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

For isolates such as *Propionibacterium*, the Coryneform group and *Bacillus* species, it may be necessary to determine whether the organism is clinically significant. Base this determination on specimen type, presence of WBC, numbers of other types of bacteria present, preliminary identification and whether the organism is isolated from more than one culture. Refer to procedure for specimen site. Refer to ASTM for susceptibility reporting.

IDENTIFICATION OF SOME ANAEROBIC GRAM-POSITIVE BACILLI:

- Refer anaerobic organism(s) to DynaLIFE for identification and susceptibility testing if required.

Test:	Result:	Next step(s):	Possible identification:
Growth	Anaerobic	Gram stain	
Gram stain	Thin rods, rare spores	Large, flat colonies, barnyard (cow manure) smell, indole negative, chartreuse fluorescence.	*Clostridium difficile*
	Boxcar, large, square rods	BRU: large irregular- shaped double zone B-hemolysis (place BRU in fridge for 2 hours).	*Clostridium perfringens*
	Thin rods, subterminal spores	Smoothly swarming, indole negative.	*Clostridium septicum*
	Thin rods, subterminal spores	Very large, lobate, irregular, flat, indole positive.	*Clostridium sordellii*
	Diphtheroidal, no spores, pleomorphic or branching	Small opaque, enamel-white circular, catalase positive, indole positive.	*Propionibacterium acnes*
		Refer to DynaLIFE if significant.	*Actinomyces* / Propioni species
	Short to long slender rods, chains common, no spores	Refer to DynaLIFE if significant.	Possible *Lactobacillus*

Notes for anaerobic Gram-positive bacilli:

- *Actinomyces, Propionibacterium and Lactobacillus* are variable or not as strict.
- *Clostridium tertium* is aerotolerant.
- Branching Gram-positive bacilli that grow best in anaerobic conditions and that show typical 'molar tooth' colonies on culture can be presumptively identified as *Actinomyces* species.

IDENTIFICATION OF AEROBIC GRAM-POSITIVE BACILLI:

Test:	Result:	Next step(s):	Possible identification:
Growth	Aerobic, facultative	Catalase	
Catalase	+	• Small rods (can be in pairs, short chains). • Narrow zone beta-hemolysis. • Vitek 2 GP card, refer to ASTM. • Catalase positive, urea negative, TSI: A/A H_2S negative. • Tumbling motility at 22˚C to 25˚C. • CAMP positive.	*Listeria monocytogenes*
		• Large rods, motile. • Refer to procedure for specimen site. • Refer to ASTM for susceptibility testing.	*Bacillus* species
		• Large rods, non-motile. • Non-hemolytic. • Send to ProvLab for confirmation.	*Bacillus anthracis*
		• Small diphtheroidal rods, no true branching. • Send to DynaLIFE for identification if required. • Refer to ASTM for susceptibility testing.	*Corynebacterium* species
		• Beaded GPB, branching at right angles. • Send to DynaLIFE for identification.	*Nocardia* species
		• Others: Send to DynaLIFE for ID.	
Catalase	-	• Vitek 2 GP card.	*Erysipelothrix*
		• Vitek 2 GP card.	*Arcanobacterium*
		• Refer to DynaLIFE for identification if indicated.	*Lactobacillus*
		• Others: Vitek 2 GP card or send to DynaLIFE.	

Refer to Primary Specimen Handling Flow Chart if Risk Group 3 organisms are suspected. All work and processing should be performed in BSC.

Notes for aerobic Gram-positive bacilli:

- Report aerobic, catalase positive, penicillin-susceptible GPB that are not *Listeria* or *Bacillus* species as CORSPP. Send to the DynaLIFE if clinically significant.
- 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 CORSPP unless physician indicates that the isolate may be significant.

Characteristics of *Nocardia*, *Actinomyces* and *Rhodococcus*:

Characteristic	*Nocardia*	*Actinomyces*	*Rhodococcus*
Gram stain reaction	• GPB, very long, thin, finely-beaded, obviously branching at right angles, usually have secondary branches	• GPB • Sulphur granules	• GPCB, arranged in zigzag fashion, coccoid and bacillary forms
Acid-fast (Kinyoun)	Weakly / partially acid-fast	Acid-fast	Weakly acid-fast
Catalase	Positive	Variable	Positive
API Nitrate	Positive	Variable	
Urea	Positive	Variable	Variable
Growth at 45˚C	Positive		
Culture	• Pigment production variable. • Usually produce aerial hyphae.	• Most: strict anaerobes. • Molar tooth colonies.	• No aerial hyphae. • 24h: pinpoint to small mucoid. • 48h: large, mucoid pale to salmon pink.

***Erysipelothrix rhusiopathiae*:**

- Has characteristics resembling Actinomyces and the Coryneform group.
- Widespread in nature. Infections are acquired from animals and enter through a wound.
- The Gram-positive cells are easily overdecolourized, with Gram-negative cells often showing Gram-positive beading.
- Blood agar under the colonies may show green decolourization after 48 hours incubation.
- Catalase negative, non-motile, H_2S positive in TSI, urease negative.
- Susceptible to most antimicrobials including penicillin and erythromycin.

Listeria monocytogenes, Mycobacteriaceae, Corynebacterium diphtheriae:

- Notify Health Protection Unit (HPU1) and Infection Control Nurse (SOHS) if inpatient.

Rothia dentocariosa:

- Normal oral flora, usually associated with dental caries and periodontal disease, implicated in infective endocarditis, bacteremia, pneumonia, peritonitis, endopthalmitis and meningitis.
- Aerobic or facultative, non-spore forming, non-motile, non-acid fast, pleomorphic coccobacilli that can form filamentous branches.
- Colonies are off-white and rough or smooth or 'spoke-wheel'.
- Catalase variable, PYR positive, urease negative, indole negative. Send to DynaLIFE for identification.

PROCEDURE NOTES:

- Potential agent of bioterrorism or Risk Group 3 organism: upon first suspicion of a possible agent of bioterrorism or risk group 3 organism, refer to Primary Specimen Handling Flow Chart.
- All work and processing should be performed in BSC.
- Notify Health Protection Unit (HPU1) and Infection Control Nurse (SOHS) if inpatient.

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