

PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC40300 – Identification of Gram-Negative Bacilli	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	

**PURPOSE/RATIONALE:**

This standard operating procedure describes the workflow and identification scheme for gram-negative bacilli isolates from clinical microbiology specimens.

**SCOPE/APPLICABILITY:**

This procedure applies to Medical Laboratory Technologists (MLTs) performing gram-negative bacilli identification on clinical microbiology specimens.

**REAGENTS and/or MEDIA:**

- VITEK 2 ANC ID, NH ID and GN ID cards
- Identification reagents: oxidase, spot indole, API 20E, urea, etc.

**SUPPLIES:**

- 0.45% Saline
- Plastic VITEK tubes and caps
- Sterile swabs

**EQUIPMENT:**

- VITEK 2

**ENVIRONMENTAL CONTROLS:**

- Store VITEK 2 cards at 2°C to 10°C in unopened package liner
- Allow the card to come to room temperature before

**QUALITY CONTROL:**

- Refer to MIC60030-VITEK 2 Quality Control for VITEK 2 QC procedures
- Record all results on MIC60032-QC Results Record-VITEK 2

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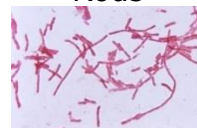
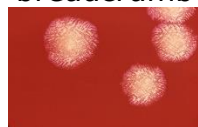





### Quick Identification Reference Chart for Common GNB Organisms:

Organism	Specimen Type	ID Tests Required
<i>E.coli</i>	Urine	<ul style="list-style-type: none"> <li>Pink colonies on UriSelect, SI (+)</li> </ul>
	All other specimen types including BC, CSF, BFC	<ul style="list-style-type: none"> <li>Perform VITEK GN card</li> <li><b>NOTE:</b> Subsequent BC bottles SI (+)</li> </ul>
<i>K. pneumoniae</i>	All specimens	<ul style="list-style-type: none"> <li>Perform VITEK GN card</li> </ul>
<i>P. aeruginosa</i>	All specimens	<ul style="list-style-type: none"> <li>Perform OX (+)</li> <li>Perform VITEK GN card</li> </ul>
<i>H. influenzae</i>	All specimens	<ul style="list-style-type: none"> <li>Perform gram (GNB, small)</li> <li>Perform catalase (+)</li> <li>Perform oxidase (+)</li> <li>Perform VITEK NH card</li> </ul>

### Minimal ID VS Full ID Reporting Names for GNB Organisms:

Organism	Minimal ID Name	Full ID Name
Anaerobic GNB	Gram Negative Bacilli Anaerobic	Genus and species
Aerobic GNB, LF	Gram Negative Bacilli LF	Genus and species
Aerobic GNB, NLF	Gram Negative Bacilli NLF	Genus and species

### IDENTIFICATION OF ANAEROBIC GRAM-NEGATIVE BACILLI:

Organism	Morphology on BRU	Gram	Indole	VITEK ID Card
<i>Bacteroides fragilis</i> grp.	Large, convex 	Rods 	Not done	ANC
<i>Fusobacterium nucleatum</i>	Opalescent, breadcrumb 	Fusiform, thin pointed 	+	ANC
<i>Porphyromonas</i> spp.	Smooth, shiny, black pigment 	Tiny coccobacilli 	+	ANC
<i>Prevotella</i> spp.	Convex, shiny, black pigment 	Tiny coccobacilli 	-	ANC

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### IDENTIFICATION OF AEROBIC GRAM-NEGATIVE BACILLI:

Step	Test	Result	Next Step
1	Growth	Aerobic	Growth on MAC -> Step 2
2	Growth on MAC	Yes	Fermentative (LF) GNB -> Table 1
		Yes	Non-fermentative (NLF) GNB -> Step 3
		No	Growth on BA -> Step 4
3	Oxidase	Negative	Refer to Table 1
		Positive	Refer to Table 2
4	Growth on BA	Yes	Refer to Table 3
		Poor	Refer to Table 4
		No	Refer to Table 5

**Table 1-Growth on MAC, LF or Oxidase Negative NLF GNB ID Table:**

Growth on MAC Oxidase negative	Indole	Motility	VITEK ID card
<i>Escherichia coli</i>	+	+	GN
<i>Klebsiella pneumoniae</i>	-	-	GN
<i>Klebsiella oxytoca</i>	+	-	GN
<i>Citrobacter</i> spp.	-	+	GN
<i>Enterobacter</i> spp.	-	+	GN
<i>Proteus mirabilis</i>	-	+	GN
<i>Proteus vulgaris</i>	+	+	GN
<i>Salmonella</i> spp.	-	+	GN
<i>Shigella</i> spp.	-	-	GN
<i>Stenotrophomonas maltophilia</i>	-	+	GN
<i>Yersinia</i> spp.	-	-	GN

**NOTE:** The VITEK 2 GN card can identify several highly pathogenic organisms including: *Brucella melitensis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Escherichia coli* O157, *Francisella tularensis*, and *Yersinia pestis*. Always use universal precautions. Refer to MIC40100-Suspect High Risk Organism Workup if Risk Group 3 organisms are identified on the VITEK 2

**NOTE:** For urine specimens on chromogenic agar, only SI needs to be performed to identify *E.coli* (SI+) and *P.mirabilis* (SI-) with correct colour of growth

**NOTE:** The API 20 E can be used to identify Enterobacterales if VITEK GN card does not give identification or is not available

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**Table 2-Growth on MAC, Oxidase Positive NLF GNB ID Table:**

Growth on MAC Oxidase positive	Indole	Motility	VITEK ID card
<i>Pseudomonas aeruginosa</i>	-	+	GN
<i>Chromobacterium violaceum</i>	-/+	+	GN
<i>Alcaligenes xylosoxidans</i>	-	+	GN
<i>Aeromonas hydrophila</i>	+	+	GN
<i>Plesiomonas shigelloides</i>	+	+	GN
<i>Vibrio</i> spp.	+	+	GN

***Pseudomonas aeruginosa:***

- Oxidase positive, typical smell (fruity/grapes), recognizable morphology (metallic or pearlescent, rough, pigmented or extremely mucoid), often strong  $\beta$ -hemolysis on blood agar

***Chromobacterium violaceum:***

- Colonies are distinctive smooth low convex with a dark violet metallic sheen (due to violacein production)

***Alcaligenes xylosoxidans:***

- Colonies are circular, flat to convex, smooth, and have an entire margin. The colonies tend to be colorless or greyish white

***Aeromonas hydrophila:***

- On blood agar forms circular colonies that are 1-3 mm in diameter that start off greyish in color due to beta-hemolysis and after three days become dark green

***Plesiomonas shigelloides:***

- Colonies are 1.5 mm in diameter, grey, shiny, smooth, opaque, and slightly raised in the center

***Vibrio* spp.:**

- Colonies are relatively large (about 3 mm in diameter), greyish white and glistening on blood agar. Some strains are beta hemolytic on blood agar

**Table 3-No Growth on MAC, Growth on BA GNB ID Table:**

NO growth on MAC Growth on BA	Catalase	Oxidase	Indole	Motility	VITEK ID card
<i>Pasteurella multocida</i>	+	+	+	-	GN
<i>Sphingomonas paucimobilis</i>	+	+	-	+ at RT	GN

***Pasteurella multocida*:**

- Colonies are small, gray, and non-hemolytic
- Gram: plump almost coccoid, bipolar staining, singly, pairs, short chains

***Sphingomonas paucimobilis*:**

- Older colonies positive for yellow (mustard) pigment
- Gram: medium to long, straight or slightly curved

**Table 4-No Growth on MAC, Poor Growth on BA GNB ID Table:**

No growth on MAC Poor growth on BA	Oxidase	Catalase	Indole	Gram	VITEK ID card
<i>Capnocytophaga</i> spp.	V	V	-	Slender rods	NH
<i>Cardiobacterium</i> spp.	+	-	+	Pleo, thin, bulbous	NH
<i>Eikenella</i> spp.	+	-	-	Small, slender straight	NH
<i>Kingella</i> spp.	+	-	-	Short, coccoid	NH

***Capnocytophaga* spp.:**

- After 48 hours, colonies are narrow, flat and smooth with spreading edges
- Colonies can be pigmented in orange or pink but when they are removed from the agar plate, they are always yellow in appearance

***Cardiobacterium* spp.:**

- Colonies attain a diameter of approximately 1 mm after 48 hours
- Colonies are circular, smooth and opaque and may pit the agar

***Eikenella corrodens*:**

- Often first recognized on chocolate agar where it tends to form flat spreading edges that extend out from the edge of the colonies. On blood agar, colonies are non-hemolytic, pit or adhere to the agar, and give off a distinct odor of bleach when the plate is first opened

***Kingella kingae*:**

- Early growth can be confused with beta-hemolytic *Streptococci*, but *Streptococci* are not oxidase positive

**Table 5-No Growth on MAC, No Growth on BA GNB ID Table:**

No growth on MAC No growth on BA	Catalase	Oxidase	Gram	VITEK ID card
<i>Haemophilus influenzae</i>	+	+	Small coccobacilli	NH
<i>Aggregatibacter aphrophilus</i>	-	+	Small coccobacilli	NH
<i>Francisella tularensis</i>	w+	-	Tiny coccobacilli	GN
<i>Brucella</i> spp.	+	+	Tiny coccobacilli	None

***Haemophilus influenzae*:**

- Can be confused with *Francisella tularensis* and *Brucella*: Growth on blood agar only around *Staphylococcus* separates *Haemophilus* from *Francisella* and *Brucella*

***Francisella tularensis*:**

- Is a category A potential bioterrorism agent
- Refer to MIC40100-Suspect High Risk Organism Workup if Risk Group 3 organisms are suspected
- Gram stain is tiny, poorly staining, pleomorphic gram-negative coccobacilli
- May have poor growth on BA, tiny growth after 48 hours
- Catalase positive, oxidase negative and urea negative

***Brucella* spp.:**

- Is a category A potential bioterrorism agent
- Refer to MIC40100-Suspect High Risk Organism Workup if Risk Group 3 organisms are suspected
- Gram stain is tiny, faintly staining gram-negative coccobacilli
- May have poor growth on BA, tiny growth after 2 to 3 days
- Oxidase positive and urea positive

**LIMITATIONS:**

1. If identification is problematic and the isolate is clinically significant, refer isolate to APL for further identification and susceptibility testing (if required)
2. Refer the following to APL as applicable for further testing:
  - Unusual or uncommon isolates for confirmation
  - Potential agents of bioterrorism

**CROSS-REFERENCES:**

- MIC60030-VITEK 2 Quality Control
- MIC60032-QC Results Record-VITEK 2
- MIC40100-Suspect High Risk Organism Workup

## REFERENCES:

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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. bioMérieux. (2021-05). *VITEK 2 GN* package insert, 044066-05
4. bioMérieux. (2021-04). *VITEK 2 NH* package insert, 043902-04
5. bioMérieux. (2021-03). *VITEK 2 ANC* package insert, 043907-04
6. CLSI. *Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition*. CLSI document M35-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008

## APPROVAL:

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Date

## REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	20 Mar 19	Initial Release	L. Steven
2.0	08 Mar 21	Procedure reviewed	L. Steven
3.0	27 Feb 23	Procedure reviewed and added to NTHSSA policy template	L. Steven
4.0	31 Mar 25	Procedure reviewed	L. Steven

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