PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC40500 – Identification of Gram-Positive 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-positive bacilli isolates from clinical microbiology specimens.

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

REAGENTS and/or MEDIA:
- VITEK 2 ANC ID card
- VITEK 2 GP ID card
- Identification reagents: oxidase, spot indole, API 20E, etc.

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

EQUIPMENT:
- VITEK 2 and supplies

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 GPB Organisms:

Organism	Specimen Type	ID Tests Required
Corynebacterium spp.	BC, BFC, CSF, Deep Eye	• Perform gram (diphtheroidal) • Catalase (+) • Perform VITEK ANC card
	All other specimen types	• Perform gram (diphtheroidal) • Catalase (+)
Bacillus spp.	BC, BFC, CSF, Deep Eye	• Perform gram (large rods) • Catalase (+) • Motility (+) • Refer to DL for full ID*
	All other specimen types	• Perform gram (large rods) • Catalase (+) • Motility (+)

*Bacillus spp. is a contaminant of blood cultures if growing in only 1 bottle in a series of sets and does not need to be referred to DL unless motility is negative

NOTE: *Bacillus* spp. that are motility (-) could potentially be *Bacillus anthracis*. Perform all testing in the BSC. Refer to MIC40100-Suspect High Risk Organism Workup if Risk Group 3 organisms are suspected

Minimal ID VS Full ID Reporting Names for GPB Organisms:

Organism	Minimal ID Name	Full ID Name
Corynebacterium spp.	*Corynebacterium* spp.	Genus and species
Bacillus spp. (motility +)	*Bacillus* species, not anthracis	Refer to APL

IDENTIFICATION OF ANAEROBIC GRAM-POSITIVE BACILLI:

Organism	Morphology on BRU	Gram	Indole	VITEK ID Card
Actinomyces spp.	"Molar tooth" 	Branching 	-	ANC
Clostridium perfringens	Double-zone hemolysis 	Large, box-shaped 	-	ANC
Propionibacterium acnes (Cutibacterium acnes)	Small opaque, enamel-white 	Diphtheroidalor branching 	+	ANC

IDENTIFICATION OF AEROBIC GRAM-POSITIVE BACILLI:

Step	Test	Result	Organism	Next Step
1	Growth	Aerobic, facultative		Catalase -> Step 2
2	Catalase	Positive		Refer to Table 1
		Negative		Refer to Table 2

Table 1-Catalase Positive GPB ID Table:

Catalase positive	Gram	Morphology	Motility	VITEK ID card
Listeria monocytogenes	Small rods	Narrow zone beta-hemolysis	Tumbling motility +	GP
Bacillus spp.	Large rods	Usually, beta hemolytic	+	Refer to APL
Bacillus anthracis	Large rods	Non-hemolytic	-	Refer to APL
Corynebacterium spp.	Diphtheroidal	Usually, dry colonies	-	ANC
NOTE: *C.urealyticum* is urea positive and will give a positive urea test in minutes				
Nocardia spp.	Beaded and branching	White or yellow or colonies	NA	Refer to DL

Table 2-Catalase Negative GPB ID Table:

Catalase negative	Gram	Morphology	Urea	VITEK ID card
Arcanobacterium spp.	Irregular gram positive bacilli	Beta hemolytic	NA	ANC
Erysipelothrix rhusiopathiae	Easily over decolorized, and often showing Gram-positive beading	Small, alpha-hemolytic, smooth colonies on BA	-	GP
Rothia dentocariosa	Pleomorphic coccobacilli that can form filamentous branches	Colonies are off-white and rough or smooth or 'spoke-wheel'	-	GP

NOTE: Blood cultures: If *Listeria, Bacillus anthracis* and *Corynebacterium jeikeium* are ruled out, AND isolate is a catalase positive, aerobic/facultative gram-positive diphtheroidal bacillus AND present in only one culture of several collected, report as *Corynebacterium* spp. with contamination comment

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:

- 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. (2021-04*). VITEK 2 GP* package insert, 043900-04
4. bioMérieux. (2021-03*). VITEK 2 ANC* package insert, 043907-04
5. 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