PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC40600 – Identification of Gram-Positive 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-positive cocci isolates from clinical microbiology specimens.

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

REAGENTS and/or MEDIA:
- VITEK 2 ANC ID and GP ID cards
- Identification reagents: catalase, Staph latex test, Strep latex test, 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

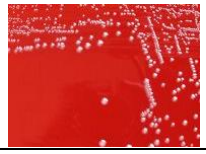
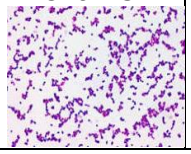
Quick Identification Reference Chart for Common GPC Organisms:

Organism	Specimen Type	ID Tests Required
S.aureus	Sterile sites	• Staphylococcus latex test (+) • Tube coagulase (+) **NOTE:** Subsequent BC bottles RS (+)
	Non-sterile sites	• Staphylococcus latex test (+)
CNST	Sterile sites	• Staphylococcus latex test (-) • Tube coagulase (-)
	Non-sterile sites	• Staphylococcus latex test (-)
S.pyogenes	Sterile sites	• Large colony, beta hemolytic on BA • Streptococcus latex test A (+) • PYR (+)
	Non-sterile sites	• Large colony, beta hemolytic on BA • Streptococcus latex test A (+)
S.pneumoniae	Sterile sites	• Perform VITEK 2 GP card
	Non-sterile sites	• TXP (>14 mm)
Streptococcus viridans grp.	Sterile sites	• Alpha hemolytic on BA • Gram (GPC) • Perform VITEK 2 GP card
	Non-sterile sites	• Alpha hemolytic on BA • TXP (<14 mm)
Enterococcus spp.	Sterile sites	• PYR (+) • Perform VITEK 2 GP card
	Non-sterile sites	• VITEK 2 GP card if full ID required • PYR if just minimal ID

Minimal ID VS Full ID Reporting Names for GPC Organisms:

Organism	Minimal ID Name	Full ID Name
Anaerobic GPC	Gram Positive Cocci Anaerobic	Genus and species from ANC
CNST	*Staphylococcus* spp. (coagulase negative)	Genus and species from GP
Viridans *Strep*.	*Streptococcus* viridans group	Genus and species from GP
Enterococcus spp.	*Enterococcus* spp.	Genus and species from GP

IDENTIFICATION OF ANAEROBIC GRAM-POSITIVE COCCI:

Organism	Morphology on BRU	Gram	Catalase	Indole	VITEK ID Card
Peptostreptococcus spp.	Grey with off-white centers 	Pairs or chains 	-	-	ANC

IDENTIFICATION OF AEROBIC GRAM-POSITIVE COCCI:

Step	Test	Result	Next Step
1	Growth	Aerobic	Catalase -> Step 2
2	Catalase	Positive	Refer to Table 1
		Negative	Hemolysis -> Step 3
3	Hemolysis	Beta	Refer to Table 2
		Alpha	Refer to Table 3
		None	Refer to Table 4

Table 1-Catalase Positive GPC ID Table:

Catalase Positive	Staph. Latex	Tube Coag.	PYR	Oxidase	VITEK ID Card
Staphylococcus aureus	+	+	-	-	
CNST	-	-	+/-	-	GP
Staphylococcus lugdunensis	+/-	-	+	-	GP
Staphylococcus saphrophyticus	-	-	-	-	GP
Micrococcus spp.	-	-	-	+	GP

NOTE: Do not report species name of coagulase negative *Staphylococcus* from VITEK unless ID is >90%

***Staphylococcus intermedius*:**

- Staph. latex test +, tube coagulase – and PYR+
- Infrequent human pathogen, associated with animal contact
- Identify with VITEK 2 GP card if clinically significant

Staphylococcus lugdunensis:

- Typically clumping factor positive and tube coagulase negative. Using a positive Staph. latex test to rule out *S. lugdunensis* will miss a significant number of isolates. Using a Staph. latex test to rule in *Staphylococcus aureus* may misidentify *S.lugdunensis* as *S. aureus*
- The tube coagulase and PYR tests should be used to confirm *Staphylococcus aureus* from CNST from sterile sites or used if growth does not morphologically resemble *Staphylococcus aureus* but is Staph. latex test positive
- Identify with VITEK 2 GP card if clinically significant

***Micrococcus* spp.:**

- Strict aerobe
- Often (but not always) oxidase +

Table 2-Catalase Negative, Beta Hemolytic GPC ID Table:

Catalase Negative Beta hemolytic	Zone of Hemolysis	Strep. Latex	PYR	VITEK ID Card
Streptococcus pyogenes	>0.5 mm	A+	+	GP
Streptococcus agalactiae	>0.5 mm	B+	N/A	GP
Streptococcus GrpC/G	>0.5 mm	C+/G+	N/A	GP
Streptococcus anginosus	<0.5 mm	See note	N/A	GP

NOTE: The majority of strains are either serologically ungroupable or belong to Lancefield group F, although strains might possess the Lancefield group A, C, or G antigen

***Streptococcus agalactiae*:**

- Usually shows a narrow zone of β-hemolysis, but non-hemolytic strains exist

***Streptococcus anginosus* grp.:**

- Species within this group (*Streptococcus anginosus, Streptococcus constellatus* and *Streptococcus intermedius)* are normal flora of the oropharyngeal, urogenital and gastrointestinal tracts and are associated with abscess formation in the soft tissues, head and neck, brain and intraabdominal sites
- Colonies of *Streptococcus anginosus* group exhibit a strong smell of caramel, butterscotch, vanilla or burnt sugar
- They may carry Lancefield A, C, F, or G antigen. Strains positive for F antigen belong to the *Streptococcus anginosus* grp. (All Group F *Streptococci* are *anginosus* group, but not all *anginosus* group Streps. are F)
- Perform VITEK 2 GP card for identification

Table 3-Catalase Negative, Alpha Hemolytic GPC ID Table:

Catalase Negative Alpha hemolytic	Gram Stain Arrangement	TXP	PYR	VITEK ID Card
Streptococcus pneumoniae	GPC in pairs	S	N/A	GP
viridans *Streptococci*	GPC in chains	R	-	GP
Aerococcus urinae	GPC in tetrads/clusters	N/A	-/+	GP
Streptococcus bovis grp.	GPC in chains	N/A	-	GP

***Aerococcus* spp.:**

- Gram morphology: GPC in tetrads and clusters
- Colonial morphology: Like viridans *Streptococci*
- *Aerococcus urinae*: PYR negative, *Aerococcus viridans*: PYR positive
- Identify with VITEK 2 GP card

Streptococcus mutans:

- viridans *Streptococci*
- Gram morphology: From broth-GPC, short chains, from solid media, GPCB, diphtheroid-like
- Colonial morphology: Tiny, white, dry colonies which may adhere to and pit the agar. Non-hemolytic, may show yellowing under the growth on blood agar
- Biochemical characteristics: PYR negative and TXP resistant
- Identify with VITEK 2 GP card if indicated in applicable procedure

Streptococcus bovis:

- Extensive taxonomic changes, six species now recognized: *S.alactolyticus*, *S.equinus*, *S.gallolyticus* subsp *gallolyticus*, *S.gallolyticus* subsp *pasteurianus*, *S.infantarius* subsp *infantarius* and *S.infantarius* subsp *coli*

Table 4-Catalase Negative, Non-Hemolytic GPC ID Table:

Catalase Negative Non-hemolytic	Yellow Pigment	PYR	Ampicillin	VITEK ID Card
Enterococcus faecalis	-	+	S	GP
Enterococcus faecium	-	+	R	GP
Enterococcus gallinarum	-	+	S	GP
Enterococcus casseliflavus	+	+	S	GP

Other Catalase Negative GPC:

***Abiotrophia* and *Granulicatella*:**

- Formerly known as nutritionally variant *Streptococcus*
- Colonial morphology: No growth on BA or satellitic around *Staphylococcus aureus* on BA, growth on CHO
- Biochemical characteristics: PYR positive
- Identify with VITEK 2 GP card if indicated in applicable procedure

***Gemella* spp.:**

- Colonial morphology: Poor growth on BA and may require 48 hours to grow
- Often alpha hemolytic and resembling viridans *Streptococci*, but may be non-hemolytic
- Biochemical characteristics: PYR positive (with a very heavy inoculum)
- Identify with VITEK 2 GP card if indicated in applicable procedure

***Lactococcus* spp.:**

- GPC in chains
- Colonial morphology: Like *Enterococcus*
- Biochemical characteristics: PYR positive, susceptible to Ampicillin
- Identify with VITEK 2 GP card if indicated in applicable procedure

***Leuconostoc* and *Pediococcus*:**

- Biochemical characteristics: Catalase negative, PYR negative, Vancomycin resistant
- Identify with VITEK 2 GP card if indicated in applicable procedure

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:

- MIC60030-VITEK 2 Quality Control
- MIC60032-QC Results Record-VITEK 2

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, 11th edition, ASM Press, Washington, D.C.
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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