PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC33500 – Gonorrhoeae Culture	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard:	

GUIDING PRINCIPLE:
Neisseria gonorrhoeae (also called GC) is mainly transmitted through sexual practices and infects the cervix, urethra, rectum, throat, and eyes. Gonorrhoea is one of the most commonly reported sexually transmitted infections. The fastidious and fragile nature of *Neisseria gonorrhoeae* requires careful consideration of proper methods of specimen collection and transport. *N.gonorrhoeae* must be properly differentiated from other saprophytic Neisseria spp. and prior to reporting must be confirmed using two reliable testing methods.

In women, the endocervix is the primary site of infection. A vaginal swab is not considered optima for the recovery of GC from woman but can be a valuable specimen for the diagnosis of gonorrhoea in preteen-aged girls.

The urethra is the primary site of infection in men but extragenital sites, including the rectum, throat and eye can act as sources of *N.gonorrhoeae.*

PURPOSE/RATIONALE:
To determine the presence or absence of Neisseria gonorrhoeae in urethra, cervix, throat, eye, and rectum specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for gonorrhoeae culture.

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal • Charcoal swabs are recommended

Source	• Urethra (male specimens only) • Cervix • Throat • Eye • Rectum **NOTE:** If gonorrhoeae culture is ordered on throat or eye specimens, full culture along with gonorrhoeae culture will be performed
Stability	If the sample is received in the laboratory and processed greater than 24 hours from collection: • Add specimen quality comment: "Delayed transport may adversely affect pathogen recovery"
Storage Requirements	Room temperature or refrigerated
Criteria for rejection	1. Unlabeled/mislabeled swabs. 2. Specimen container label does not match patient identification on requisition.

REAGENTS and/or MEDIA:

- Chocolate agar (CHO) and Thayer Martin agar (TM)
- Identification reagents: catalase, oxidase, API NH, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Wooden sticks

EQUIPMENT

- Biosafety cabinet
- 35° CO_2 incubator
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential infectious materials or cultures.

- Ensure that appropriate hand hygiene practices be used.
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures

PROCEDURE INSTRUCTIONS:

Step	Action
Processing swabs for gonorrhoeae culture	
1	In the biosafety cabinet: • Inoculate CHO and TM with the swab • Ensure all surfaces of swab make contact with the agar • Streak for isolated growth using a disposable inoculation needle • If applicable, prepare smear by rolling the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements • If specimen is from the eye, inoculate BA with the swab • IF specimen is from the throat, inoculate BA with the swab
2	Incubate the media: • Place CHO and TM in the CO_2 incubator
3	If applicable, allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain Procedure.

INTERPRETATION OF RESULTS:

Step	Action	
1	Ensure growth on culture media correlates with gram stain results if applicable. If discordant results are found between the gram stain and growth: • Re-examine smear and culture plates • Check for anaerobic growth • Re-incubate media to resolve • Consider re-smearing or re-planting specimen	
2	• Observe CHO and TM plates at 24 hours, 48 hours, and 72 hours • Examine for colonies resembling gonorrhoeae	
3	**IF**	**THEN**
	No colonies resembling *Neisseria gonorrhoeae* at 24 hours	• Record observations in the LIS • Re-incubate plates in CO_2 incubator on the "Old wound culture" shelf
	No colonies resembling *Neisseria gonorrhoeae* at 48 hours	• Record observations in the LIS • Re-incubate plates in CO_2 incubator on the "Old wound culture" shelf
	No colonies resembling *Neisseria gonorrhoeae* at 72 hours	• Perform flood oxidase if any growth present on plates • Record observations in the LIS • *Neisseria gonorrhoeae* not isolated
	Colonies resembling *Neisseria gonorrhoeae* present	• Record observations in the LIS • Subculture to CHO plate • From CHO sub plate, perform catalase, oxidase, and gram stain

		IF	THEN
		Gram stain = GRAM NEGATIVE DIPLOCOCCI Catalase = POSITIVE Oxidase = POSITIVE	• Perform Vitek NH card • Perform API NH **NOTE:** Ensure there are sufficient colonies for send out the following day for susceptibility testing
4	• Two identification methods must be used to report an identification of *Neisseria gonorrhoeae* • Beta lactamase test must also be performed on all isolates of *Neisseria gonorrhoeae*		

REPORTING INSTRUCTIONS:

IF	REPORT
No *Neisseria gonorrhoeae* isolated	• Report: "**No Neisseria gonorrhoeae isolated"** • Add culture comment **{GENP**
No *Neisseria gonorrhoeae* isolated and plates overgrown with yeast	• Report: "**No Neisseria gonorrhoeae isolated"** • Add culture comment **{GCY**
Neisseria gonorrhoeae isolated	• Add organism: "**Neisseria gonorrhoeae"** • List quantification as: "**Isolated"** • Add Beta-lactamase result if positive • Add isolate comment **&REF6** • Refer isolate to APL for susceptibility testing • Freeze isolate(s) and log into stored isolates log

NOTE:

- Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to LQM70620-Laboratory Critical Results List-Microbiology for results that need to be phoned to ordering location
- Refer to MIC36100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Prevention and Control
- Refer to MIC36300-Referral of Category B Specimens to APL for sending isolates to APL

LIMITATIONS:

1. The presence of yeast may inhibit the growth of *Neisseria gonorrhoeae*. Although Thayer Martin agar contains Nystatin to inhibit the growth of yeast, inhibition of *Neisseria gonorrhoeae* should be considered on CHOC if culture is positive for yeast species.

2. A single negative result produced by any of the confirmatory tests does not rule out an identification of *N.gonorrhoeae*. Further confirmatory testing using at least one different method should be performed.
3. False-negative results can be caused by delay in transport.

CROSS-REFERENCES:

- 15-10-V1 Laboratory Critical Results Procedure
- MIC20115-Gram Stain Procedure
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36300-Referral of Category B Specimens to APL

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th ed.) Washington, D.C.: ASM Press
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*. Washington, D.C: ASM Press

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	27 Nov 17	Initial Release	L. Steven
2.0	30 Nov 18	Updated to include new Vitek 2 instrument and Vitek NH card	L. Steven
3.0	5 Mar 21	Procedure reviewed and added to NTHSSA policy template	L. Steven