

PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC40100 – Suspect High Risk Organism Workup	Policy Number: 15-203-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 17/04/2025	Next Review Date: 17/04/2027
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 17/04/2025
Accreditation Canada Applicable Standard: NA	

**GUIDING PRINCIPLE:**

The STH Microbiology Laboratory is a Containment Level 2 facility licensed to safely process and handle Risk Group 2 organisms. However, as the laboratory processes unknown specimens, risk exists to isolate organisms with a high biosafety risk.

**PURPOSE/RATIONALE:**

This standard operating procedure describes the method to safely identify and handle organisms of high biosafety risk.

**SCOPE/APPLICABILITY:**

This standard operating procedure applies to Medical Laboratory Technologists (MLTs) performing the identification and workup on clinical microbiology specimens.

**SPECIAL SAFETY PRECAUTIONS:**

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

### WHEN TO SUSPECT HIGH BIOSAFETY RISK AGENTS

A. Presumptive diagnosis provided:

- **SUSPECT** relevant agents listed in this procedure

B. Gram stain results:

- Small, gram-negative bacilli or coccobacilli from sterile sites
- **SUSPECT** *Brucella* spp. and *Francisella* spp.

C. Gram stain results:

- Gram-negative diplococci from sterile sites
- **SUSPECT** *Neisseria meningitidis*

**NOTE:** *Neisseria meningitidis* is a Risk Group 2 organism, but given the potential for serious infection, culture should be treated like a Risk Group 3 organism

D. Culture results:

- Slow-growing, gram-negative bacilli/coccobacilli from all sites
- **SUSPECT** *Brucella* spp., *Francisella* spp., *Yersinia pestis*, *Burkholderia pseudomallei* or *Burkholderia mallei*

E. Culture results:

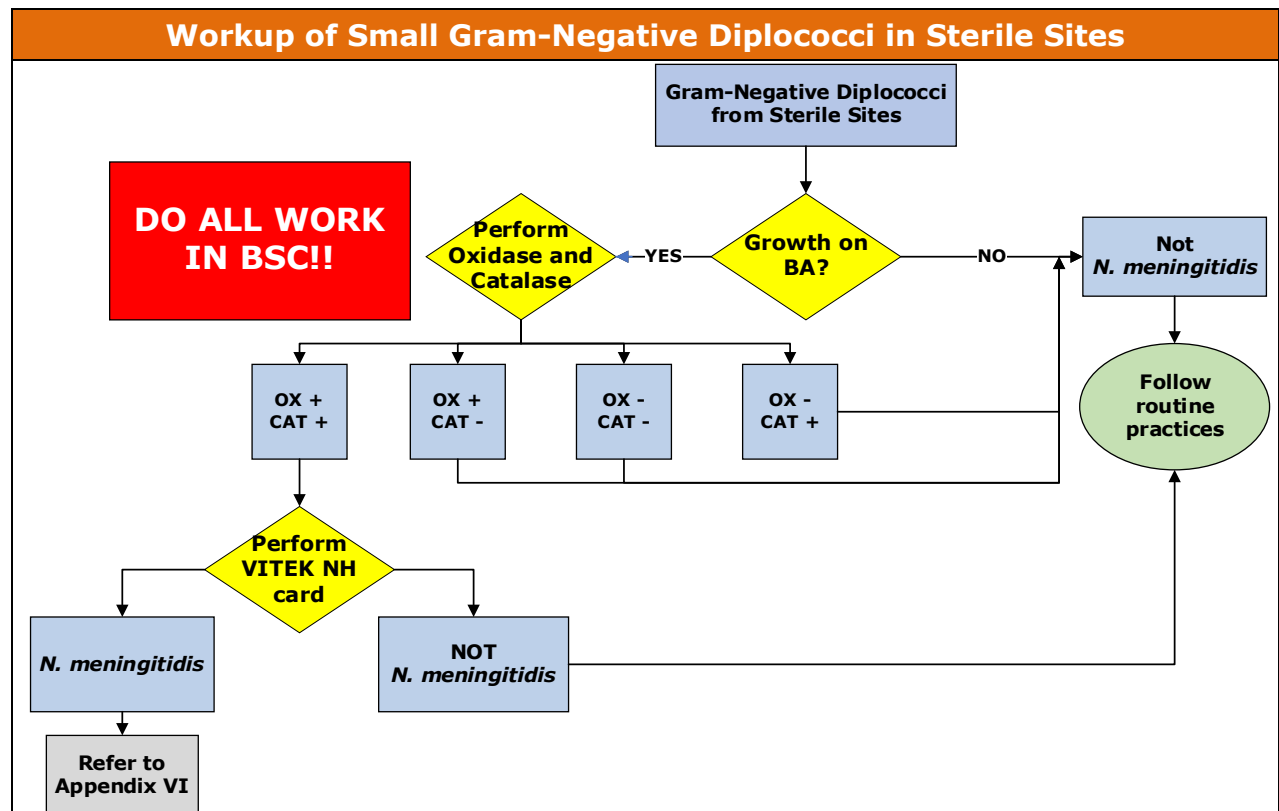
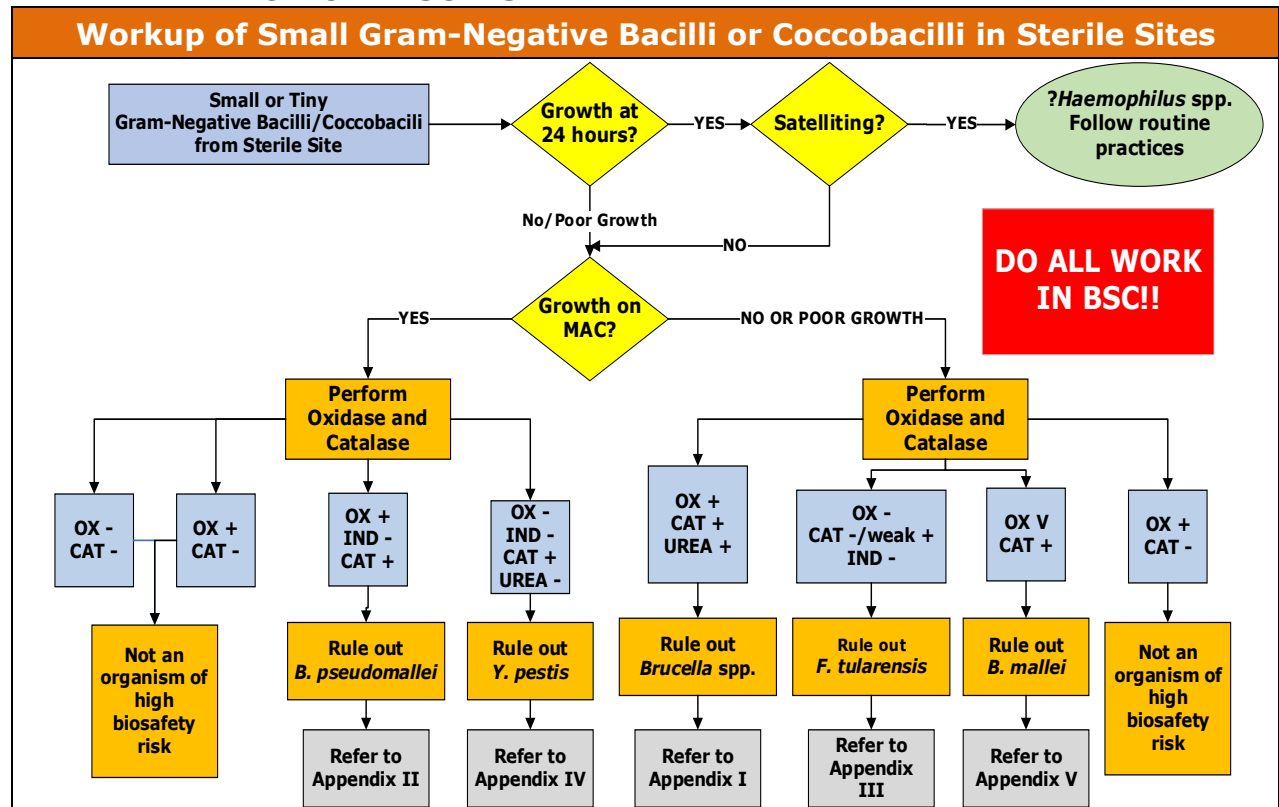
- Rapid-growing, non-hemolytic colonies with ground-glass appearance often exhibiting comma-shaped protrusions from colony edge ("Medusa head" colonies)
- **SUSPECT** *Bacillus anthracis*

## PROCEDURE INSTRUCTIONS:

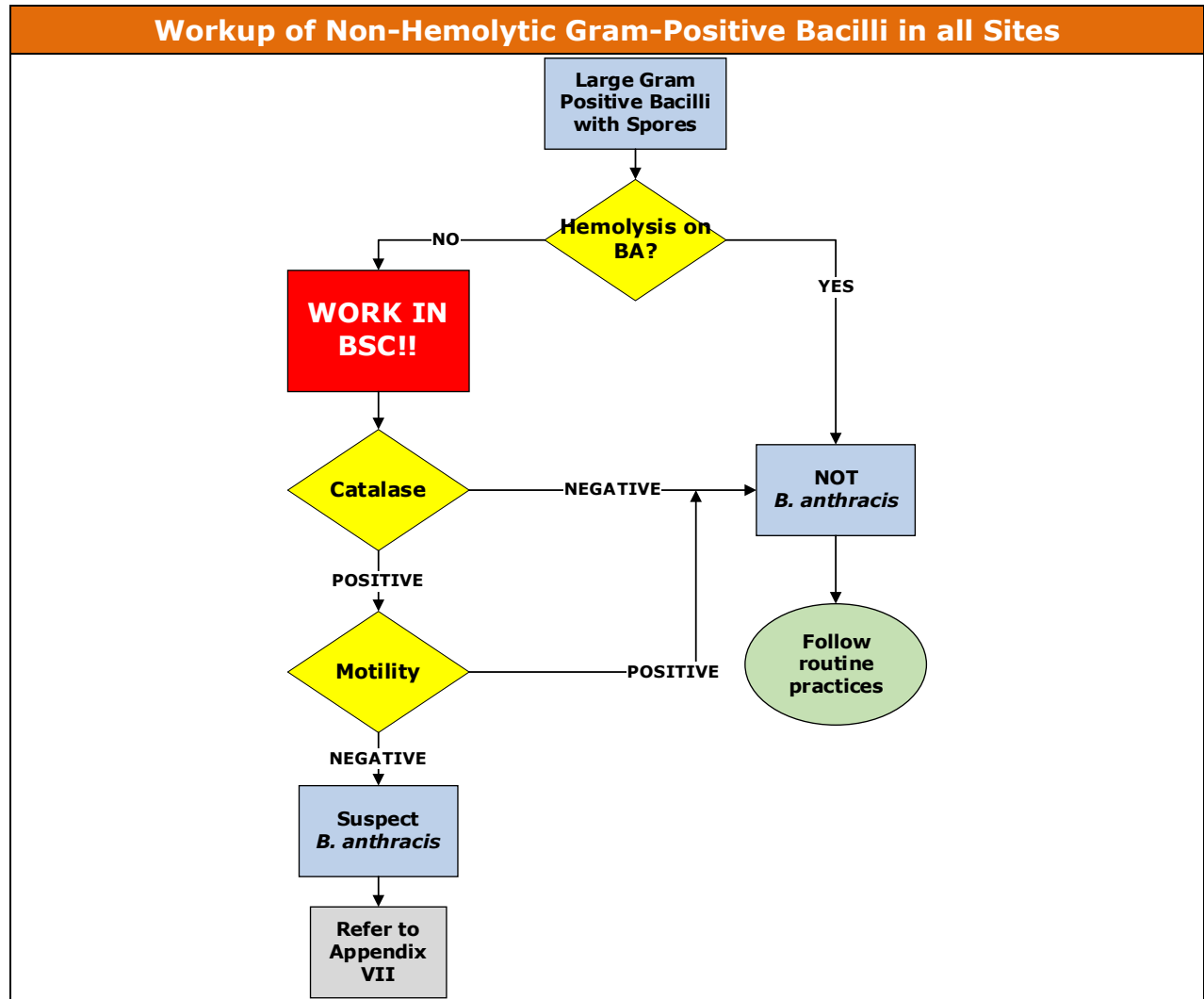
Step	Action
<b>What to do if a high biosafety risk organism is suspected?</b>	
<b>1</b>	<u>If gram stain result from any sterile site is small, gram-negative bacilli or coccobacilli:</u> <ul style="list-style-type: none"> <li>➤ Add the Potential High Biosafety Risk Organism label to all media</li> <li>➤ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out</li> <li>➤ All workup should be done in the BSC until high risk organisms are excluded</li> </ul>
<b>2</b>	<u>If gram stain result from any sterile site is gram-negative diplococci:</u> <ul style="list-style-type: none"> <li>➤ Add the Potential High Biosafety Risk Organism label to all media</li> <li>➤ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out</li> <li>➤ All workup should be done in the BSC until high risk organisms are excluded</li> </ul>
<b>3</b>	<u>If culture result from any site is slow-growing, gram-negative bacilli/coccobacilli:</u> <ul style="list-style-type: none"> <li>➤ Add the Potential High Biosafety Risk Organism label to all media</li> <li>➤ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out</li> <li>➤ All workup should be done in the BSC until high risk organisms are excluded</li> </ul>
<b>4</b>	<u>If culture result from any site is rapid-growing, non-hemolytic, large spore-forming gram-positive bacilli:</u> <ul style="list-style-type: none"> <li>➤ Add the Potential High Biosafety Risk Organism label to all media</li> <li>➤ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out</li> <li>➤ All workup should be done in the BSC until high risk organisms are excluded</li> </ul>
<b>5</b>	Notify the Technical Supervisor, Microbiology or designate if any of the above are encountered.
<b>6</b>	Ensure all plates are labelled with the precaution label and are sealed with parafilm or tape.
<b>7</b>	Any further handling of sealed plates must be done in the BSC with a N95 mask and gloves until growth is determined to not be high risk.
<b>8</b>	If suspicious growth is observed, proceed as per below.

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## INTERPRETATION OF RESULTS:



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## REPORTING INSTRUCTIONS:

IF	REPORT
<p>Slow growing gram-negative bacilli where RG3 organism is suspected</p>	<ul style="list-style-type: none"> <li>• Contact the APL microbiologist immediately at (825) 394-1835</li> <li>• If the APL microbiologist confirms that the organism is a potential Risk Group 3 (RG3) agent:                         <ul style="list-style-type: none"> <li>➢ Notify the Biological Safety Officer or alternate immediately by email</li> <li>➢ Notify the APL Advisor, Contracts and Consulting Sandra Lemke by email at Sandra.Lemke@albertaprecisionlabs.ca</li> <li>➢ Phone results to ordering location</li> <li>➢ Phone results to OCPHO (HPU1) and send a copy of the report</li> <li>➢ Phone the APL RG3 laboratory at (780) 407-7680 to notify them of the incoming shipment and provide details of its contents</li> <li>➢ Copy results to STHIPAC so they are aware that this organism was isolated in the STH microbiology lab</li> </ul> </li> <li>• Report: "<b>Gram negative bacilli/coccobacilli</b>"</li> <li>• List quantitation as "<b>Isolated</b>"</li> <li>• Add isolate comment <b>&amp;REF2</b></li> <li>• Do NOT freeze the isolate</li> <li>• Package isolate as per TDG CAT A regulations. Refer to MIC36200-Referral of Category A Specimens to APL</li> <li>• Ensure that all media that is not forwarded to APL is autoclaved along with the patient swab and any other supplies that were used during the testing process</li> </ul>

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IF	REPORT
<p><i>Neisseria meningitidis</i> isolated from sterile site</p>	<ul style="list-style-type: none"> <li>• Contact the APL microbiologist immediately at 825-394-1835</li> <li>• If the APL microbiologist confirms organism is a potential RG3 organism:                         <ul style="list-style-type: none"> <li>➢ Notify the Biological Safety Officer or alternate immediately by email</li> <li>➢ Notify the APL Advisor, Contracts and Consulting Sandra Lemke by email at Sandra.Lemke@albertaprecisionlabs.ca</li> <li>➢ Phone results to ordering locations</li> <li>➢ Phone results to OCPHO (HPU1) and send a copy of the report</li> <li>➢ Phone the APL RG3 laboratory at to (780) 407-7680 to make them aware that the shipment is coming to them and provide details of what is being sent</li> <li>➢ Copy results to STHIPAC so they are aware that this organism was isolated in the STH microbiology lab</li> </ul> </li> <li>• Report: <b>"Neisseria meningitidis"</b></li> <li>• List quantitation as <b>"Isolated"</b></li> <li>• Add isolate comment <b>&amp;Ref5</b></li> <li>• Do NOT freeze the isolate</li> <li>• Package isolate as per TDG CAT A regulations. Refer to MIC36200-Referral of Category A Specimens to APL</li> <li>• Ensure that all media that is not forwarded to APL is autoclaved along with the patient swab and any other supplies that were used during the testing process</li> </ul>

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IF	REPORT
<p>Non-hemolytic, non-motile, large gram-positive bacilli isolated from any site</p>	<ul style="list-style-type: none"> <li>• Contact the APL microbiologist immediately at 825-394-1835</li> <li>• If the APL microbiologist confirms organism is a potential RG3 organism:                         <ul style="list-style-type: none"> <li>➢ Notify the Biological Safety Officer or alternate immediately by email</li> <li>➢ Notify the APL Advisor, Contracts and Consulting Sandra Lemke by email at Sandra.Lemke@albertaprecisionlabs.ca</li> <li>➢ Phone results to ordering locations</li> <li>➢ Phone results to OCPHO (HPU1) and send a copy of the report</li> <li>➢ Phone the APL RG3 laboratory at to (780) 407-7680 to make them aware that the shipment is coming to them and provide details of what is being sent</li> <li>➢ Copy results to STHIPAC so they are aware that this organism was isolated in the STH microbiology lab</li> </ul> </li> <li>• Report: "<b>Bacillus species</b>"</li> <li>• List quantitation as "<b>Isolated</b>"</li> <li>• Add isolate comment <b>&amp;REF2</b></li> <li>• Do NOT freeze the isolate</li> <li>• Package isolate as per TDG CAT A regulations. Refer to MIC36200-Referral of Category A Specimens to APL</li> <li>• Ensure that all media that is not forwarded to APL is autoclaved along with the patient swab and any other supplies that were used during the testing process</li> </ul>

#### CROSS REFERECES:

NA

#### REFERENCES:

1. Clinical Microbiology Procedures Handbook, 4<sup>th</sup> edition, ASM Press, 2016
2. Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11<sup>th</sup> edition, ASM Press, Washington, D.C.
3. CLSI. *Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition*. CLSI document M35-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008

APPROVAL:

\_\_\_\_\_

Date

\_\_\_\_\_

Director, Laboratory and Diagnostic Imaging Services

REVISION HISTORY:

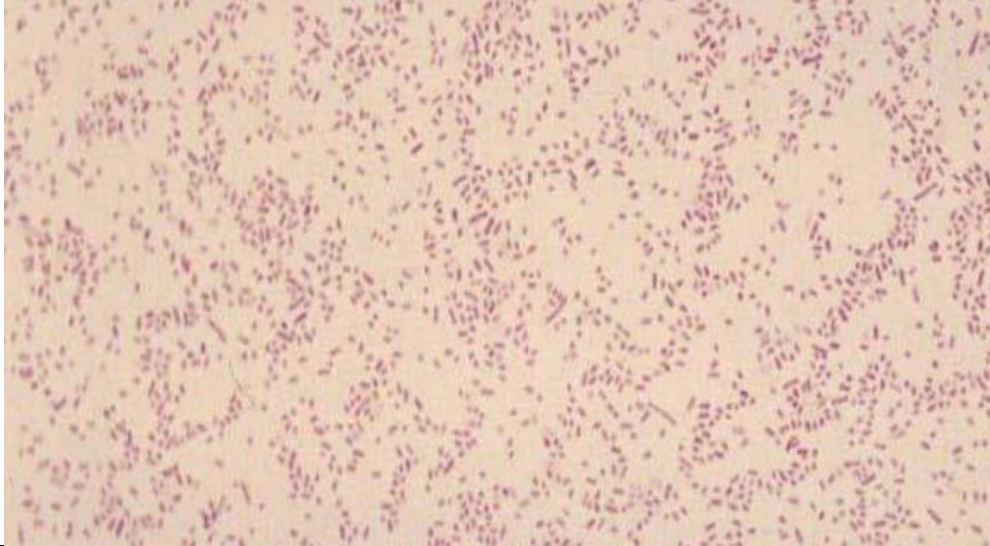
REVISION	DATE	Description of Change	REQUESTED BY
1.0	05 Nov 24	Initial Release	L. Steven

## APPENDIX:

### Appendix I-*Brucella* spp.

#### 1. Gram Stain:

- Tiny, faintly staining, gram-negative coccobacilli



#### 2. Culture:

- On BA, small (0.5 to 1.0 mm) glistening, non-hemolytic, non-pigmented colonies at 24 to 48 hours



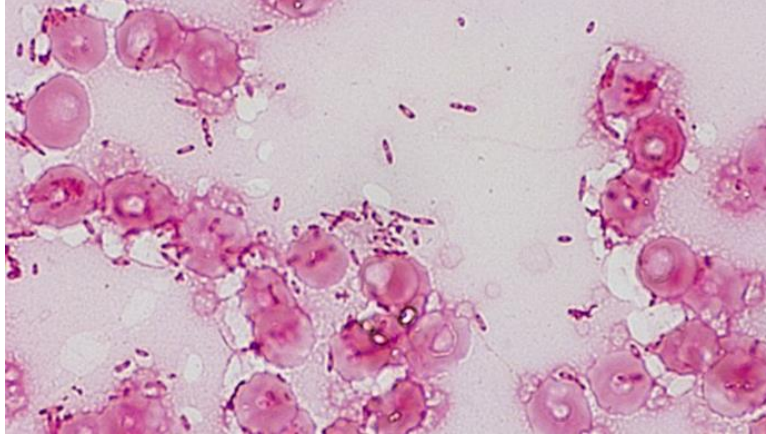
#### 3. Presumptive Identification:

- Gram stain is tiny, gram-negative coccobacilli
- Colonial morphology on BA
- No growth on MAC
- Oxidase positive
- Catalase positive
- Urea positive

## Appendix II-*Burkholderia pseudomallei*

### 1. Gram Stain:

- Small gram-negative bacilli with bipolar staining ("safety pin" appearance)



### 2. Culture:

- On BA, smooth, creamy, white colonies growing at 24 hours, may become wrinkled at 48 hours
- On MAC, variably lactose-fermenting or colorless colonies at 24 to 48 hours and colonies are wrinkled and have a metallic appearance



### 3. Presumptive Identification:

- Gram stain is small gram-negative bacilli
- Colonial morphology on BA
- Colonial morphology on MAC
- Colonies often produce a distinctive, musty or earthy odour that is very pronounced when opening the agar plate or even when opening the incubator

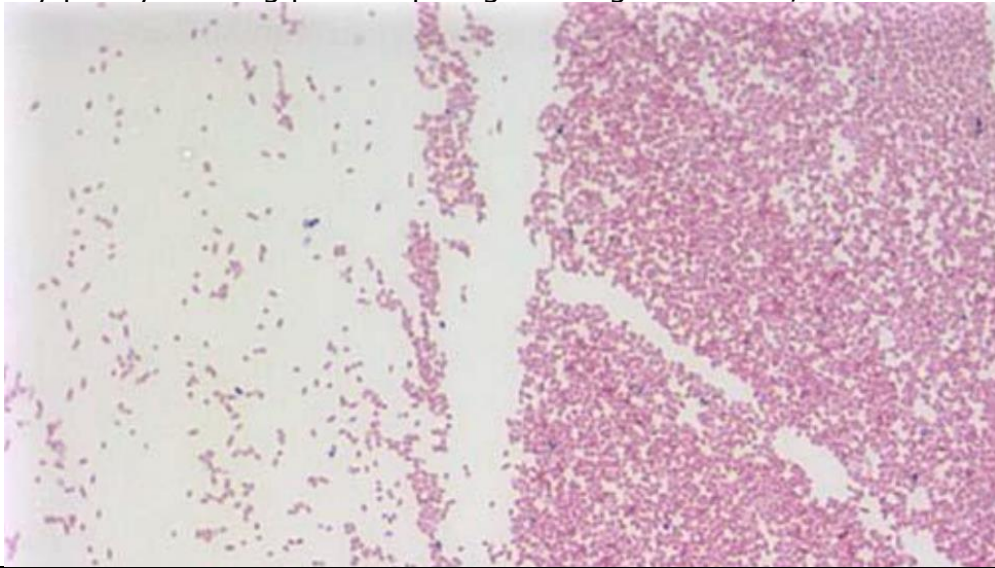
**NOTE:** Sniffing of plates containing *B. pseudomallei* is dangerous and should not be done. The odour will be present without sniffing

- Oxidase positive
- Catalase positive
- Spot indole negative

### Appendix III-*Francisella tularensis*

1. Gram Stain:

- Tiny poorly staining pleomorphic gram-negative bacilli/coccobacilli



2. Culture:

- On BA, non-hemolytic, grey-white colonies, 1 to 2 mm after 48 hours



3. Presumptive Identification:

- Gram stain is pleomorphic gram-negative bacilli/coccobacilli
- Colonial morphology on BA
- No growth on MAC
- Oxidase negative
- Catalase negative or weak positive
- Urea negative
- Satelliting negative

## Appendix IV-*Yersinia pestis*

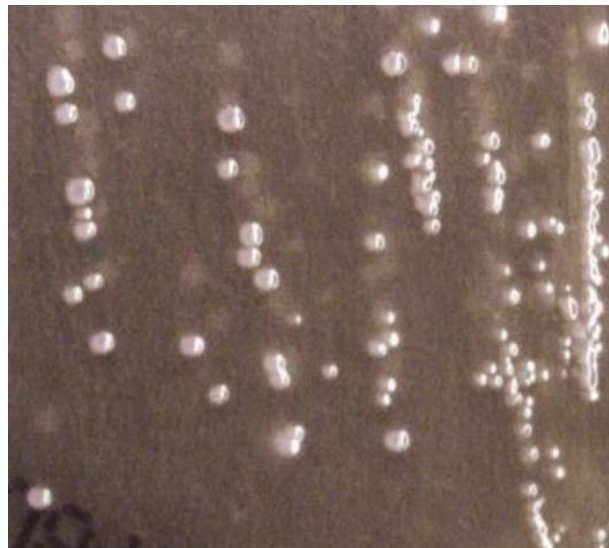
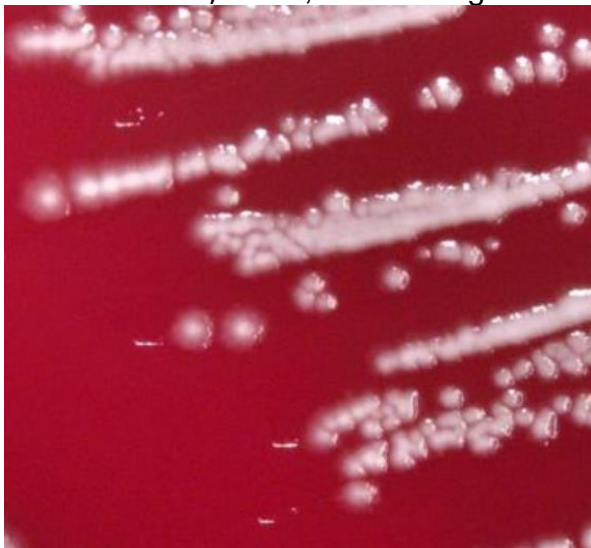
### 1. Gram Stain:

- Small gram-negative bacilli that are seen mostly in single cells or pairs and short chains



### 2. Culture:

- On BA, grey/white/ translucent colonies usually too small to see at 24 hours. At 48 hours, colonies are 1 to 2 mm in diameter, grey-white to slightly yellow and opaque
- On MAC, small, lactose negative colonies after 24 hours



### 3. Presumptive Identification:

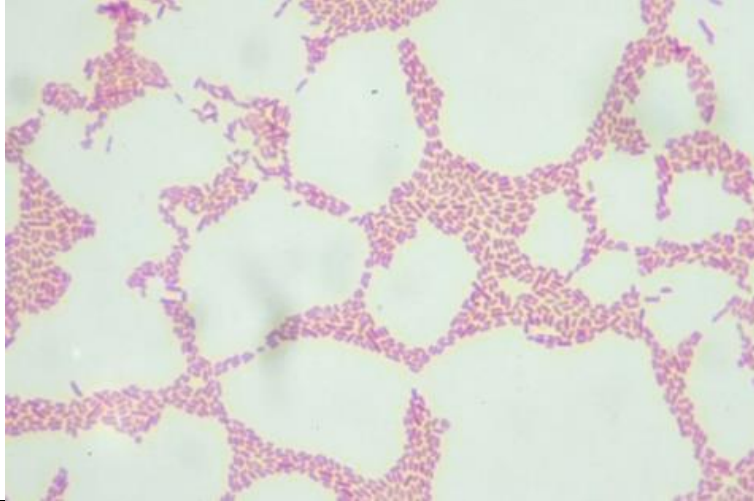
- Gram stain is small gram-negative bacilli
- Colonial morphology on BA
- Colonial morphology on MAC
- Oxidase negative
- Catalase positive
- Spot indole negative
- Urea negative

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## Appendix V- *Burkholderia mallei*

### 1. Gram Stain:

- Small, straight or slightly curved gram-negative coccobacilli arranged in pairs, bundles or the Chinese-letter form



### 2. Culture:

- On BA, smooth, grey, translucent colonies at 48 hours



### 3. Presumptive Identification:

- Gram stain is small, gram-negative coccobacilli
- Colonial morphology on BA
- Organism grows weakly or not at all in 48 hours on MAC
- Oxidase negative or variable
- Catalase positive
- Spot indole negative
- Motility negative

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## Appendix VI-*Neisseria meningitidis*

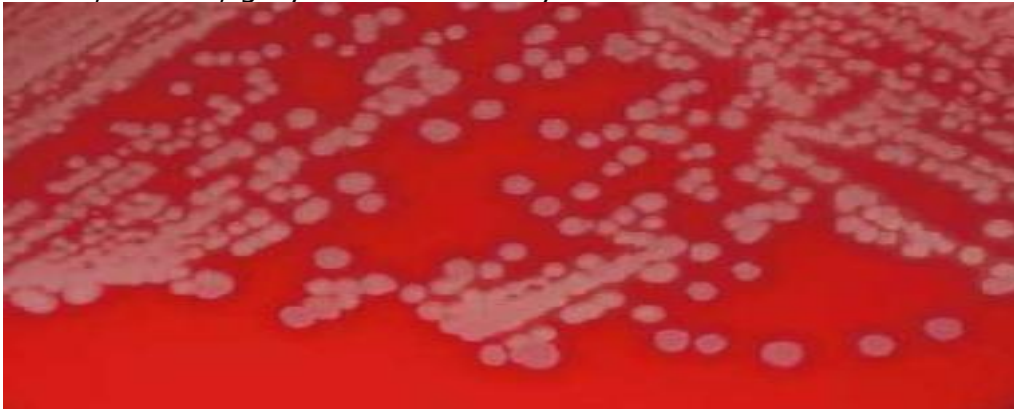
### 1. Gram Stain:

- Small gram-negative, non-spore forming, encapsulated diplococci which appear in kidney bean shape

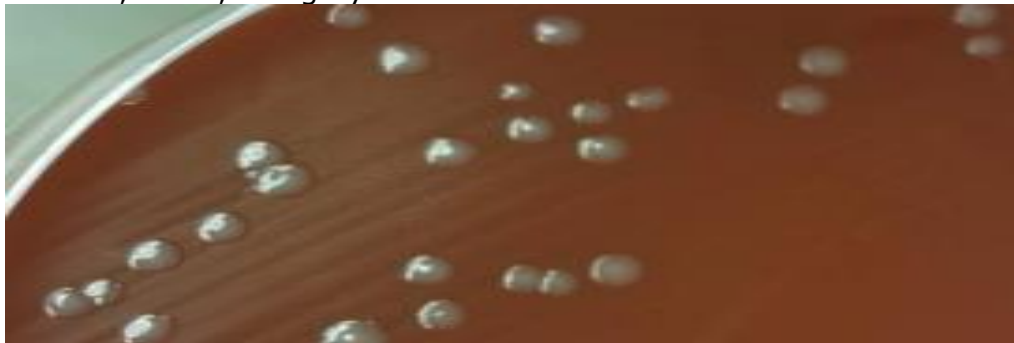


### 2. Culture:

- On BA, convex, grey and non-hemolytic



- On CHO, small, tan-grey smooth colonies



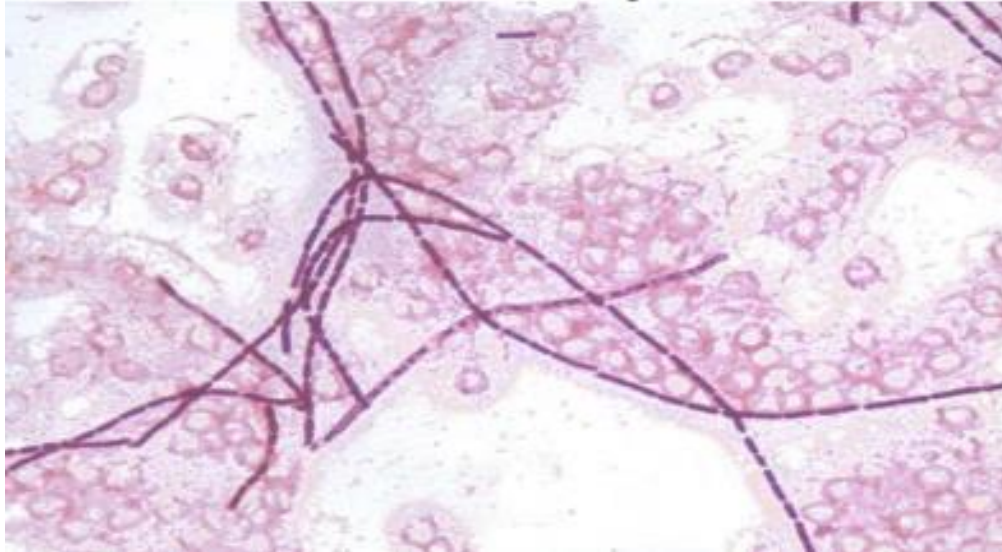
### 3. Presumptive Identification:

- Gram stain is small, gram-negative diplococci in kidney bean shape
- Colonial morphology on BA and CHOC
- No growth on MAC
- Oxidase positive
- Catalase positive

## Appendix VII-*Bacillus anthracis*

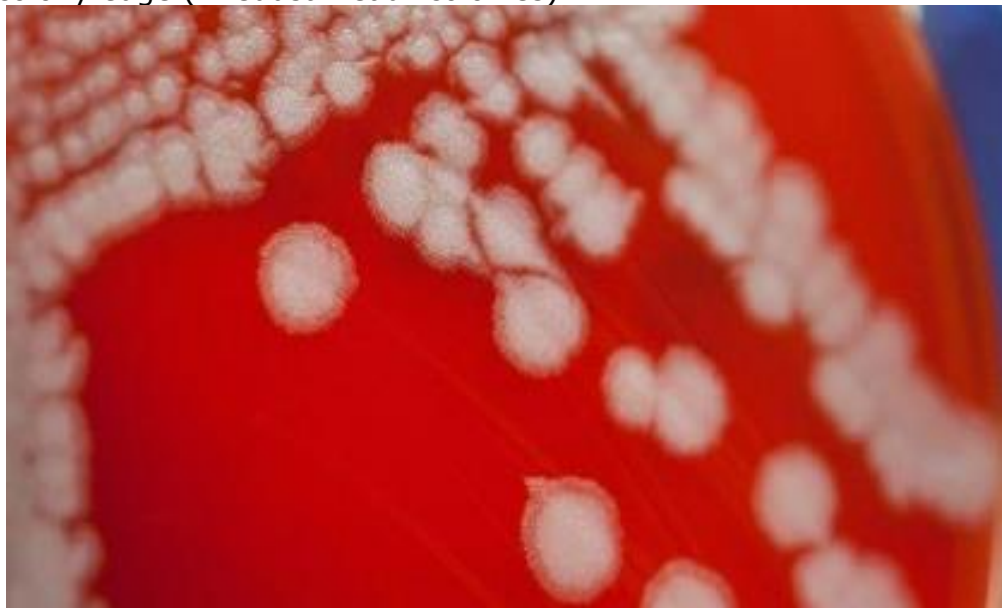
### 1. Gram Stain:

- Large encapsulated gram-positive bacilli in short chains. Can demonstrate clear zones (capsules) around bacilli



### 2. Culture:

- On BA, non-hemolytic, flat or slightly convex with ground-glass appearance. Colonies often exhibit comma-shaped protrusions from colony edge ("Medusa head" colonies)



### 3. Presumptive Identification:

- Gram stain is large, gram-positive bacilli, spores not normally observed
- Colonies on BA are non-hemolytic, ground-glass appearance
- No growth on MAC
- Catalase positive
- Motility negative