PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC33500 – *Neisseria gonorrhoeae* Culture	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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GUIDING PRINCIPLE:

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

PURPOSE/RATIONALE:

This standard operating procedure describes the screening for *Neisseria gonorrhoeae* in urethra, cervix, throat, eye, and rectum specimens.

SCOPE/APPLICABILITY:

This standard operating procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for *Neisseria gonorrhoeae* culture.

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal • Charcoal swabs are recommended **NOTE:** If charcoal swab is not received, add specimen quality comment **NGON**
Source	• Urethra (male specimens only) • Cervix • Throat • Eye • Rectum

	NOTE: *Neisseria gonorrhoeae* will be performed on vaginal swabs with the specimen quality comment VAGGC added
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 potentially 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 *Neisseria gonorrhoeae* culture	
1	In the biosafety cabinet: • Inoculate CHO and TM with the swab • Ensure all surfaces of the swab make contact with the agar • Streak for isolated growth using a disposable inoculation needle • If applicable, prepare a smear by rolling the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements
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	If applicable, ensure growth on culture media correlates with gram stain results. 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 *Neisseria gonorrhoeae*	
3	If no colonies resembling *Neisseria gonorrhoeae* are seen at 24 hours: • Record observations in the LIS • Re-incubate plates in CO_2 incubator on the "Old urine culture" shelf	
4	If no colonies resembling *Neisseria gonorrhoeae* are seen at 48 hours: • Record observations in the LIS • Re-incubate plates in CO_2 incubator on the "Old urine culture" shelf	
5	If no colonies resembling *Neisseria gonorrhoeae* are seen at 72 hours: • Perform flood oxidase if any growth is present on plates • Sub any oxidase positive organisms to chocolate agar immediately • Record observations in the LIS • *Neisseria gonorrhoeae* not isolated	
6	If colonies resembling *Neisseria gonorrhoeae* are seen: • Record observations in the LIS • Subculture to CHO plate • From CHO sub plate, perform catalase, oxidase, and gram stain:	
7	**IF**	**THEN**
	Gram stain = GRAM NEGATIVE DIPLOCOCCI Catalase = POSITIVE Oxidase = POSITIVE	• Perform VITEK NH card • Perform API NH

8	Ensure there are sufficient colonies for send out the following day for susceptibility testing.
9	Two identification methods must be used to report an identification 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 **{GENP** • Add culture comment **{GCY**
Neisseria gonorrhoeae isolated	• Add organism: "**Neisseria gonorrhoeae"** • List quantification as: "**Isolated"** • Report susceptibility results as per ASTM • Add isolate comment **&REF6** • In order entry, copy report to OCPHO (HPU1) • Refer isolate to APL for susceptibility testing • Freeze isolate 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 MIC36300-Referral of Category B Specimens to APL for sending category B 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 *Neisseria gonorrhoeae*. Further confirmatory testing using at least one different method should be performed.
3. False-negative results can be caused by delays in transport.

CROSS-REFERENCES:

- MIC20115-Gram Stain Procedure
- MIC36300-Referral of Category B Specimens to APL
- LQM70620-Laboratory Critical Results List-Microbiology

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	22 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	27 Feb 23	Procedure reviewed	L. Steven