PURPOSE: To provide a workflow and identification scheme for Gram-positive cocci from clinical specimens.

IDENTIFICATION OF ANAEROBIC GRAM- POSITIVE COCCI:

Test	Result	Organism	Next step
Growth	Anaerobic	*Peptostreptococcus* (cocci in pairs / chains, small peaked circular colonies) *	Send to DynaLIFE if significant.

* Anaerobic Gram-positive cocci are usually either *Peptostreptococcus* species or a member of one of the newly named genera (*Peptoniphilus, Schleiferella, Anaerococcus, Finegoldia, Micromonas)*.

IDENTIFICATION OF AEROBIC GRAM-POSITIVE COCCI:

Test	Result	Organism	Next step
Growth	Aerobic		Catalase
Catalase	+	*Staphylococcus* *Micrococcus*	Staph latex POS, tube coag (if applicable), Vitek 2 AST-GP card. Staph latex NEG, tube coag (if applicable), Vitek 2 GP card, AST-GP card.

Procedure notes for catalase positive Gram-positive cocci:

- Oxacillin-resistant *Staphylococcus aureus*: Notify HPU1 and SOHS if inpatient.
- *Staphylococcus* coagulase negative: Report as coagulase staphylococcus unless Vitek >90% certain of ID.
- *Staphylococcus intermedius*: slide coagulase positive, tube coagulase negative, PYR positive, infrequent human pathogen, associated with animal contact, organism is in Vitek 2 database.
- *Micrococcus species*: strict aerobe, often (but not always) oxidase positive.

Test	Result	Organism	Next step
Growth	Aerobic		Catalase
Catalase	-	*Streptococcus* / *Enterococcus* / *Gemella* / *Lactococcus*, others	Hemolysis
Hemolysis	Beta		Strep grouping, PYR if sterile site.
	Alpha		Optochin, GPI card if sterile site.
	None		GPI card, GPS card
Strep grouping <u>Large colonies:</u> >0.5mm after 24h incubation <u>Small colonies:</u> <0.5mm after 24h incubation	A, large colonies	*Streptococcus pyogenes*	Refer to ASTM
	A, small cols, PYR+	*Streptococcus pyogenes*	Refer to ASTM
	A, small cols, PYR -	*Strep viridans* group	Refer to ASTM
	B	*Streptococcus agalactiae*	Refer to ASTM
	Large colonies, C or G	*Streptococcus dysgalactiae* subsp. *equisimilis*	Refer to ASTM
	Small colonies, C or F or G or ungroupable	*Streptococcus viridans group* (includes *anginosus* group)	Optochin, GPI card, Refer to ASTM
	D	*Enterococcus*	PYR +, GPI card, GPS card
		Streptococcus gallolyticus (formerly *bovis*, see below)	PYR -, GPI card, Refer to ASTM
Optochin	Susceptible	*Strep pneumoniae*	GPI card if sterile site Refer to ASTM
	Resistant	*Strep viridans* group	GPI card if sterile site Refer to ASTM
		Aerococcus urinae	GPC tetrads, GPI card

NOTE: viridans group *Streptococci* can be minimally identified with colonial morphology (alpha hemolytis), catalase test (negative) and gram stain (gram-positive cocci).

<u>Enterococcus identification: Gram-positive cocci in pairs (and chains), not clusters:</u>

Test	*E. faecalis*	*E. faecium*	*E. gallinarum*	*E. casseliflavus*	*Strep bovis*
Yellow pigment	-	-	-	+	n/a
PYR	+	+	+	+	-
Ampicillin	S	R	S	S	n/a

NOTE: VRE *Enterococcus faecalis* and *Enterococcus faecium*: Notify Health Protection Unit (HPU1) and Infection Control Nurse (SOHS) if in-patient.

***Abiotrophia* and *Granulicatella*:**
- Formerly known as nutritionally variant *Streptococcus.*
- No growth on BA or satellitic around *Staphylococcus aureus* on BA, growth on CHO, PYR positive.
- Identify with Vitek 2 GP card if clinically significant.
- Refer to ASTM for susceptibility testing requirements.

***Aerococcus* species:**
- Colonial appearance is like viridans *Streptococcus*.
- Identify with Vitek 2 GP card. GPC in tetrads and clusters, catalase negative, Van S.
- *Aerococcus urinae*: PYR negative, *Aerococcus viridans*: PYR positive.

***Gemella* species:**
- Poor growth on BA and may require 48 hours to grow. Often α-haemolytic and resembling viridans *Streptococc*i, but may be non-haemolytic.
- PYR positive (with a very heavy inoculum).
- In Gram-stained smear, *Gemella haemolysans* forms pairs with adjacent flattened sides and is more likely to overdecolourize, while *Gemella morbillorum* forms pairs and short chains and is more consistently Gram-positive.

***Lactococcus* species:**
- Colonial appearance is like *Enterococcus*.
- GPC in chains, PYR positive. Susceptible to Ampicillin.
- Identify with Vitek 2 GP card if clinically significant.

***Leuconostoc* and *Pediococcus*:**
- Catalase negative, PYR negative, Vancomycin resistant.
- Identify with Vitek 2 GP card if clinically significant.
- Refer to ASTM for susceptibility testing requirements.

***Streptococcus agalactiae*:**
- Usually shows a narrow zone of β-hemolysis, but non-hemolytic strains exist.
- CAMP positive.

***Streptococcus anginosus* group:**
- Species within this group (*Streptococcus anginosus, Streptococcus constellatus* and *Streptococcus intermedius)* are normal flora of the oropharyngeal, urogenital and gastrointestinal tracts. They are also strongly associated with abscess formation in the soft tissues, pleuropulmonary sites, head and neck, brain and intraabdominal sites.
- Identification to species level within this group, when isolated from sterile site, may guide diagnostic evaluation and help assess the need to search for occult abscesses.
- Colonies are <0.5mm, and α-, β-, or non-haemolytic. They may carry Lancefield A, C, F, or G antigen. Strains positive for F antigen belong to the *Streptococcus anginosus* group. (All Group F *Streptococci* are *anginosus* group, but not all *anginosus* group Streps, are F). Colonies of *Streptococcus anginosus* group exhibit a strong smell of caramel, butterscotch, vanilla or burnt sugar. (Not all Microbiologists can recognize the odour as that ability is variable.)
- Perform Vitek 2 GP card for identification.

Streptococcus bovis:

- Extensive taxonomic changes, four DNA clusters now recognized:
  - DNA cluster I: animal strains of *Streptococcus bovis* and *Streptococcus equinus*, which were shown to belong to a single species. The earlier name, ***Streptococcus equinus*** has been formally adopted.
  - DNA cluster II: ***Streptococcus gallolyticus"***
    Streptococcus gallolyticus has three subspecies:
    Subspecies *gallolyticus* is associated with GI cancer when isolated from blood
    Subspecies *pasteurianus* is associated with meningitis when isolated from blood
    Subspecies *macedonius*
  - DNA cluster III: ***Streptococcus infantarius***, with two subspecies***; infantarius*** and ***coli***
  - DNA cluster IV: ***Streptococcus alactolyticus***

Streptococcus iniae:

- Beta or alpha or no hemolysis, PYR positive.
- Not in Vitek 2 database.

Streptococcus mutans:

- viridans *Streptococcus*
- Gram morphology: from broth: GPC, short chains, from solid media: GPCB, almost diphtheroid-like.
- Colonial morphology: requires increased CO_2 for growth, may grow better anaerobically on first isolation. Becomes aerotolerant after 1 to 2 transfers. Tiny, white, dry colonies which may adhere to and pit the agar. Non-haemolytic, may show yellowing under the growth on Blood agar.
- Biochemical characteristics: PYR negative, Strep group D negative, Optochin resistant, API tests: Gelatin negative, Glucose positive, Mannitol positive, Sorbitol positive, Melibiose positive, Arabinose negative.
- Identify with Vitek 2 GP card if clinically significant.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- 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.
- Vitek 2 Systems product information
- CLSI. *Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition.* CLSI document M35-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L.Steven