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

IDENTIFICATION OF ANAEROBIC GRAM-NEGATIVE COCCI:

Test	Result	Organism	Next step
Growth	Anaerobic	*Veillonella** / others	• Send to DynaLIFE for identification if significant. • Refer to ASTM for susceptibility testing requirements.

***Veillonella* species:**

- Tiny diplococci. On Brucella agar, colonies are small, transparent or opaque and fluoresce brick-red, indole negative.

IDENTIFICATION OF AEROBIC GRAM-NEGATIVE COCCI:

Test	Result	Organism	Next step
Growth	Aerobic		Oxidase
Oxidase	+	*Neisseria* *Moraxella catarrhalis*	• Refer to *Neisseria*/ *Moraxella* identification table.
	-	*Acinetobacter*	• Vitek 2 GN card, Vitek 2 AST-GN card.

***Neisseria / Moraxella* Identification table:**

Test	*N. gonorrhoeae*	*N. meningitidis*	*M. catarrhalis*
Gram stain reaction	Gram-negative diplococci		
Oxidase Catalase	+ +	+ +	+ +
Growth on BA (CO2)	Usually no growth	Glistening, non-hemolytic	Yes
Growth on TM (CO2)	Usually growth	No growth	No growth
API NH	Set up	Set up	
Vitek 2 NH card	Set up	Set up if sterile site	Set up if sterile site
Mueller-Hinton (CO2 ,35°C)	No growth	No growth	Growth
Catarrhalis disk (butyrate)			++

NOTE: If *Neisseria meningitidis* is suspected in a sterile site, perform all testing and processing in the BSC. Refer to Primary Specimen Handling Flow Chart.

***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.
- Acid is not produced from carbohydrates but weak positive reactions with glucose may occur with some commercial kits that detect rapid carbohydrate fermentation.
- Susceptible to colistin, and usually does not grow on selective gonococcal media such as Thayer Martin.

***Neisseria gonorrhoeae*:**

- Identify with API NH **AND** Vitek NH.
- Send organism to DynaLIFE confirmation and susceptibility testing.
- Notify Health Protection Unit (HPU1).
- Report as Presumptive *Neisseria gonorrhoeae*.

Neisseria lactamica:

- Gram-negative diplococci, oxidase positive, greyish non-haemolytic colonies on Blood agar.

Neisseria meningitidis:

- Identify with Vitek NH. Refer to ASTM for susceptibility testing procedure.
- Notify Health Protection Unit (HPU1) and Infection Control Nurse on inpatient (SOHS) if isolated from sterile site.
- Send isolates from sterile sites (e.g. CSF or blood) to the ProvLab 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 ProvLab 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.
- Refer to ASTM for susceptibility testing procedure.

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