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

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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 pseuodomallei* or *Burkholderia mallei*

E. Culture results:

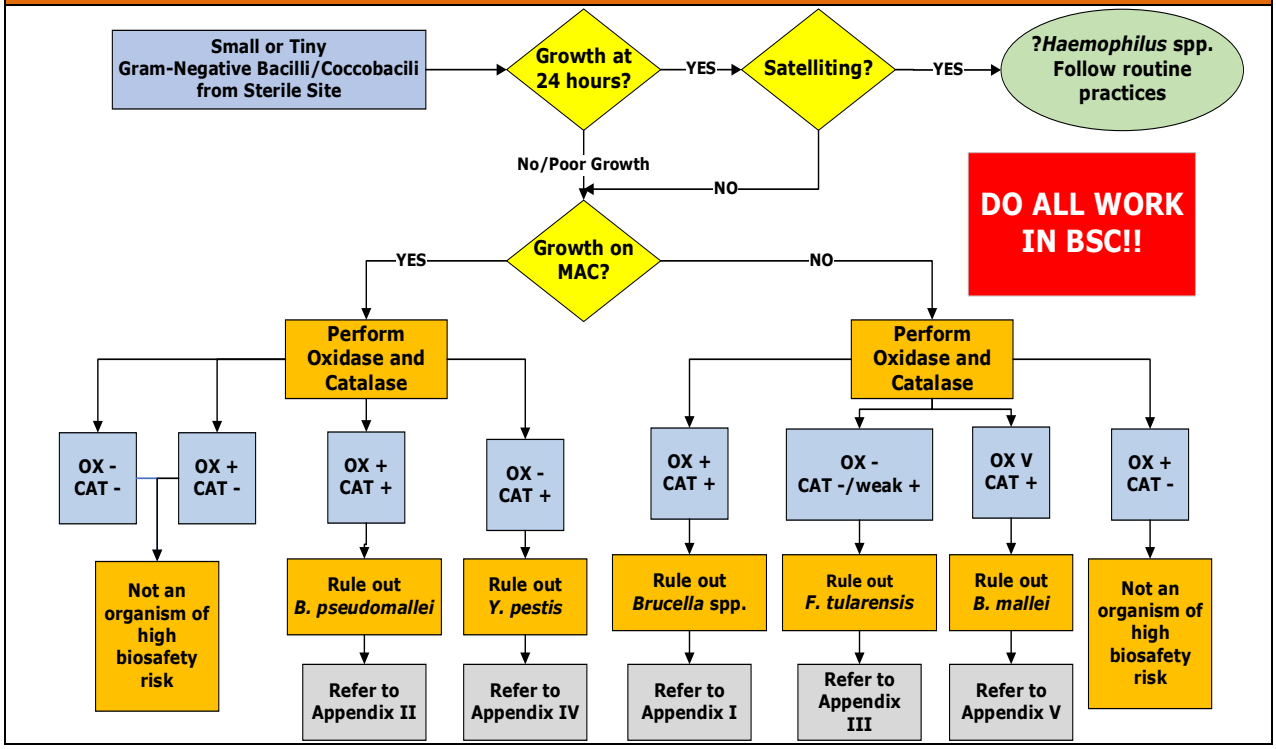
- Rapid-growing, non-hemolytic colonies with ground-glass appearance often exhibiting comma-shaped protrusions from colony edge ("Medusa head" colonies)
- **SUSPECT** *Bacillus anthracis*

PROCEDURE INSTRUCTIONS:

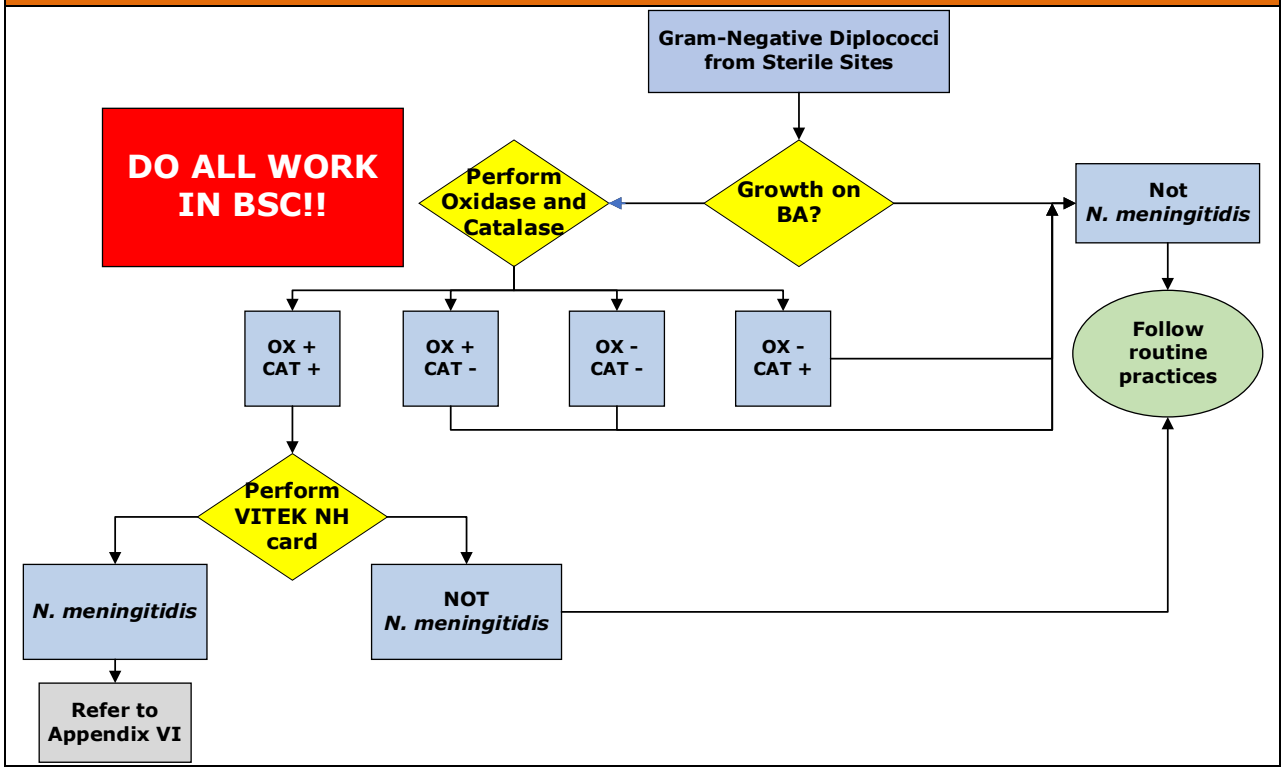
Step	Action
What to do if a high biosafety risk organism is suspected?	
1	If gram stain result from any sterile site is small, gram-negative bacilli or coccobacilli: ➢ Add the Potential High Biosafety Risk Organism label to all media ➢ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out ➢ All workup should be done in the BSC until high risk organisms are excluded
2	If gram stain result from any sterile site is gram-negative diplococci: ➢ Add the Potential High Biosafety Risk Organism label to all media ➢ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out ➢ All workup should be done in the BSC until high risk organisms are excluded
3	If culture result from any site is slow-growing, gram-negative bacilli/coccobacilli: ➢ Add the Potential High Biosafety Risk Organism label to all media ➢ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out ➢ All workup should be done in the BSC until high risk organisms are excluded
4	If culture result from any site is rapid-growing, non-hemolytic, large spore-forming gram-positive bacilli: ➢ Add the Potential High Biosafety Risk Organism label to all media ➢ Seal all plates with parafilm to ensure all workup is done in BSC until high risk organism is ruled out ➢ All workup should be done in the BSC until high risk organisms are excluded
5	Notify the Technical Supervisor, Microbiology or designate if any of the above are encountered.
6	Ensure all plates are labelled with the precaution label and are sealed with parafilm or tape.
7	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.
8	If suspicious growth is observed, proceed as per below.

INTERPRETATION OF RESULTS:

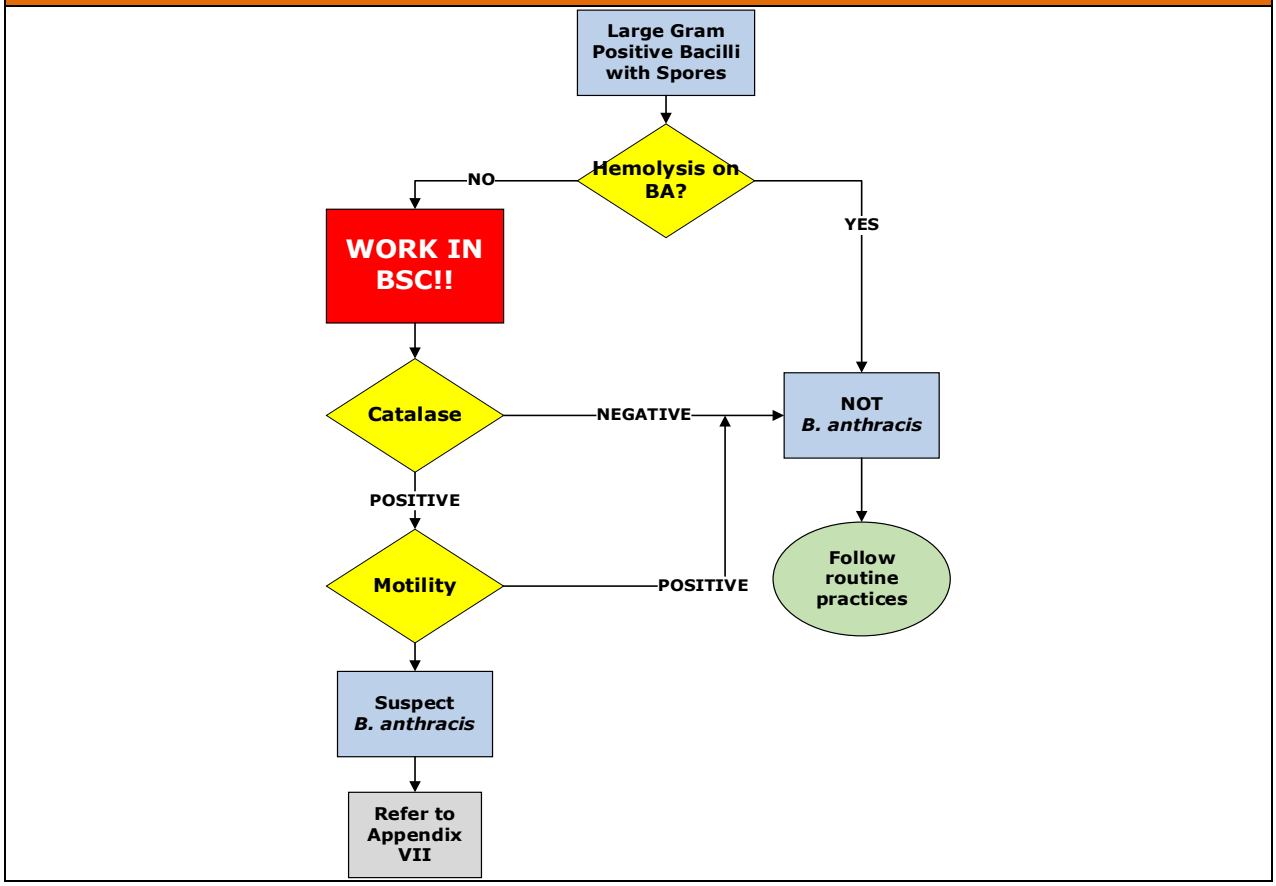
Workup of Small Gram-Negative Bacilli or Coccobacilli in Sterile Sites



Workup of Small Gram-Negative Diplococci in Sterile Sites



Workup of Non-Hemolytic Gram-Positive Bacilli in all Sites



REPORTING INSTRUCTIONS:

IF	REPORT
Slow growing gram-negative bacilli where RG3 organism is suspected	• Report: "**Gram negative bacilli/coccobacilli"** • List quantitation as **"Isolated"** • Add isolate comment **&REF2** • Contact the APL microbiologist immediately at 825-394-1835 • Notify the Technical Supervisor, Microbiology or designate and Biological Safety Officer immediately • Package isolate as per TDG CAT A regulations. Refer to MIC36200-Referral of Category A Specimens to APL
Neisseria meningitidis isolated from sterile site	• Report: **"Neisseria meningitidis"** • List quantitation as **"Isolated"** • Add isolate comment **&Ref5** • Contact the APL microbiologist immediately at 825-394-1835 • Notify the Technical Supervisor, Microbiology or designate and Biological Safety Officer immediately • Package isolate as per TDG CAT A regulations. Refer to MIC36200-Referral of Category A Specimens to APL

Non-hemolytic, non-motile, large gram-positive bacilli isolated from any site	• Report: "**Bacillus species"** • List quantitation as **"Isolated"** • Add isolate comment **&REF2** • Contact the APL microbiologist immediately at 825-394-1835 • Notify the Technical Supervisor, Microbiology or designate and Biological Safety Officer immediately • Package isolate as per TDG CAT A regulations. Refer to MIC36200-Referral of Category A Specimens to APL

CROSS REFERECES:
NA

REFERENCES:

1. Clinical Microbiology Procedures Handbook, 4th 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, 11th 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

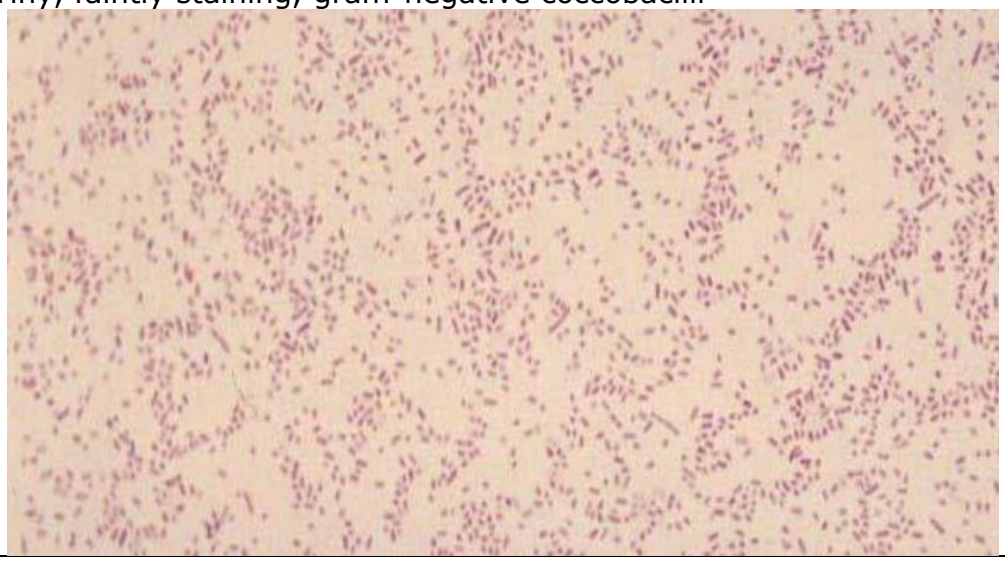
REVISION HISTORY:

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

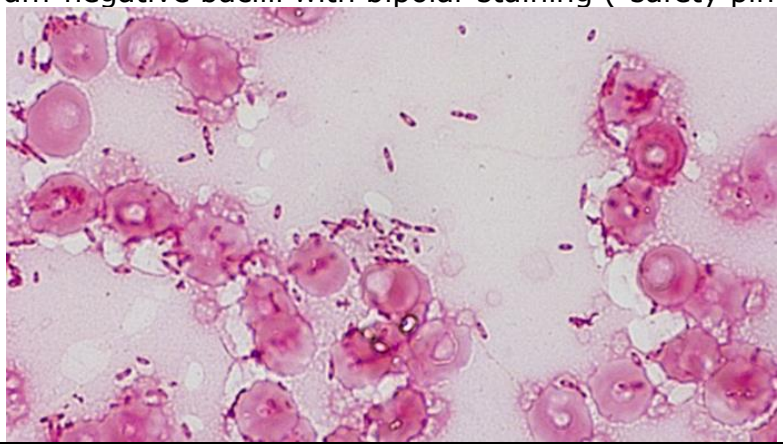


2. Culture:
   - On BA, small (0.5 to 1.0 mm) glistening, non-hemolytic, non-pigmented colonies at 24 to 48 hours

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

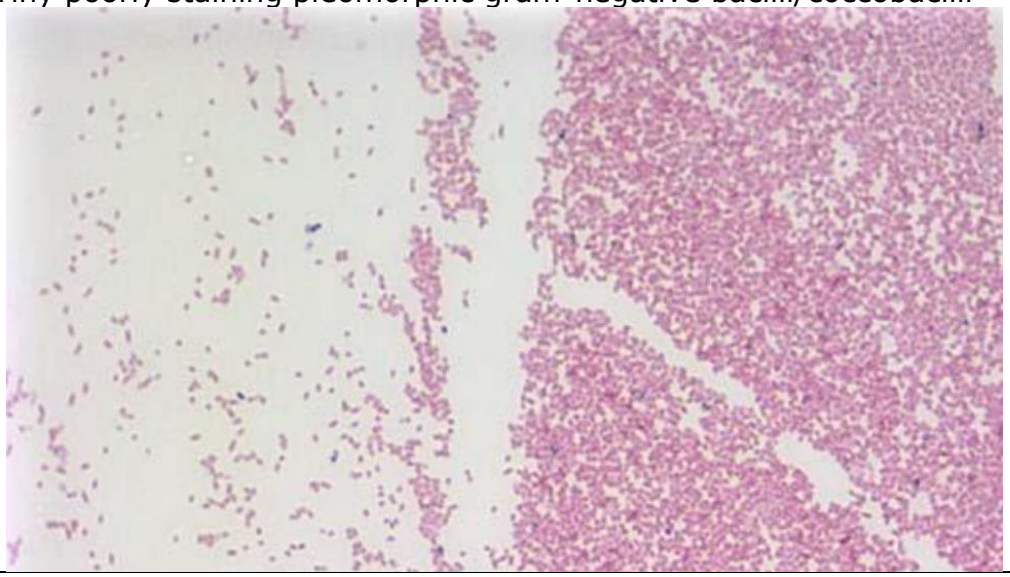
3. Presumptive Identification:
   - Gram stain is small gram-negative bacilli
   - Colonial morphology on BA
   - Colonial morphology on MAC
   - Colonies often produce a distinctive, musty or earthy odour that is very pronounced when opening the agar plate or even when opening the incubator

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

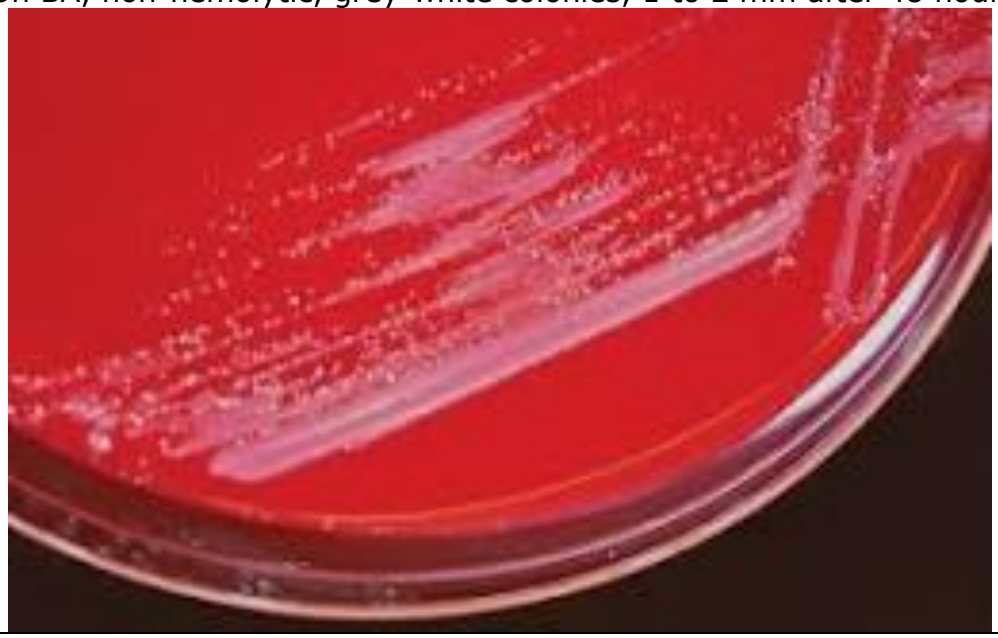
   - Oxidase positive
   - Catalase positive
   - Spot indole negative

Appendix III-*Francisella tularensis*

1. Gram Stain:
   - Tiny poorly staining pleomorphic gram-negative bacilli/coccobacilli



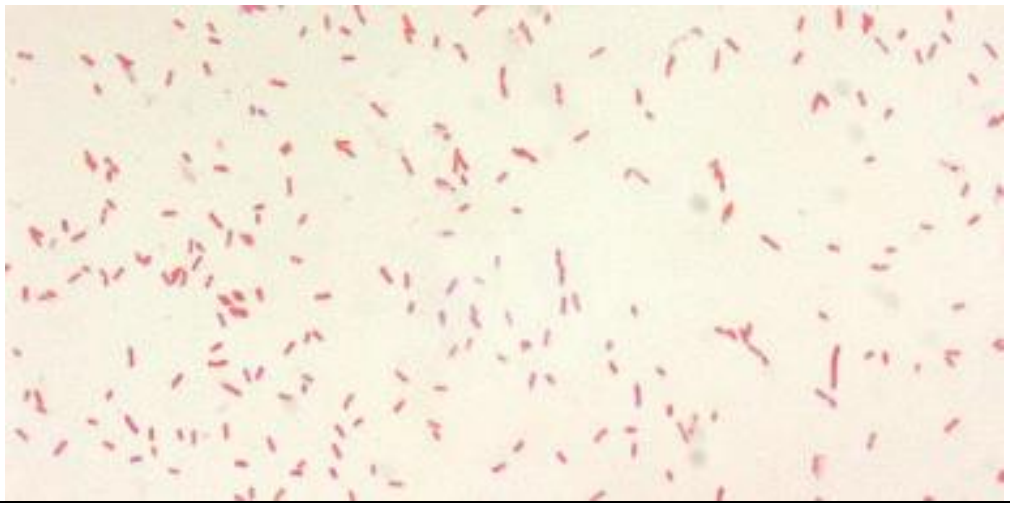
2. Culture:
   - On BA, non-hemolytic, grey-white colonies, 1 to 2 mm after 48 hours



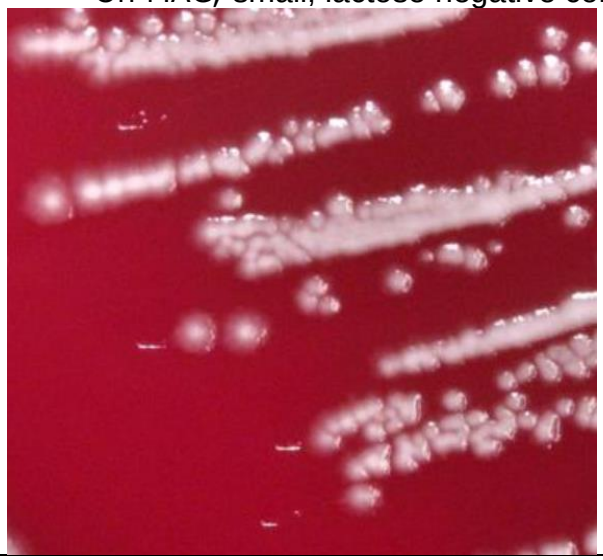
3. Presumptive Identification:
   - Gram stain is pleomorphic gram-negative bacilli/coccobacilli
   - Colonial morphology on BA
   - No growth on MAC
   - Oxidase negative
   - Catalase negative or weak positive
   - Urea negative
   - Satelliting negative

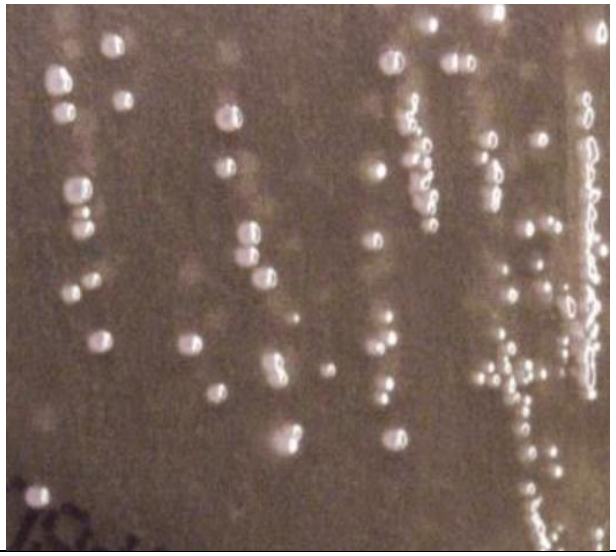
Appendix IV-*Yersinia pestis*

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

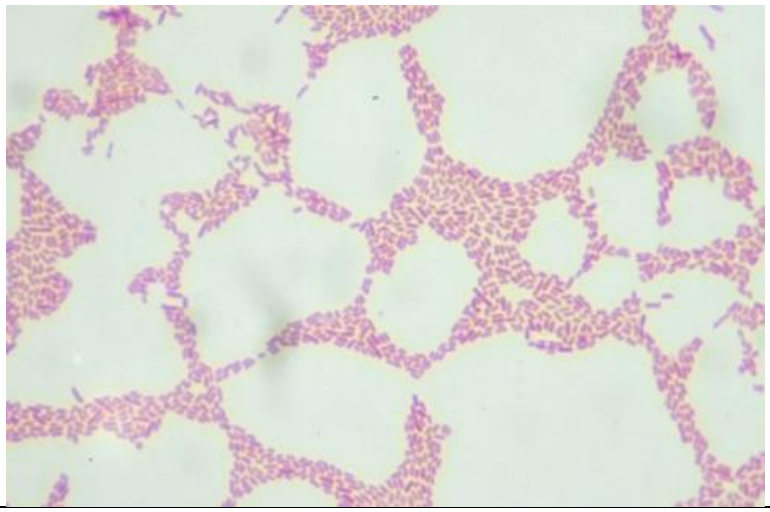




3. Presumptive Identification:
   - Gram stain is small gram-negative bacilli
   - Colonial morphology on BA
   - Colonial morphology on MAC
   - Oxidase negative
   - Catalase positive
   - Spot indole negative
   - Urea negative

Appendix V- *Burkholderia mallei*

1. Gram Stain:
   - Small, straight or slightly curved gram-negative coccobacilli arranged in pairs, bundles or the Chinese-letter form

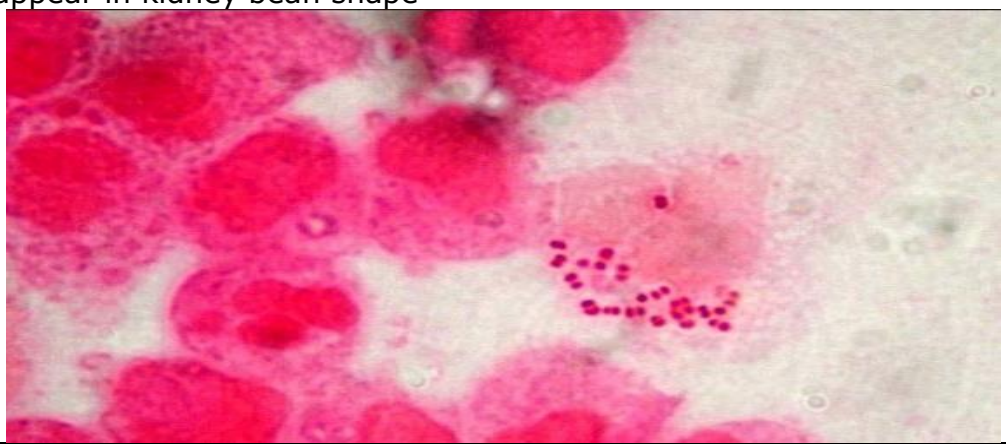


2. Culture:
   - On BA, smooth, grey, translucent colonies at 48 hours

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

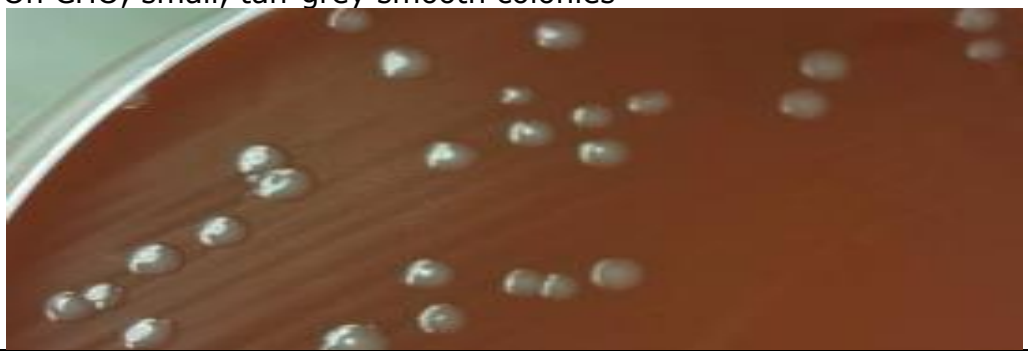
Appendix VI-*Neisseria meningitidis*

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

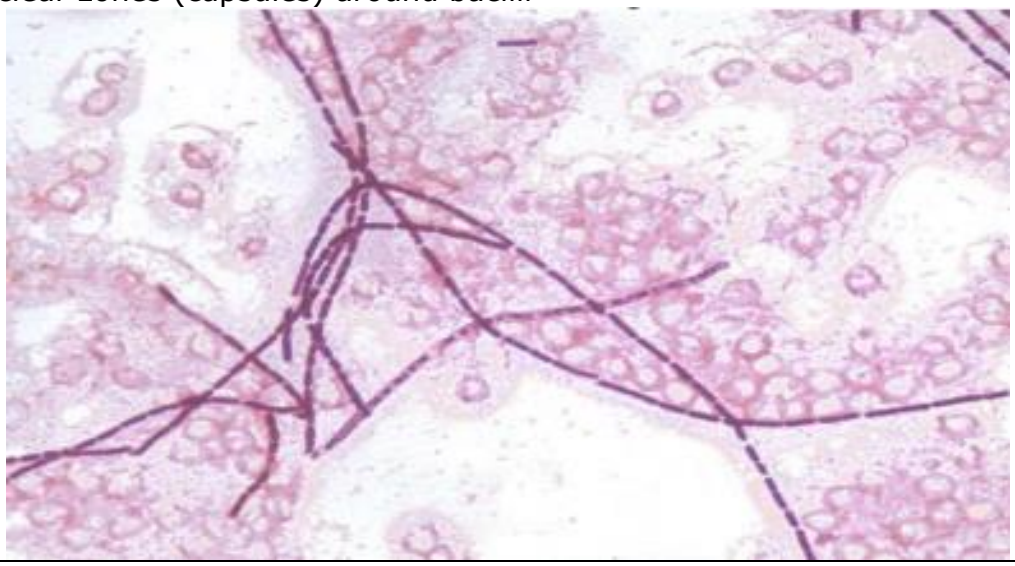
   - On CHO, small, tan-grey smooth colonies



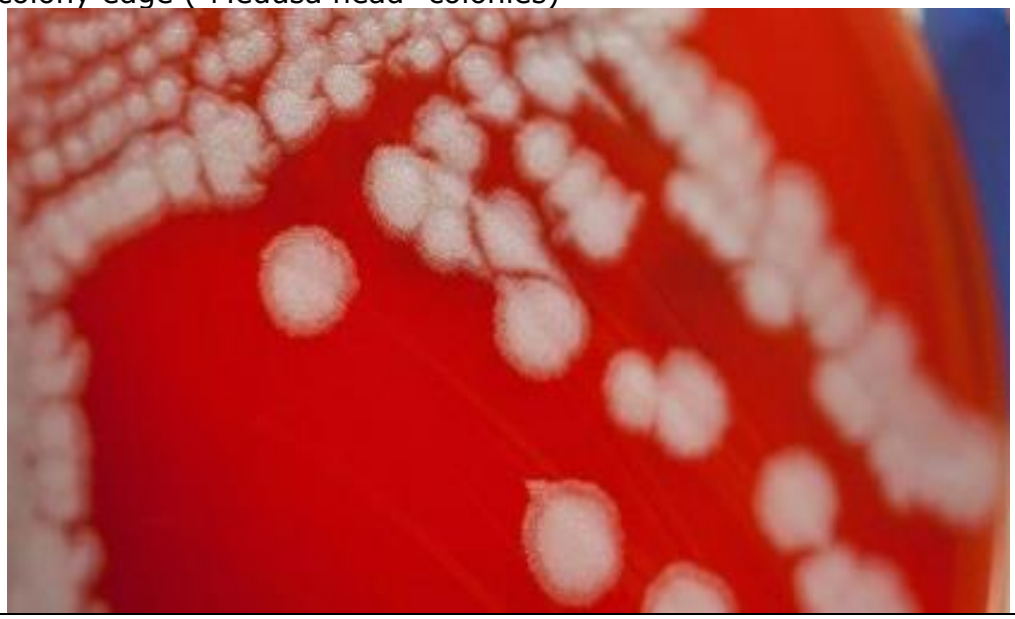
3. Presumptive Identification:
   - Gram stain is small, gram-negative diplococci in kidney bean shape
   - Colonial morphology on BA and CHOC
   - No growth on MAC
   - Oxidase positive
   - Catalase positive

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