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	

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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

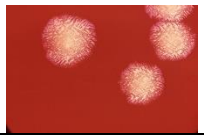
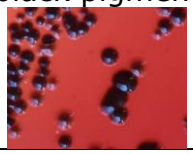
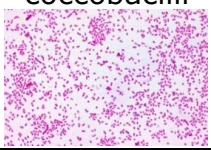
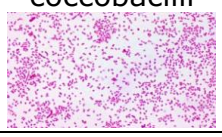
Quick Identification Reference Chart for Common GNB Organisms:

Organism	Specimen Type	ID Tests Required
E.coli	Urine	• Pink colonies on Uri*Select*, SI (+)
	All other specimen types including BC, CSF, BFC	• Perform VITEK GN card **NOTE:** Subsequent BC bottles SI (+)
K.pneumoniae	All specimens	• Perform VITEK GN card
P.aeruginosa	All specimens	• Perform OX (+) • Perform VITEK GN card
H.influenzae	BC, BFC, CSF, Deep Eye	• Perform gram (GNB, small) • Perform VITEK NH card
	All other specimen types	• Perform gram (GNB, small) • Perform satellite test (sat +) • Perform ALA (-)

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
Bacteroides fragilis grp.	Large, convex 	Rods 	Not done	ANC
Fusobacterium nucleatum	Opalescent, breadcrumb 	Fusiform, thin pointed 	+	ANC
Porphyromonas spp.	Smooth, shiny, black pigment 	Tiny coccobacilli 	+	ANC
Prevotella spp.	Convex, shiny, black pigment 	Tiny coccobacilli 	-	ANC

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
Escherichia coli	+	+	GN
Klebsiella pneumoniae	-	-	GN
Klebsiella oxytoca	+	-	GN
Citrobacter spp.	-	+	GN
Enterobacter spp.	-	+	GN
Proteus mirabilis	-	+	GN
Proteus vulgaris	+	+	GN
Stenotrophomonas maltophilia	-	+	GN
Yersinia spp.	-	-	GN

NOTE: The VITEK 2 GN card can identify several highly pathogenic organisms including: *Brucella melitensis, Burholderia 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

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

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

***Pseudomonas aeruginosa*:**

- Oxidase positive, typical smell (fruity/grapes), recognizable morphology (metallic or pearlescent, rough, pigmented or extremely mucoid), often strong β-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 grayish white

***Aeromonas hydrophilia*:**

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

***Plesiomonas shigelloides*:**

- Colonies are 1.5 mm in diameter, gray, 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
Pasteurella multocida	+	+	+	-	GN
Sphingomonas paucimobilis	+	+	-	+ at RT	GN

***Pasteurella multocida*:**

- 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
Capnocytophaga spp.	V	V	-	Slender rods	NH
Cardiobacterium spp.	+	-	+	Pleo, thin, bulbous ends	NH
Eikenella spp.	+	-	-	Small, slender straight	NH
Kingella 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	Sat.	ALA	Oxidase	Gram	VITEK ID card
Haemophilus influenzae	+	-	Varies	Small coccobacilli	NH
Aggregatibacter aphrophilus	+	+	Varies	Small coccobacilli	NH
Francisella tularensis		-	-	Tiny coccobacilli	GN
Brucella spp.		N/A	+	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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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

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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