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

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal • Charcoal swabs are recommended
Source	• Urethra (male specimens only) • Cervix • Throat • Eye • Rectum **NOTE:** Vaginal specimens are not considered optimal for the diagnosis of gonorrhoeae in women and should be reserved only for the evaluation of preteen-aged girls (<12 yrs.) with suspected sexually transmitted diseases due to presumed sexual abuse or assault. Refer to MIC30350 Genital Culture-Lower/Upper Genital Tract. **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. 3. Dry swabs.

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
- Biosafety cabinet
- 37° CO_2 incubator
- Wooden sticks
- 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.

- 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. Universal precautions 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 specimens for Neisseria gonorrhoeae culture	
1	In the biosafety cabinet, inoculate Chocolate agar and Thayer Martin agar from the swab. Make gram stain on male urethral specimens only.
2	Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
3	Place CHO and TM plates in the CO_2 incubator.
4	If applicable, allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
5	Examine plates after 24 hour incubation. Record observations in the LIS.
6	Re-incubate plates for an additional 48 hours.
7	At 48 hours, examine plates and record observations in the LIS.
8	At 72 hours, examine plates and record observations in the LIS.

INTERPRETATION OF RESULTS:

Step	Action
1	Confirm gram stain on male urethra specimens have been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.
2	Examine plates daily for growth of typical gonorrhoeae colonies: small, translucent, raised, gray and mucoid with entire margins. When picked from the agar surface, they tend to come off as whole colonies.
3	Perform initial identification testing on colonies morphologically resembling *Neisseria gonorrhoeae* including: oxidase (positive), catalase (positive) and gram stain (Gram-negative diplococci).
4	Perform Vitek 2 NH card and API NH on oxidase positive, catalase positive, and gram-negative diplococci colonies. If there is insufficient growth, subculture organism to chocolate plate. As well, ensure there are sufficient colonies for send out the following day for susceptibility testing. **NOTE:** Two testing methods must be used to report an identification of *Neisseria gonorrhoeae.*
5	Beta lactamase testing must be performed on all isolates of *Neisseria gonorrhoeae*.
6	Re-incubate and re-examine CHO and TM plates for a total of 3 days and assess as in steps 1 to 3 daily. Prior to discarding plates on day 3, flood with oxidase reagent. If a purple color colony is observed, immediately subculture to CHO, since oxidase reagent is toxic to bacteria.

REPORTING RESULTS:

IF	REPORT
No *Neisseria gonorrhoeae* isolated after 3 days	• Report: **"No Neisseria gonorrhoeae isolated"** • If only swab received add culture comment **{GENP** to state: **"GenProbe or Aptima nucleic acid amplification test is the optimum test for detection of N.gonorrhoeae, as well as Chlamydia trachomatis"**
No *Neisseria gonorrhoeae* isolated after 3 days and plates overgrown with yeast	• Report: **"No Neisseria gonorrhoeae isolated"** • Add culture comment **{GCY** to state: **"Specimen contaminated with yeast cells, which may be inhibitory to *Neisseria gonorrhoeae.* Please recollect if clinically indicated"**
Neisseria gonorrhoeae isolated	• Add organism: **"Neisseria gonorrhoeae"** • List quantification as: **"Presumptive"** • Add Beta-lactamase result if positive. • Add isolate comment **&REF5** to state: **"This organism has been referred for confirmation and susceptibility testing"** • In Order Entry; copy report to Chief Medical Officer of Health (HPU1). • Refer organism to DynaLIFE for confirmation and susceptibility testing as per MIC10510 - Referral of Category B Specimens to DynaLIFE. • Freeze isolate and log into stored isolates binder.

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.

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.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	27 Nov 2017	Initial Release	L. Steven
2.0	30 Nov 2018	Updated to include new Vitek 2 instrument; Updated to include Vitek NH card; Updated to include requirement of 2 tests for identification of *N.gonorrhoeae*	L. Steven