PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC40400 – Identification of Gram-Negative Cocci	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 cocci isolates from clinical microbiology specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) performing gram-negative cocci identification on clinical microbiology specimens.

REAGENTS and/or MEDIA:
- VITEK 2 GN ID card
- VITEK 2 NH ID card
- Identification reagents: oxidase, spot indole, API NH, etc.

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

EQUIPMENT:
- VITEK 2

QUALITY CONTROL:
- Refer to MIC60030-VITEK 2 Quality Control for VITEK 2 QC procedures
- Record all results on MIC60032-QC Results Record-VITEK 2
- Refer to Test Manual for reagent quality control procedures

Quick Identification Reference Chart for Common GNC Organisms:

Organism	Specimen Type	ID Tests Required
Moraxella catarrhalis	BC, BFC, CSF, Deep Eye	• Gram (GNDC) • Catalase (+) • Oxidase (+) • Perform VITEK NH card
	All other specimen types	• Gram (GNDC) • Catalase (+) • Oxidase (+) • Catarrhalis disk (+)
Neisseria gonorrhoeae	All specimens	• No growth on BA • Gram (GNDC) • Catalase (+) • Oxidase (+) • Perform VITEK NH card **AND** API NH
Neisseria meningitidis	All specimens	• No growth on TM • Growth on BA • Gram (GNDC) • Catalase (+) • Oxidase (+) • Perform VITEK NH card

IDENTIFICATION OF ANAEROBIC GRAM-NEGATIVE COCCI:

Organism	Morphology on BRU	Gram	Indole	VITEK ID Card
Veillonella spp.	Colonies fluoresce brick red	Tiny, diplococci	-	ANC

IDENTIFICATION OF AEROBIC GRAM-NEGATIVE COCCI:

Step	Test	Result	Organism	Next Step
1	Growth	Aerobic		Oxidase
2	Oxidase	-	• *Acinetobacter* spp.	Refer to Table 1
		+	• *Neisseria* spp. • *Moraxella catarrhalis*	Refer to Table 2

Table 1-Oxidase Negative GNC ID Table:

Growth on MAC Oxidase negative	Type of growth on MAC	Catalase	VITEK ID card
Acinetobacter spp.	NLF	+	GN

Acinetobacter spp.:

- The negative oxidase test is important for rapid presumptive identification to differentiate the genus *Acinetobacter* from other similar non-fermentative organisms

Table 2-Oxidase Positive GNC ID Table:

Test	*N.gonorrhoeae*	*N.meningitidis*	*M.catarrhalis*
Gram stain	Gram-negative diplococci		
Catalase	+	+	+
Oxidase	+	+	+
Growth on BA	No	Yes	Yes
Growth on TM	Usually yes	Usually no	No
API NH	Yes	No	No
VITEK NH	Yes	Yes	Yes, if sterile site
Catarrhalis disk	No	No	Yes, if non-sterile site

NOTE: If *Neisseria meningitidis* is suspected in a sterile site, perform all testing in the BSC. Refer to MIC40100-Suspect High Risk Organism Workup if *Neisseria meningitidis* from a sterile site is identified on the VITEK 2

***Neisseria cinerea*:**
- Growth on blood agar and Mueller-Hinton agar at 35°C but may produce colonies that resemble those of *Neisseria gonorrhoeae* on chocolate agar
- Susceptible to colistin, and usually does not grow on Thayer Martin

Neisseria gonorrhoeae:
- Identify with API NH **AND** VITEK NH card
- Send organism to Alberta Precision Labs for susceptibility testing
- Notify Health Protection Unit (HPU1)

Neisseria lactamica:
- Gram-negative diplococci, oxidase positive, greyish non-hemolytic colonies on blood agar

Neisseria meningitidis:
- Identify with VITEK NH
- Notify Health Protection Unit (HPU1) and IPAC if isolated from sterile site
- Send isolates from sterile sites (e.g., CSF or blood) to Alberta Precision Labs for serotyping
- Send isolates from sterile sites (e.g., CSF or blood) to NML for the International Circumpolar Surveillance Program

***Neisseria meningitidis* isolates must be sent to APL immediately after identification is confirmed. Ensure there is a purity plate made that can be used for this purpose**

Moraxella catarrhalis:
- Characterized by a positive "hockey puck" test in which a plastic loop is used to gently nudge the colony across the agar: the entire colony should glide intact

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

CROSS-REFERENCES:

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

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. 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