category B Shipping Instructions
Transportation of Dangerous Goods Regulations

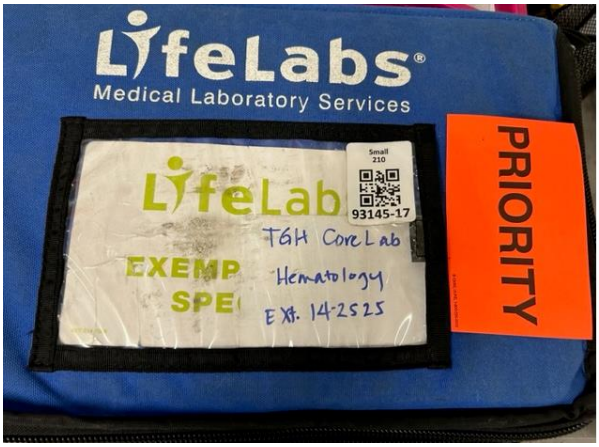
FOR CJD
Send to NML by FEDEX with Dry Ice same day to NML otherwise put vial in freezer MIFY to send next shipping day.

Prion Diseases Section
National Microbiology Laboratory Public Health Agency of Canada
1015 Arlington Street Winnipeg, MB R3E 3R2
T: 204-789-6078 F: 204-789-5009



FOR MSH CSF Cell count

Send to TGH-CORE LAB STAT by KJV



CJD Reporting Instructions

Preliminary done by MLA:

i) CJD test : INTERIM result as
"This specimen has been sent to the National Microbiology Laboratory, 1015 Arlington, Winnipeg, MB.,
Results will be reported when available."

ii) Other microbiology tests: PRELIM result as }CJDD :
"This test has been deferred until CJD is ruled out. Please contact the microbiologist-on-call with any questions."

iii) Rejected Samples**:** FINAL result as }CDJDB:
"Test not processed as CSF received is xanthochromic and/or has visible blood and is unsuitable for testing. Please recollect sample if test still indicated or contact the Microbiologist on call."

FINAL resulting and extra processing done by MLT:

CJD test results should be resulted from NML report as per keypad in LIS

For POSITIVE CJD results:

i) Email microbiologist-on –call, BSO and IPAC

ii) Other microbiology tests: result as }CJDX : "This test was CANCELLED for biosafety reasons given positive CJD test results. Please contact the microbiologist-on-call with any questions."

iii) Email hematology and chemistry a copy of the original requisition with their tests that were requested (ie glucose, total protein, lactate dehydrogenase) and a copy of our positive results (LIS report).

iv) Dispose of remaining sample(s) from fridge or freezer according to protocol

For NEGATIVE CJD results:

i) Take off ESO flag in LIS

ii) Other microbiology tests can now be processed, retrieve from fridge (bacterial, fungal cultures) or freezer (virology) and distribute accordingly

Result with canned message }CJDR : "Testing was delayed until CJD was ruled out which may reduce the sensitivity of this test. Please take this into consideration when interpreting this result. Please contact the microbiologist-on-call with any questions.

iii) For other non – microbiology tests, email haematology and chemistry and microbiology.specialqueries@sinaihealth.ca
With a copy of the original requisition with their tests that were requested and a copy of our negative results (LIS report) so they can respond if they want our frozen aliquot(s) to run their tests.

For non CJD testing, if CJD was ordered then Cancelled /Error in Ordering by MRP, add comment

> }CJDCX "Testing was delayed as CJD testing was ordered but has since been cancelled. The delay may reduce the sensitivity of this test. Please take this into consideration when interpreting this result. Please contact the microbiologist-on-call with any questions."

Profile of Relevant Risk Group 3 Organisms

Bacillus anthracis

Direct Gram stain from clinical samples:

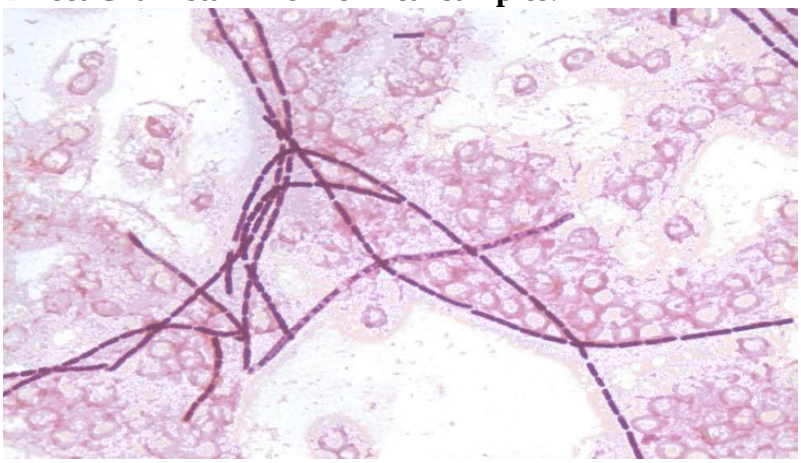


Photo courtesy of Dr. James Rudrick, Michigan Department of Community Health
https://www.asm.org/images/PSAB/LRN/Anthrax%20LRN%20091217.pdf

- large (1.0 to 1.5 μm by 3 to 5 μm) encapsulated Gram-positive bacilli in short chains.
- Gram stain can demonstrate clear zones (capsule) around bacilli.
- Spores usually not present in clinical specimens unless exposed to atmospheric O_2.

Gram stain from Sheep Blood Agar (SBA) or other routine nutrient medium

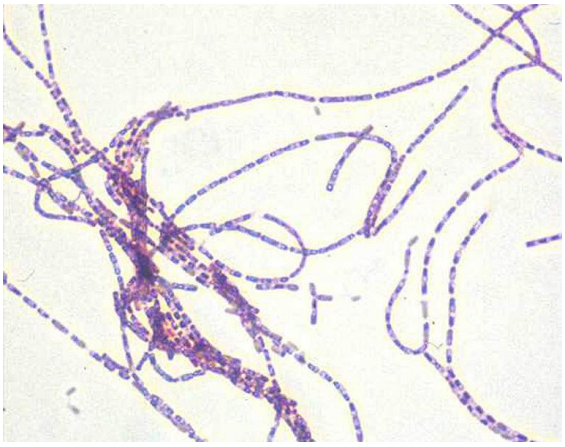
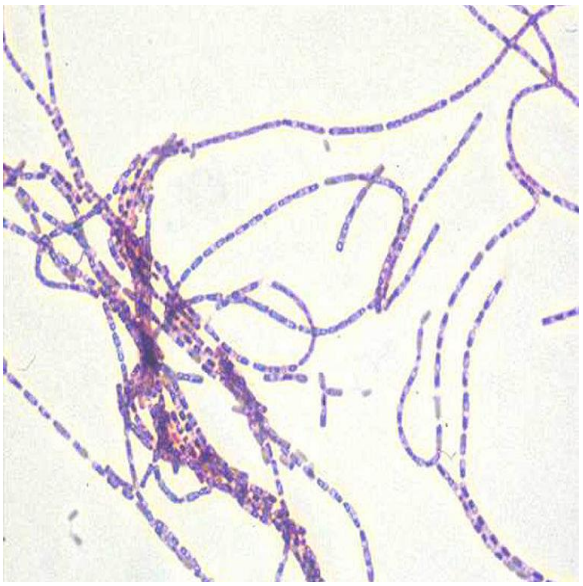


Photo curtesy of U.S. Army Medical Research Institute of Infectious Diseases, 2009.



- Large Gram-positive bacilli in long chains, usually non-encapsulated.
    - Oval, central to subterminal spores: 1 x 1.5 µ with no significant swelling of cell.

Culture:

Photo courtesy of APHL
https://www.asm.org/images/PSAB/LRN/Anthrax%20LRN%20091217.pdf

On Sheep Blood Agar (SBA):

- *Rapid growth*. Heavily inoculated areas may show growth on a blood agar plate within 6-8 h and individual colonies may be detected within 12-15 h. This trait can be used to isolate *B. anthracis* from mixed cultures containing slower-growing organisms.
- Colonies are nonhemolytic (hemolysis on SBA excludes B. anthracis), flat or slightly convex, with ground-glass appearance and tenacious consistency.
- Colonies often exhibit comma-shaped protrusions from colony edge ("Medusa head" colonies).

If isolate is non-hemolytic, perform motility test using motility test media. Presumptive identification of *B. anthracis* is based on identification of large Gram-positive bacilli that are **nonhemolytic** on SBA and **non-motile**. If presumptive diagnosis of *B. anthracis*, proceed as WHAT TO DO IF A RISK GROUP 3 ORGANISM IS SUSPECTED. Otherwise, report as "Bacillus species isolated" (from sterile sites) or as part of "Commensal flora" (from non-sterile sites such as wounds).

If a presumptive *B. anthracis* colony is identified and suspected as a bioterrorism threat agent, **preserve original specimens** pursuant to a potential criminal investigation.

N.B. *B. anthracis* does not typically grow on McConkey (MAC) agar containing crystal violet, but the MAC plate used at our microbiology lab does not contain crystal violet, hence this characteristic is not particularily useful here. (This is why we do not include MAC as a media for primary isolation to avoid confusion).

Francisella tularensis

Gram stain:

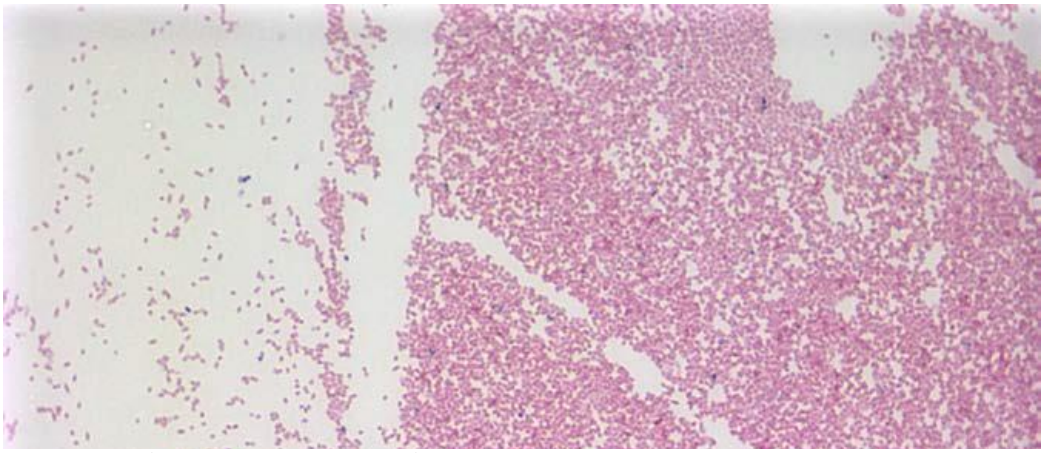


Photo courtesy of Cheryl Gauthier, MA Dept. of Public Health
https://www.asm.org/images/PSAB/LRN/Tularemia316.pdf

Tiny (0.2 to 0.5 μm by 0.7 to 1.0 μm), poorly staining pleomorphic Gram-negative bacilli / coccobacilli.

Culture:

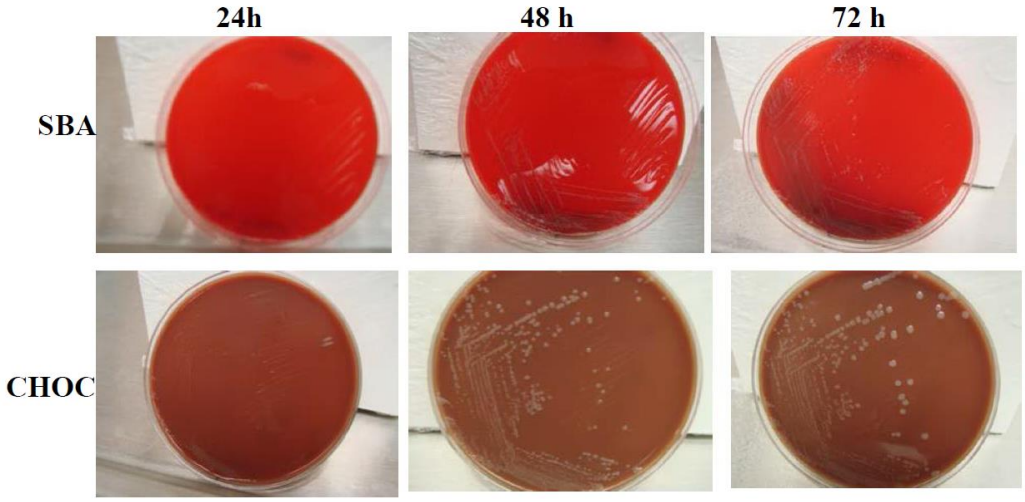


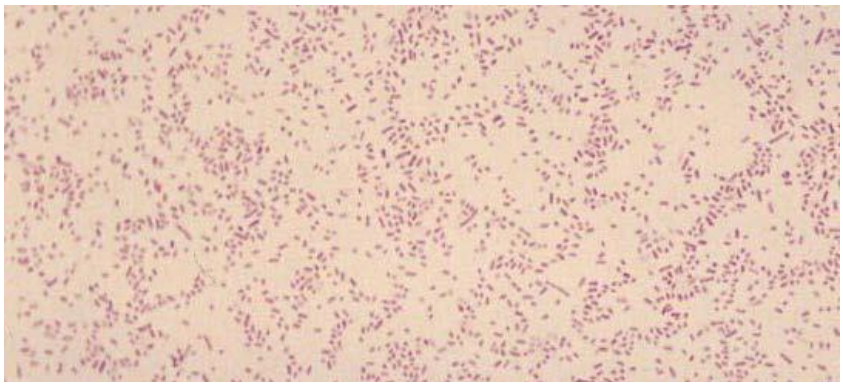
Photo courtesy of: MAJ Todd Kijek, USAMRIID
https://www.asm.org/images/PSAB/LRN/Tularemia316.pdf

- On Sheep Blood Agar (SBA): Non-hemolytic, gray-white colonies, 1-2 mm after 48h.
- On MacConkey agar (MAC): No growth.

When tiny gram negative bacilli/coccobacilli are identified, follow Small/tiny Gram-negative bacilli/cocco-bacilli from all sites.

Report as "Gram negative bacillus / coccobacillus isolated. Further identification to follow".

***Brucella* spp.**



https://www.asm.org/images/PSAB/LRN/Brucella316.pdf

Gram Stain:
Tiny (0.5 to 0.7 µm by 0.6 to 1.5 µm), faintly staining, Gram-negative coccobacilli

Culture:

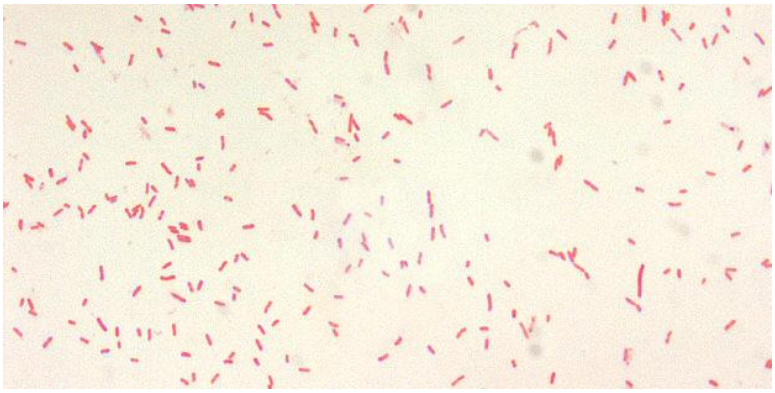
- On Sheep Blood Agar (SBA): Small (0.5 to 1.0 mm) glistening, non-hemolytic, non-pigmented colonies after 2 to 3 days incubation.

- On MacConkey agar (MAC): Some strains may grow slowly.

Courtesy Larry Stauffer, Oregon State Public Health Laboratories, Image #1902
https://www.asm.org/images/PSAB/LRN/Brucella316.pdf

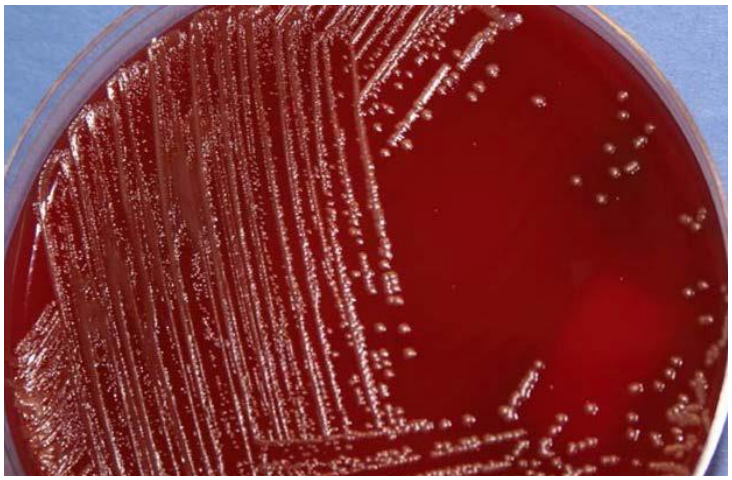
When tiny gram negative bacilli/coccobacilli are identified, follow Small/tiny Gram-negative bacilli/cocco-bacilli from all sites. Report as "Gram negative bacillus / coccobacillus isolated. Further identification to follow".

Yersinia pestis



Gram Stain:
Gram-negative bacilli (1.0 by 0.5 μm) that may exhibit bipolar staining

CDC/ Courtesy of Larry Stauffer, Oregon State Public Health Laboratory
https://phil.cdc.gov/details_linked.aspx?pid=1915



Culture:

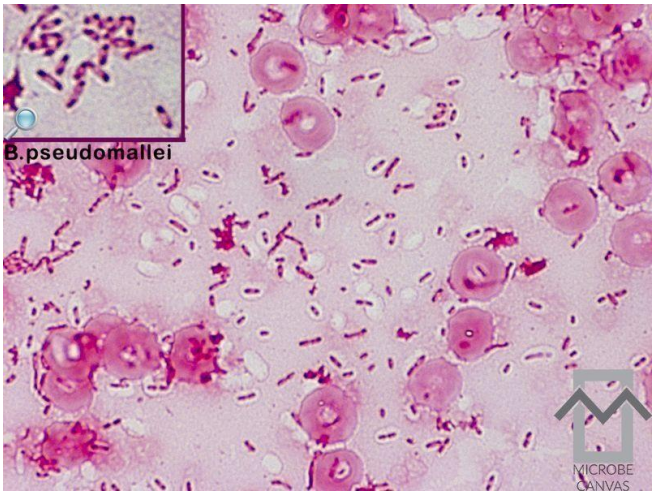
- On Sheep Blood Agar (SBA): gray/white/slightly yellow opaque colonies after 48 h incubation, with little or no hemolysis. Colonies develop fried egg appearance beyond 48 h incubation.

- On MacConkey agar (MAC): small, lactose negative colonies after 24 h incubation.

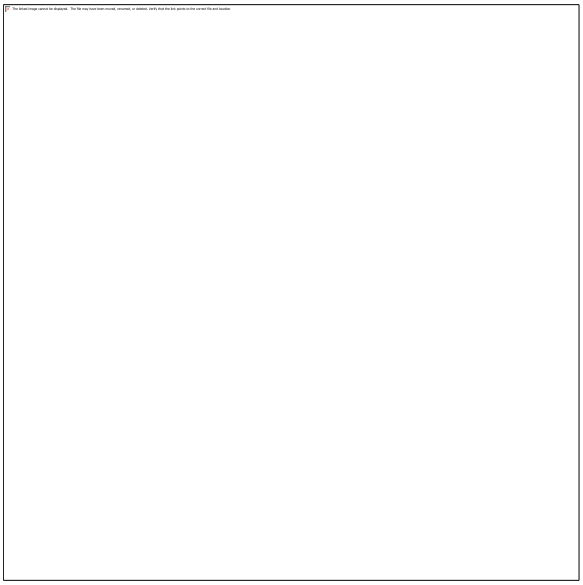
Photo courtesy of APHL
https://www.asm.org/images/PSAB/LRN/Ypestis316.pdf

When slow growing gram negative bacilli as per growth characteristics are described, follow Small/tiny Gram-negative bacilli/cocco-bacilli from all sites. Report as "Gram negative bacillus / coccobacillus isolated. Further identification to follow".

Burkholderia pseudomallei

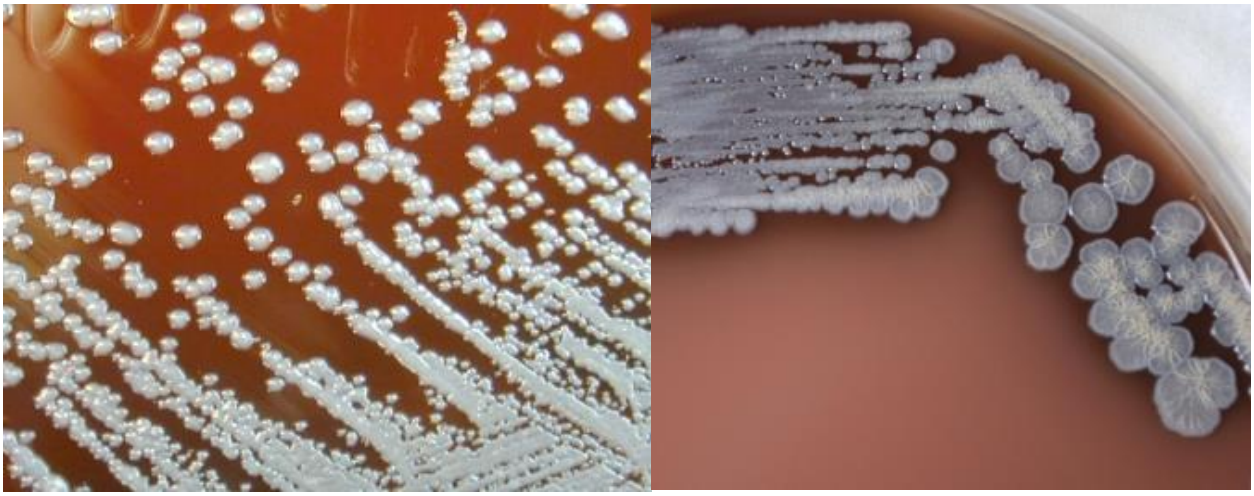


Gram:

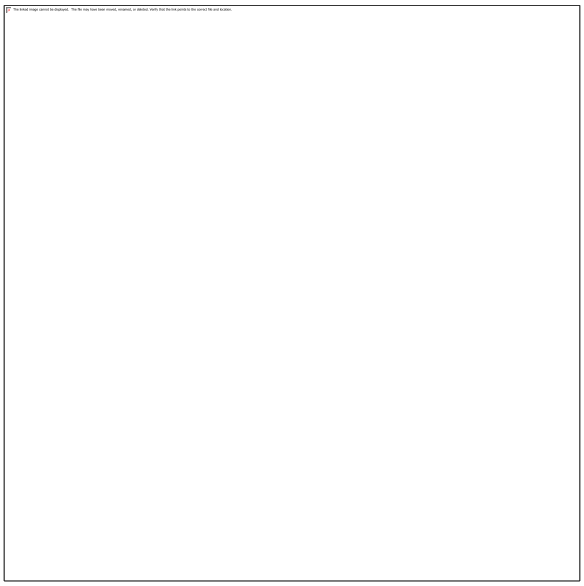


- Small (1-3 µm) Gram-negative bacilli with bipolar staining (“safety pin” appearance) with irregular arrangement (occasionally as long thin bundles).

Photo curtesy of Erasmus MC University Medical Center Rotterdam Dept. Medical Micro. and Inf. Dis.



Culture:
Photo curtesy of US CDC. Left, colonies on SBA at 48 h. Right, colonies on CHOC at 72 h.



- Sheep Blood Agar (SBA): Variable, smooth, creamy, white colonies growing within 24 h of incubation; may become wrinkled (“cornflower” appearance), metallic, and dry with purple hue over time.

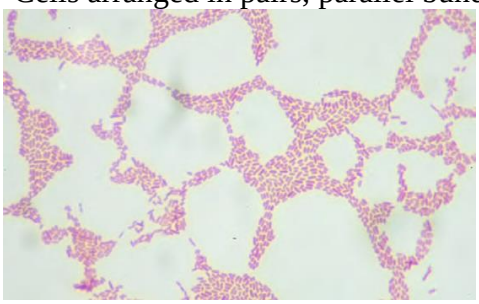
- MacConkey agar (MAC): Variably lactose-fermenting or colorless colonies at 24-48 h of incubation.

Burkholderia mallei

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

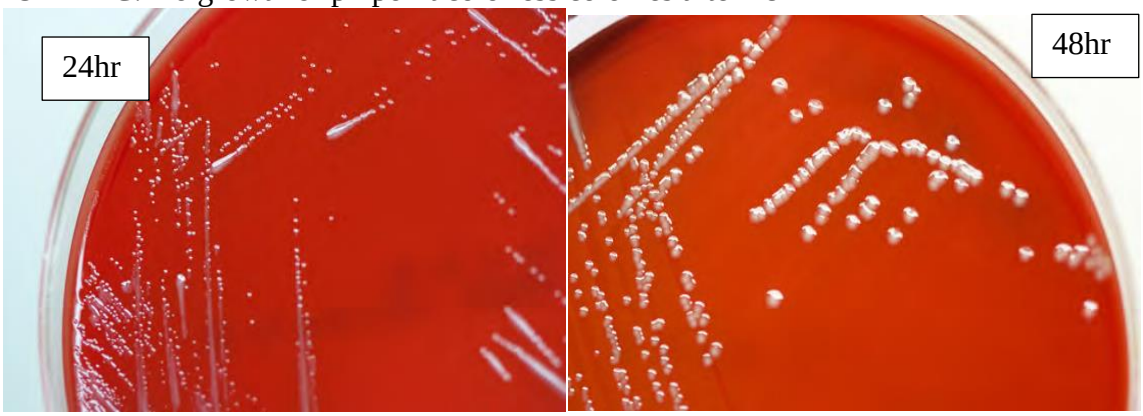


Reference:
https://www.aphl.org/aboutAPHL/publications/Documents/2018_BiothreatAgents_SentinelLab_BenchCards_WEB.pdf

Culture:

-On BA: 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

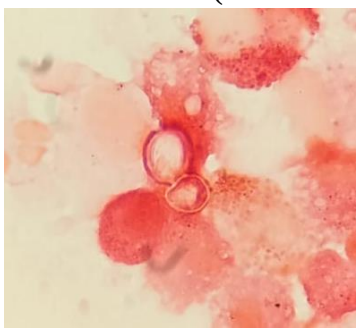


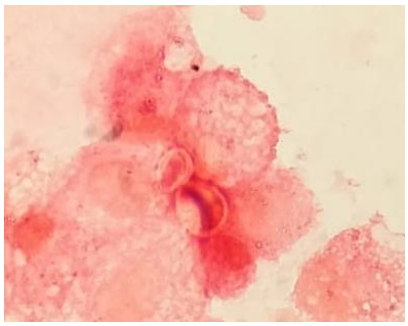
Reference:

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

Blastomyces dermatitidis

Gram stain (from clinical samples, or after incubation at 37˚C):





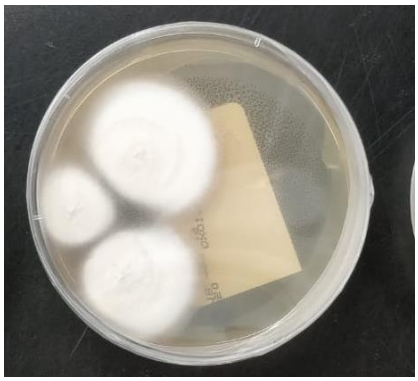
Large (8-15 µm), globose, thick-walled ("double wall", refractile) yeast cell, often with single broad-based (4-5 µm) budding daughter cell.

Photo curtesy of Yin Ping Tse, Mount Sinai Hospital Dept. of Microbiology

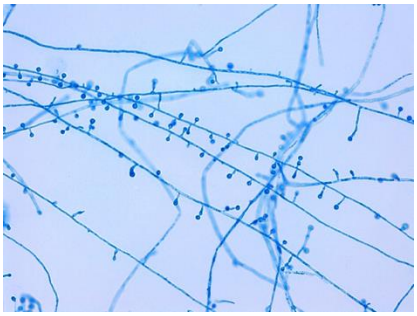
Culture:

- BHIM or BAP at 37˚C: White or beige, wrinkled, pasty, and moist colonies seen at 3 days to 4 weeks of incubation.

Photos curtesy of Yin Ping Tse, Mount Sinai Hospital Department of Microbiology



- IMA at 25˚C: Floccose, white mold (turning tan to yellow with age) with tan to brown reverse seen at 3 days to 4 weeks of incubation.



Lactophenol cotton blue stain (after incubation at 25˚C):

Septate hyaline hyphae with microconidia (2 – 10 µm) at right angles. No macroconidia.

Photo curtesy of Yuri, Fun With Microbiology Blog (http://thunderhouse4-yuri.blogspot.com)

Histoplasma capsulatum

Gram stain (from clinical samples, or after incubation at 37˚C):

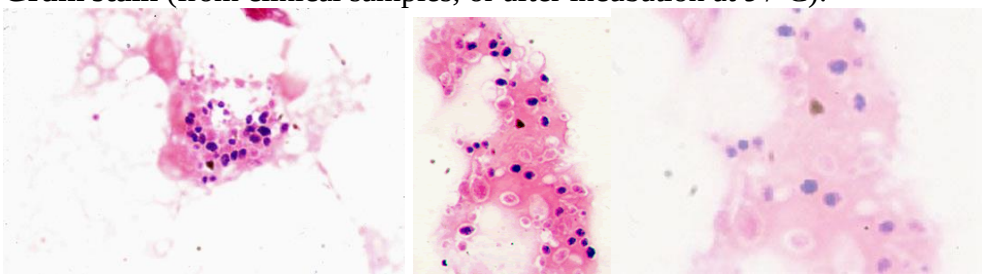


Photo curtesy of Subhash Mohan, Mount Sinai Hospital Department of Microbiology

Small (2 -4 µm) elongate, narrow budding yeast cells, variably stained, occasionally encapsulated, and often intracellular (e.g. within macrophages).

Culture:

- BHIM or BAP at 37˚C: White, creamy, smooth, moist, and round colonies seen at 3 days to 4 weeks of incubation.

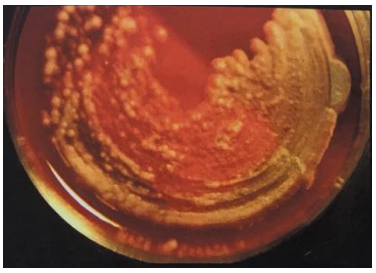
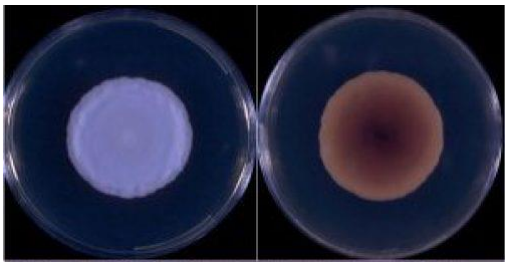
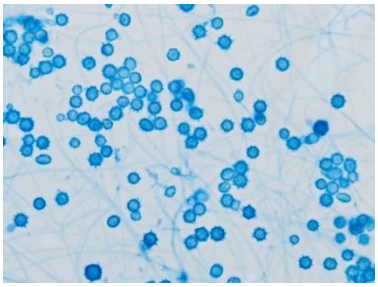


Photo curtesy of Subhash Mohan, Mount Sinai Hospital Dept. of Microbiology

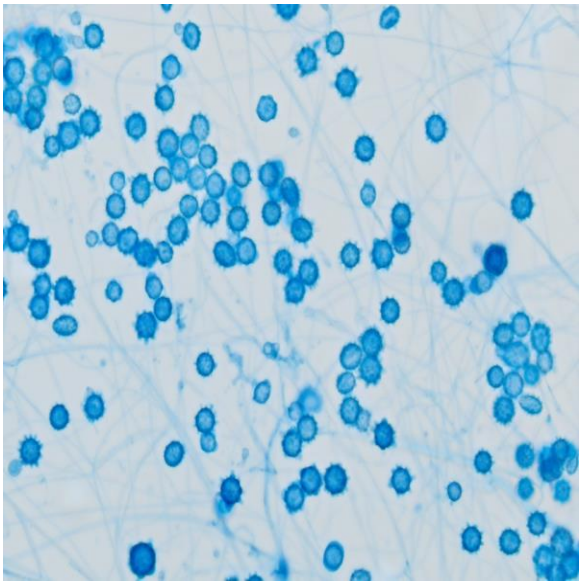


- IMA at 25˚C: Suede, white mold ((turning tan to buff-brown with age) with yellow to tan reverse seen at 3 days to 4 weeks of incubation.

Photo curtesy of Sarah Kidd et al, National Mycology Reference Centre, University of Adelaide 2016



Lactophenol cotton blue stain (after incubation at 25˚C)



Septate hyaline hyphae with large, tuberculate, thick-walled, round, and unicellular macroconidia having finger-like projections on the surface. Also has round, unicellular microconidia with smooth or rough wall.

Photo curtesy of Joy King and Lisa Stempak, University of Mississippi Medical Centre

Coccidioides immitis

Gram strain (from clinical samples, or after incubation at 37˚C):

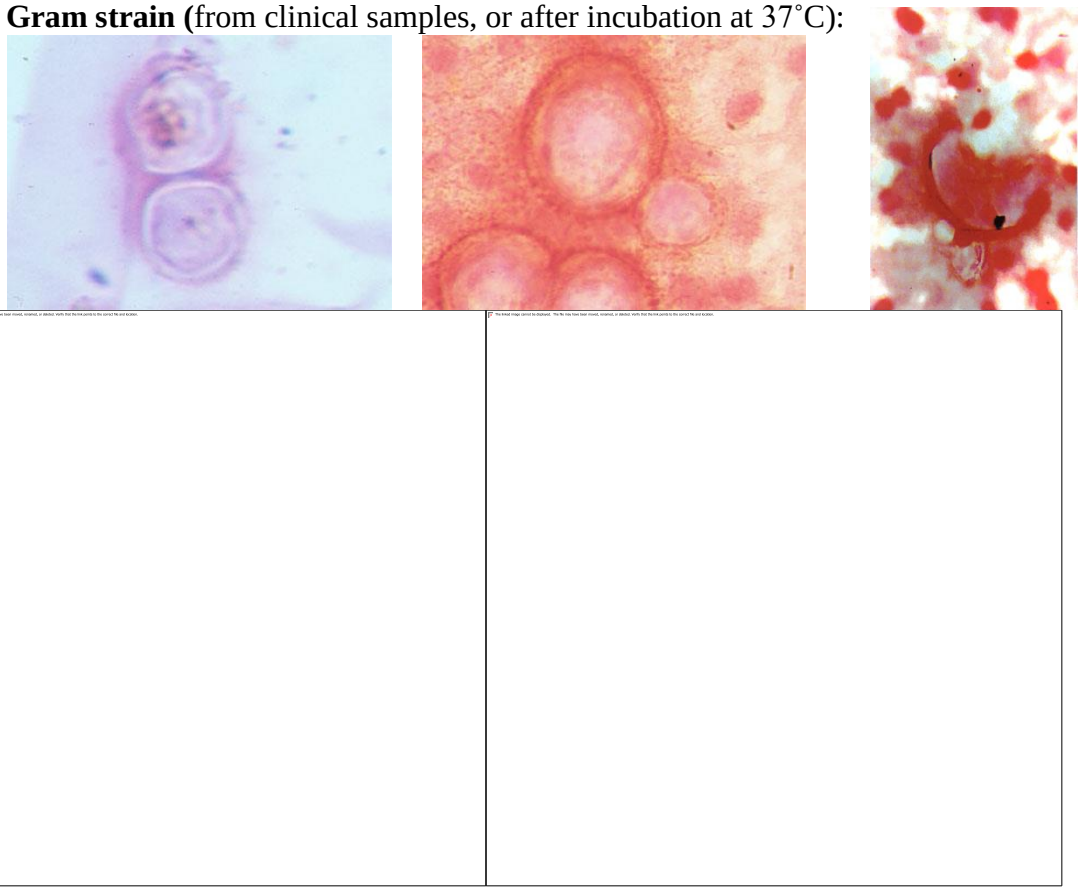
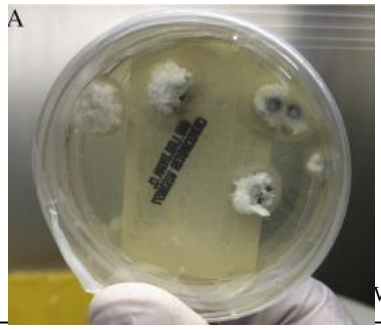


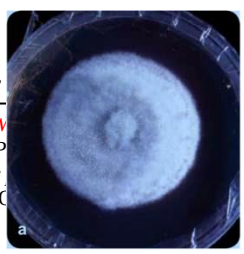
Photo curtesy of Subhash Mohan, Mount Sinai Hospital Department of Microbiology. Left and middle immature spherules; right, ruptured mature sphere with released endospores.

Large, tick-walled spherules (10-80 µm) containing multiple endospores (2-5 µm); immature spherules with no endospores may mimic *Blastomyces.* No true budding.

- **Culture:**



BHIM or BAP at 37˚C, or IMA at 25˚C: Variable morphology from greyish, moist, glabrous, and membranous colonies to abundant, floccose, and white mold (turning tan to red with age) with pale brown to orange reverse seen at 3 days to 4 weeks of incubation.



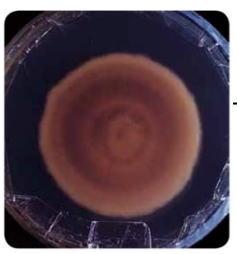
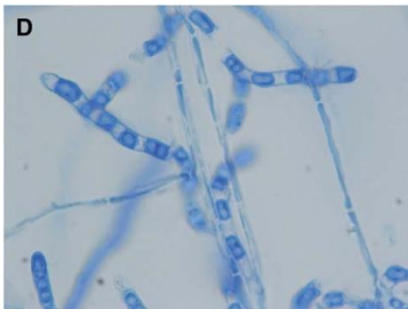


Photo curtesy of Lorena Porte et al, J of Hosp Inf 2019

Photo curtesy of Sarah Kidd et al, National Mycology Reference Centre, Adelaide 2016

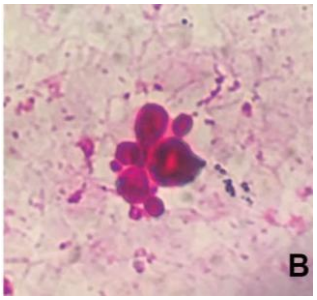
x 

Lactophenol cotton blue stain (after incubation at 25˚C)

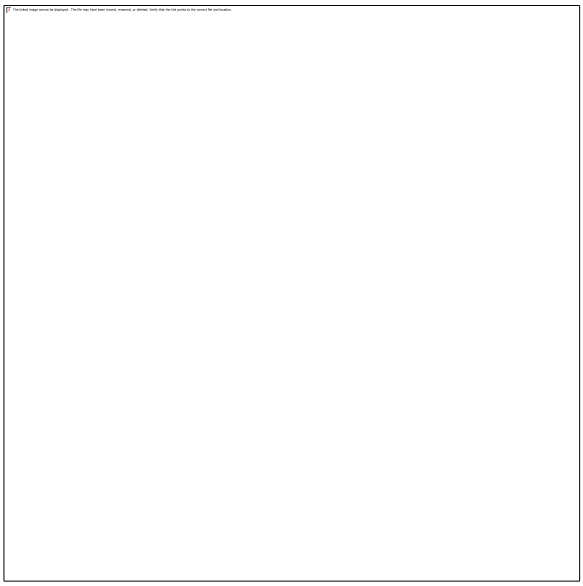
Thin, septate hyaline hyphae, with one-celled, cylindrical to barrel-shaped, thick and smooth-walled arthroconidia (2-8 3-5 µm) alternating with thin-walled empty disjunctor cells. True arthroconidia eventually fragment and disperse.

Photo curtesy of Audrey Schuetz et al, Diagn Cytopathol 2012

Paracoccidioides brasiliensis

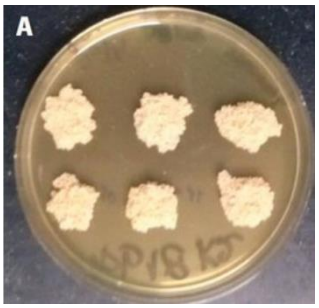


Gram strain (from clinical samples, or after incubation at 37˚C):



Large (4-60 µm), thick-walled (1 µm), double-countered, globose cells with multilateral narrow budding of daughter cells ("steering wheel" appearance) which may produce smaller secondary buds.

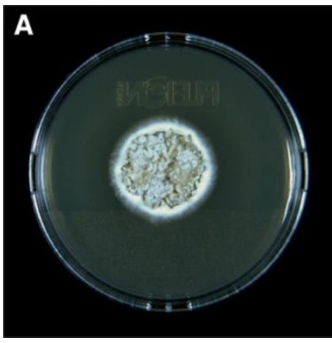
Photo curtesy of Priscila Marques de Macedo et al, Rev Soc Bras Med Trop 2018



Culture:

- BHIM or SBA at 37˚C: White, heaped, wrinkled or folded colonies seen at 10 days to 4 weeks of incubation.

Photo curtesy of Jessica Gomes-Rezende thesis 2017



- IMA at 25˚C: White cream filamentous, leathery, flat to wrinkled, wolly or cottony or glabrous mold (turning to tan or brown with age) with yellowish to brown reverse seen at 10 days to 4 weeks of incubation. Similar appearance to *Blastomyces*.

Photo curtesy of Rosane Christine Hahn et al, Am J Trop Med 2014

Lactophenol cotton blue (after incubation at 25˚C):

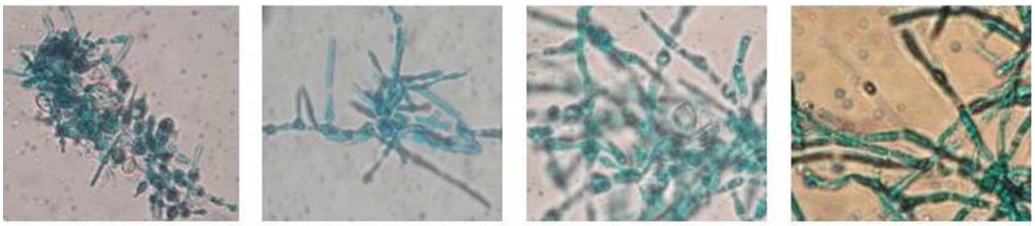


Photo curtesy of R. C. Cruz et al, J Clin Microbiol 2012

Hyaline septate hyphae, often sterile, or with rare oval, unicellular, truncate conidia with broad base and round apex. Arthroconidia and intercalary chlamydospores may also be observed.
Cladophialophora bantiana (previously *Cladosporium bantiana*)

Culture:

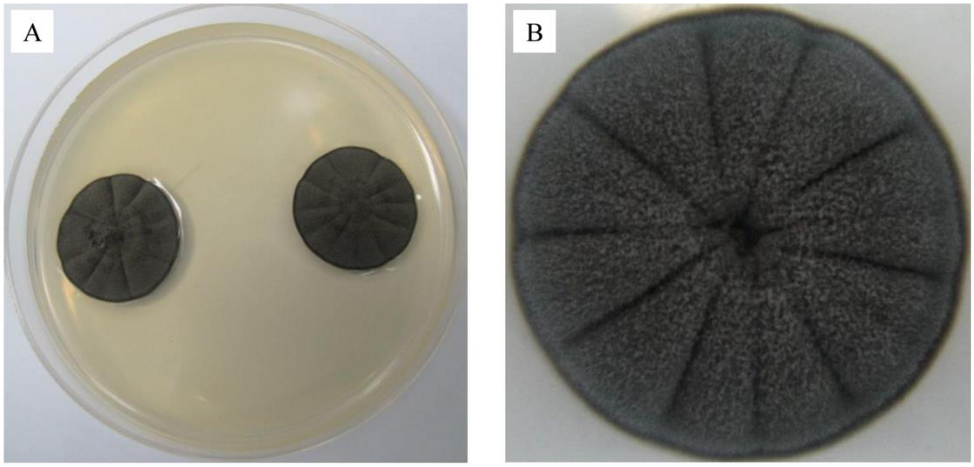
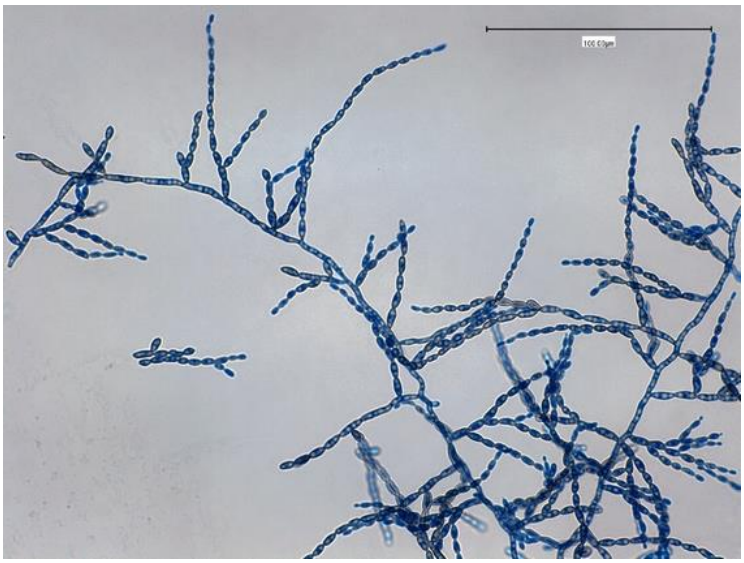
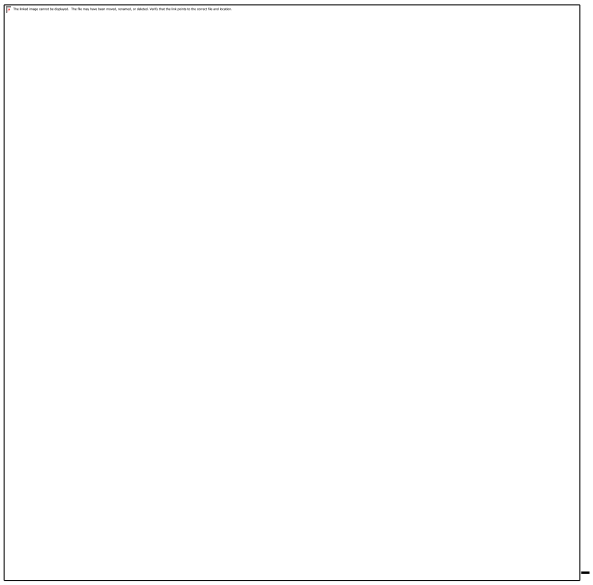


Photo curtesy of Sian Kuan et al, PLoS One 2016

- IMA at 30˚Ç: Powdery, woolly, or velvety, olivaceous green to black with black reverse mould often growing after 2 weeks of incubation. Supports growth at 42˚C.

Lactophenol cotton blue stain (after incubation at 30˚Ç):



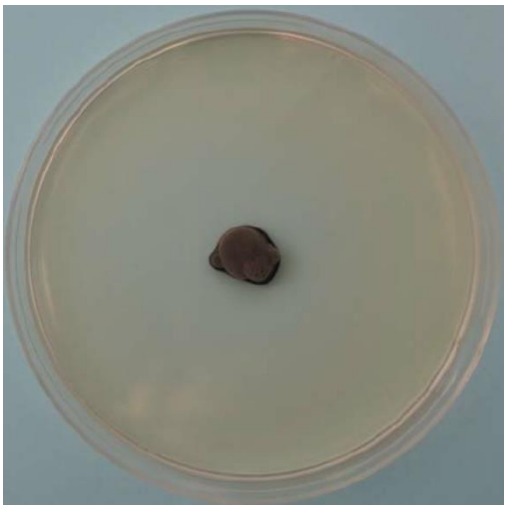


- Septate brown hyphae with long, smooth, lemon-shaped unicellular conidia (6-11 x 2.5-5 µm) in sparsely-branched chains emerging from undifferentiated conidiophores, and occasional chlamydoconidia. The youngest conidia are found at the apex of the chain (acropetal conidium formation). No attachment scars, comparatively to other *Cladophialophora* species. No shield cells, comparatively to *Cladosporium* species.

Photo curtesy of Yuri, Fun With Microbiology Blog (http://thunderhouse4-yuri.blogspot.com)

Rhinocladiella mackenziei (previously *Ramichloridium mackenziei*):

Culture:



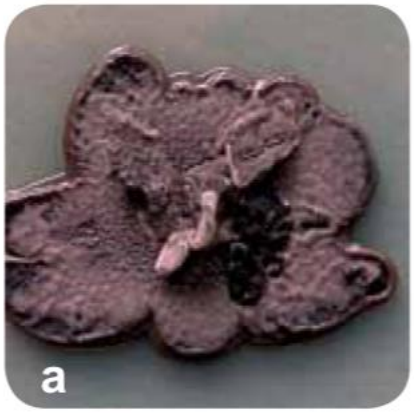
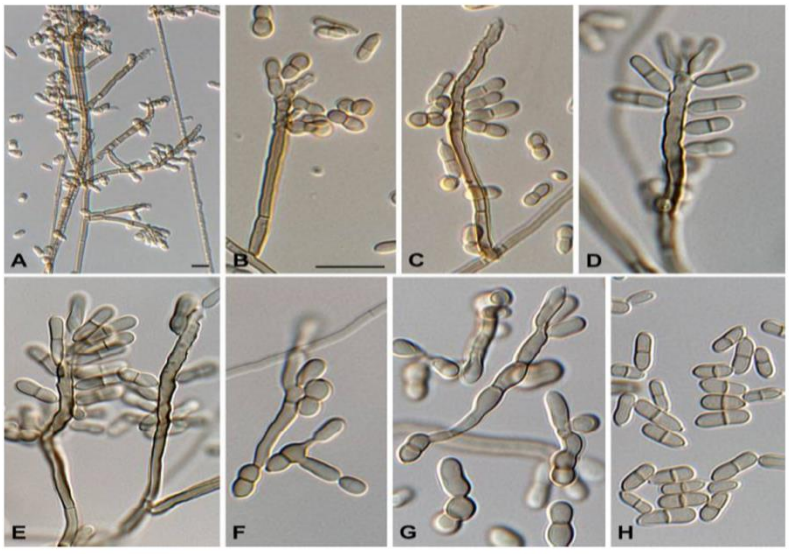


Photo curtesy of Sarah Kidd et al, National Mycology Reference Centre, Adelaide 2016

Photo curtesy of Taj-Aldeen et al, Med Mycol 2010

- IMA at 30˚C: Velvety, olivaceous to brown with olivaceous black reverse with occasionally elevated center mould often growing after 2 weeks of incubation.

Lactophenol cotton blue stain (after incubation at 30˚C)



- Dark-pigmented, smooth septate hyphae with short, thick, brown conidiophores at right angles leading to cylindrical denticles producing oval sympodial conidia (8-10 x 4-5 µm, “Mickey Mouse” appearance) with prominent basal scar.

Photo curtesy of Arzanlou M, University of Tabriz.

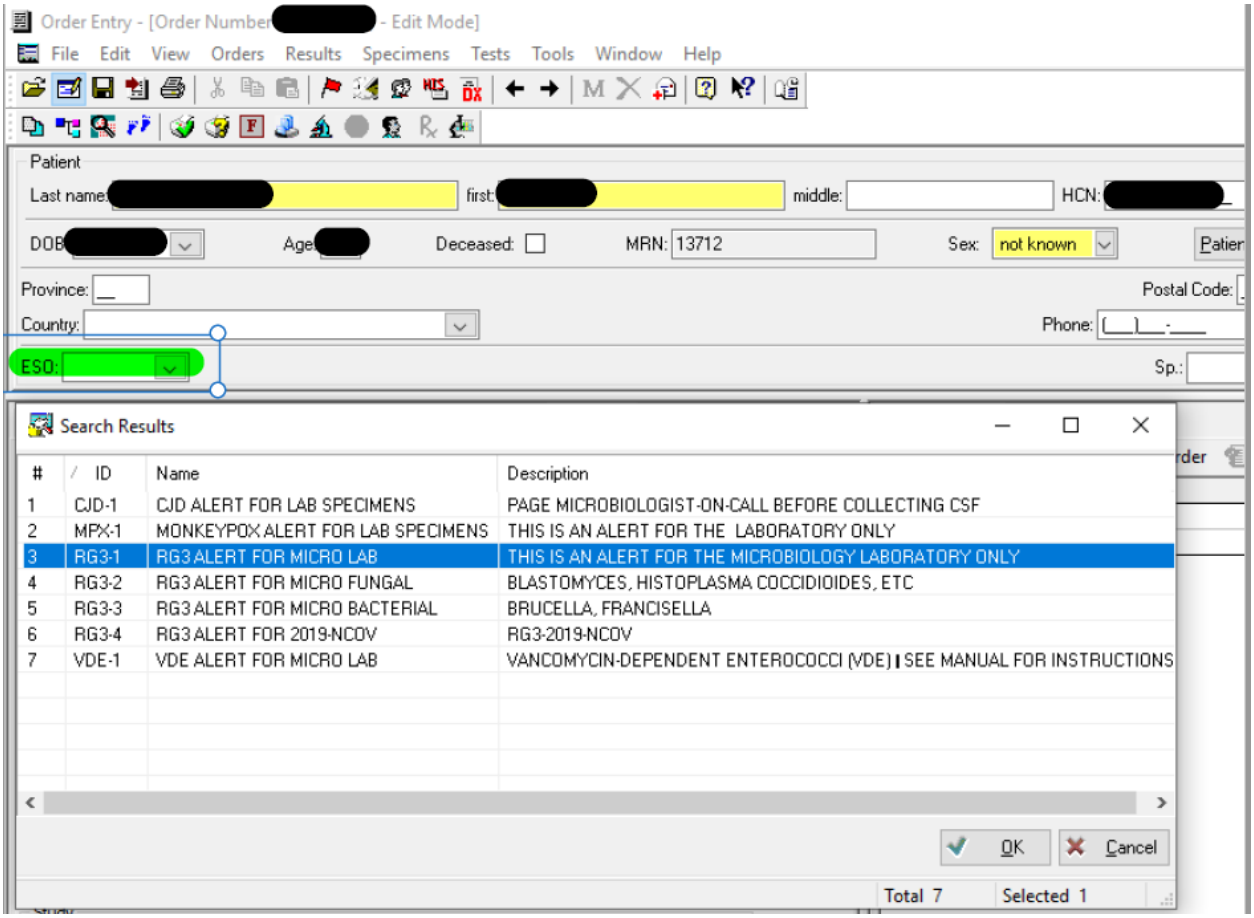
HOW TO ADD ESO FLAG

Access to ESO flag change

- All senior and charge technologists and LIS officers will have access to add an ESO flag.
- During shifts where there are seniors working, senior technologists (charge technologists as back-up) will add the flag.
- During shifts where there are no senior and charge technologists working, the acting senior or microbiologist will contact the LIS officer on call through locating for adding an ESO flag.

Steps to add an ESO flag

- Go to the order entry of the patient report involved.
- Click the drop down button under ESO field.
- Choose the appropriate ESO code, eg: RG3-1 (RG3 ALERT FOR MICRO LAB).
- Save the change



PANDEMIC SPECIMEN SAFETY

Emergence of novel microorganisms causing pandemic pose an increased risk to public health and healthcare workers alike. Due to the likely ease of human to human transmissions, it is essential to have protocols in place to protect employees working with these related specimens.

General Safety practices:

- The use of N95 or surgical masks for general processes within the laboratory will be determined on a case by case outbreak occurrence followed published guidelines (eg. MOH, WHO, CDC)
- Increase of surface disinfection by hospital supported disinfectants, paying close attention to high touch, high traffic areas.

Sample collection:

- Infection control to prescribe collection protocol and required PPE
- Samples shall be double bagged in two sealed clean biosafety bags
- Label outside of outer bag with name of infection suspected

Sample transport:

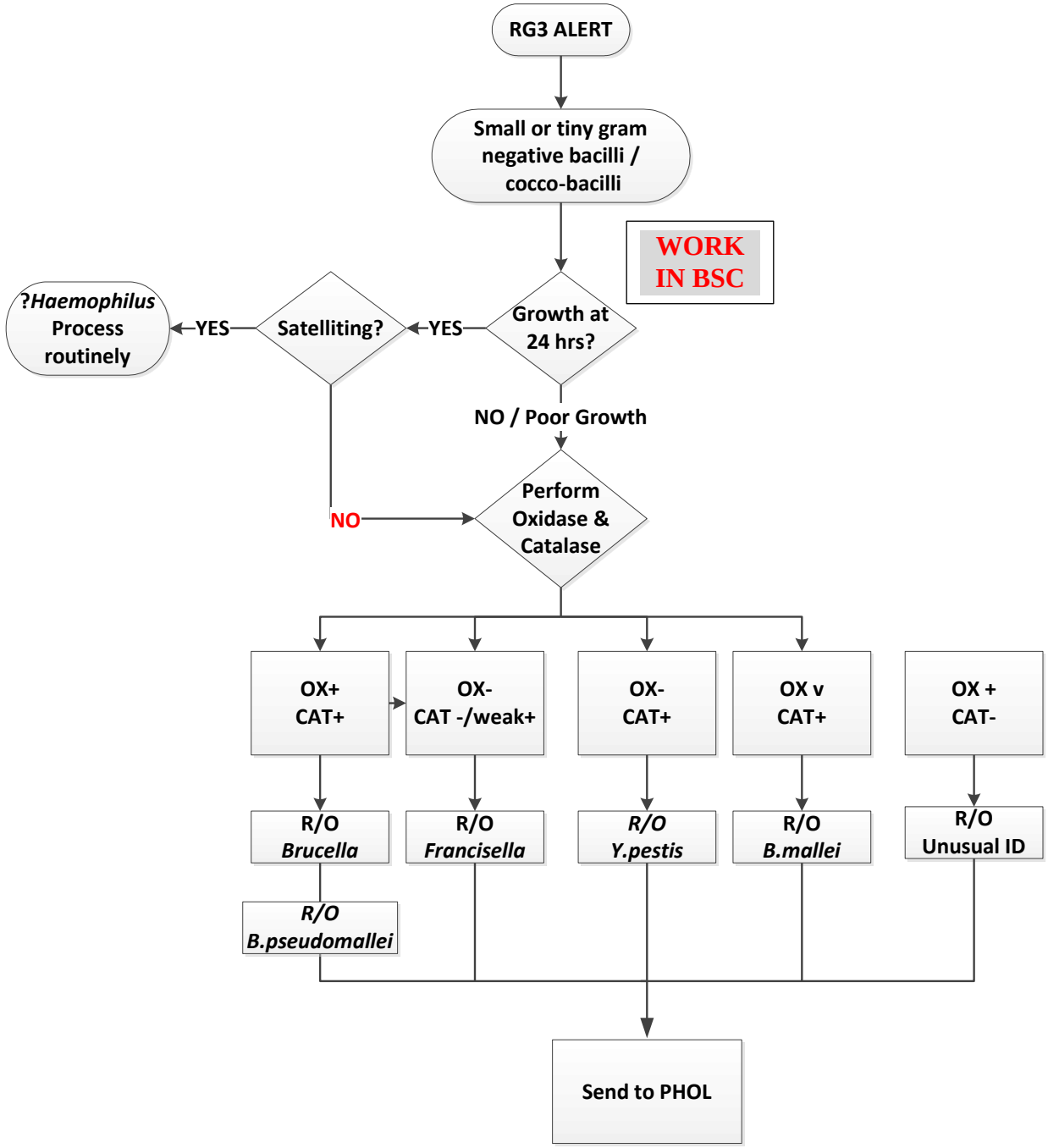
- Transport any specimen type by porter
- Do NOT use the pneumatic tube system under any circumstances

Specimen processing:

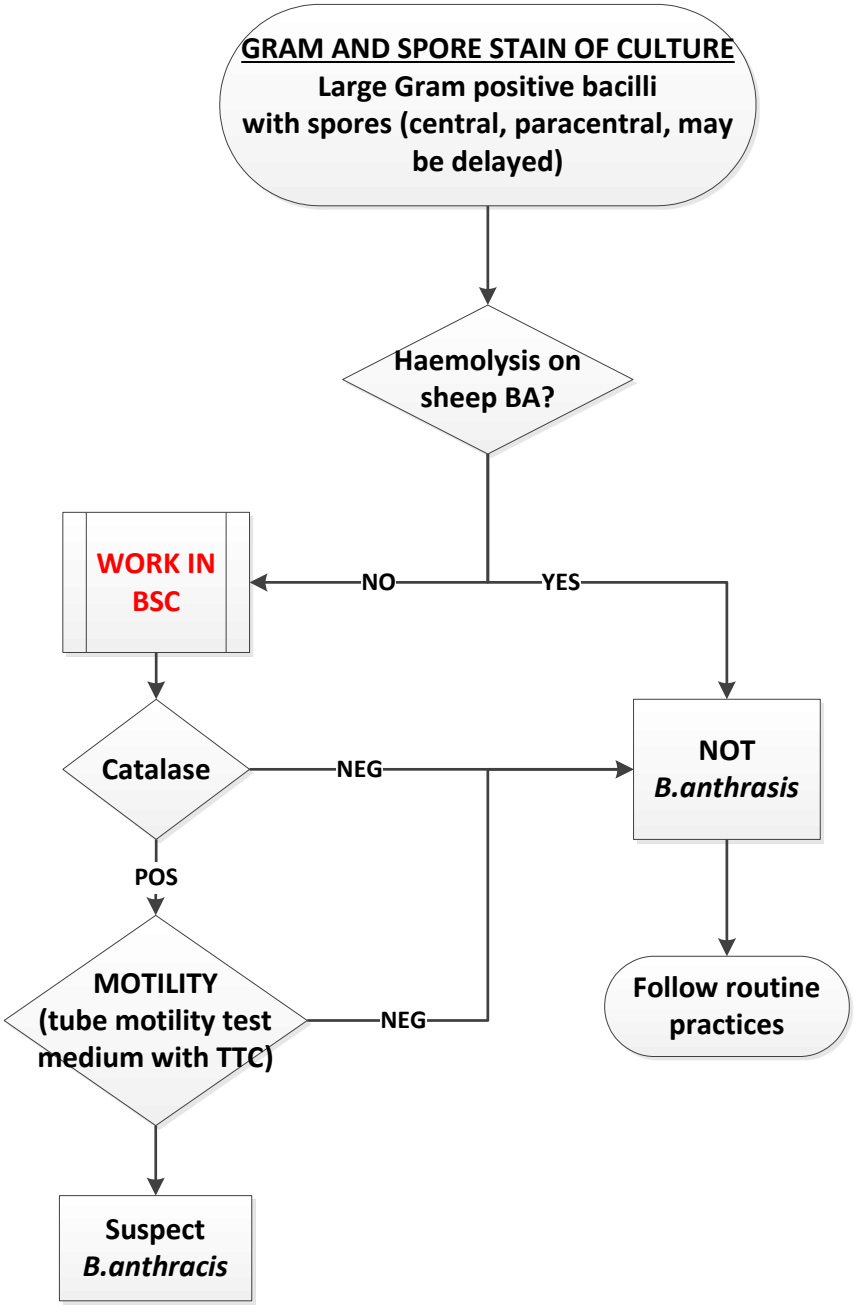
- Enhanced level 2 practices are recommended for pandemic related samples.
- PPE will be reviewed per specific pandemic outbreak but can include
    - All manipulation of primary sample in a PPE with
        - Water impermeable gown
        - Double gloves
        - N95 mask / surgical mask / rounded face shield as applicable
        - Other
- Disinfection of samples after manipulation of primary sample
- When possible, using an organism inactivation method of samples prior to testing.

:

APPENDIX I: Flowchart work-up for suspect small or tiny gram negative bacilli / coccobacilli potential Risk Group 3 organisms



APPENDIX II: Flowchart of *Bacillus* sp. work-up for suspect *B. anthracis*



APPENDIX III – Disposal of confirmed RG3/4 isolates and samples

Once an isolate has been confirmed as RG3 or RG4, all culture media, primary specimens and aliquots should be collected and discarded as soon as possible via waste management (Daniels Health).

Paperwork required for Daniels Health (F3-10-6010, F3-10-6011) are located in T drive: T:\microbiology\Health and Safety\Biological Waste Disposal

1) Double bag all waste.
2) Place waste into a red Daniel's plastic waste container.
3) If needed, add absorbent powder to the drum to absorb any free liquid in the bags.
4) Securely fasten lid to the plastic waste container.
5) Disinfect surface of the container.
6) Fill and Sign both forms:
    a. Healthcare Facility Confirmation (F3-10-6010), and
    b. Generator certification of proper waste packaging (F3-10-6011)
7) Bring container with signed forms to wash-up room for removal. If required, notify housekeeping of waste container to be discarded.
8) Make a copy of both signed forms and lab reports and provide all copies to the Manager.

References:
Daniels SOP – 3-10-6018 Handling & packaging biomedical waste V1 2021.pdf
Daniels SOP – 3-10-6019 Packaging guidelines – infectious substance category A V1 2021.pdf

REFERENCES

1. Murray PR, Baron EJ, et al. 1999. Manual of Clinical Microbiology. 7th Ed. ASM Press, Washington, DC.

2. CDC Guidelines for State Health Departments (Revised October 14, 2001)

3. CDC Basic protocol for the presumptive identification of Bacillus anthracis

4. Guideline and Fact sheet form Ontario Ministry of Health October 15, 2001

5. PHAC guidelines for CJD **http://www.phac-aspc.gc.ca/cjd-mcj/index-eng.php**

6. ASM. Sentinel level clinical laboratory protocols for suspected biological threat agents and emerging infectious diseases. http://www.asm.org/index.php/guidelines/sentinel-guidelines

Record of Edited Revisions

Manual Section Name: Bioterrorism Procedure Manual

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	March 1 2002	Dr. T. Mazzulli
Annual Review	May 12 2003	Dr. T. Mazzulli
Annual Review	May 26 2004	Dr. T. Mazzulli
Annual Review	May 12 2005	Dr. T. Mazzulli
Annual Review	July 23 2006	Dr. T. Mazzulli
Annual Review	August 13 2007	Dr. T. Mazzulli
Annual Review	August 17 2008	Dr. T. Mazzulli
Annual Review	August 20, 2009	Dr. T. Mazzulli
Annual Review	August 20, 2010	Dr. T. Mazzulli
Annual Review	May 31, 2011	Dr. T. Mazzulli
Annual Review	May 31, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli
Pathology & Laboratory Medicine - Emergency Preparedness Plan D0004273.doc Link Added Annual Review	September 17, 2014	Dr. T. Mazzulli
Annual Review	April 14, 2015	Dr. T. Mazzulli
Annual Review Update MSH logo in header	April 2, 2016	Dr. T. Mazzulli
Annual Review Update MSH logo in header	April 7, 2017	Dr. T. Mazzulli
Annual Review	April 4, 2018	Dr. T. Mazzulli
Addition of Biosafety procedures: How to Identify and what to do when potential RG3 organism are suspected. Addition of Flowchart of suspect RG3 organisms. Addition of gram and culture images. Removal of instruction to perform any testing including oxidase, catalase, urease on suspect RG3 organisms.	January 28, 2019	Dr. T. Mazzulli
Entire document: update title to RG 3/4 organisms clarify procedures for safe handling Appendix I, II - change "rule out" to "suspect"	01Nov2019	Dr. T. Mazzulli
Annual Review Update CJD procedure with MSH core lab instructions, emphasize cannot send xanthochromic samples.	Feb 6, 2020	Dr. T. Mazzulli

-Addition of Burkholderia mallei -added fungal smear to WHEN TO SUSPECT HIGH BIOSAFETY RISK AGENTS - added Refer to Isolate Notification and Freezing Table	May 01,2020	Dr. T. Mazzulli
Changed senior responsibilities after RG3/4 notification by the staff to: Notify seniors, who shall: Ensure notification of Biological Safety Officer, Microbiologist (if concern for exposure, notify microbiologist-on-call in real-time; otherwise notify once report provided to Health Canada and isolate/plates discarded, and Infection Control (as appropriate). addition of Smears suggestive of Blastomyces: Call ward (was already added to Isolate Notification table July 26, 2020)	Aug 20, 2020	Dr. T. Mazzulli
Removed instruction to autoclave RG3 and RG4 samples and plates.	Jan 29, 2021	Dr. T. Mazzulli
Added appendix III – disposal of rg3/4, added hyperlinks	June 14, 2021	Dr. T. Mazzulli
Added *C. diphtheriae to RG3*	April 28, 2023	Dr. T. Mazzulli

Full document review included in all updates. Annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Updated entire CJD handing procedure	December 6, 2023	Jessica Bourke
Updated Appendix III – Disposal of confirmed RG3/4 isolates and samples procedure: inserted “a red Daniel’s plastic waste container.” Deleted step number 5, “Ensure Biohazard labels are affixed to the container.” And added last step, in the procedure “Make a copy of both signed forms and lab reports and provide all copies to the Manager.”	December 11, 2023	Jamaal Pratt
Added “HOW TO ADD ESO FLAG” • Access to ESO flag change • Steps to add an ESO flag	June 20, 2024	Oliver Li