PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC33200 – Genital Culture-Lower Genital Tract	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:
Organisms which are associated with infection or disease of the genital tract include *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), *Haemophilus ducreyi*, yeasts, *Trichomonas vaginalis* and viruses such as Herpes simplex virus (HSV). Isolation or detection of other organisms such as Group A streptococcus, Group B streptococcus, *Staphylococcus aureus*, and others may be associated with certain specific clinical syndromes or risk of infection in the neonate (e.g. Group B streptococcus). Proper handling, transport, processing and plating of specimens with selective, non-selective and enriched media, and incubating under specific environmental conditions will facilitate the recovery of fastidious genital tract pathogens such as *Neisseria gonorrhoeae*.

PURPOSE/RATIONALE:
To determine the presence or absence of bacterial pathogens in lower genital tract specimens.

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

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal • Charcoal swabs are recommended
Source	• Vaginal vault • Vagina or vaginal orifice • Vulva • Penis

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
Criteria for rejection	1. Unlabeled/mislabeled swabs 2. Specimen container label does not match patient identification on requisition 3. Do not accept vaginal swabs from women >12 years of age for genital culture unless significant clinical information is provided. 4. Do not process vaginal swabs for yeast culture unless significant clinical information is provided.

NOTE:

- Genital culture is performed on vaginal specimens if <12 years old

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO), Thayer Martin agar (TM), Sabouraud Dextrose agar (SAB), Colistin Nalidixic Acid agar (CNA) and MacConkey agar (MAC)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Wooden sticks

EQUIPMENT

- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators
- 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 hang 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 specimens for lower genital tract culture	
1	In the biosafety cabinet: • Inoculate BA, CHO, TM, and MAC with the swab • Ensure all surfaces of swab make contact with the agar • Streak for isolated growth using a disposable inoculation needle • Prepare smear by rolling the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements
2	Incubate all media: • Place BA, CHO, and TM in the CO_2 incubator • Place MAC in the O_2 incubator
3	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain Procedure.

Probable Pathogens	Possible Pathogens
• *Neisseria gonorrhoeae* • *Streptococcus pyogenes* • *Streptococcus agalactiae* • *Listeria monocytogenes* • *Candida* spp. • *Shigella* spp. • *Salmonella* spp. • *Aeromonas* spp. • *Yersinia* spp.	• *Haemophilus influenzae* • *Staphylococcus aureus* **NOTE:** If clinical history indicates suspected Toxic Shock Syndrome (TSS), consider a pathogen • *Streptococcus pneumoniae* • *Neisseria meningitidis* • *Pseudomonas* spp. and other non-glucose fermenting Gram-negative bacilli

INTERPRETATION OF RESULTS:

Step	Action
1	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 BA, CHO and TM plates at 24 hours, 48 hours, and 72 hours • Observe MAC plate at 24 hours

3	• In prepubescent females, diptheroids and coagulase-negative staphylococci are predominant • In the adult female, lactobacilli are predominant • In postmenopausal women, fewer lactobacilli are present and a greater number of Enterobacteriaceae are predominant
4	• <u>If organism is a pathogen</u>: ➢ Perform full identification and report all pathogens ➢ Perform and report susceptibility testing as per ASTM
5	• <u>If organism is a potential pathogen</u>: ➢ Perform full identification and report all potential pathogens if both are true: ○ Growth is heavy ○ Growth is predominant ➢ Perform and report susceptibility testing if any of the following are true: ○ 3 to 4+WBC in the gram stain ○ Organism is intracellular in the gram stain ○ Clinical diagnosis is infection ○ Patient is immunocompromised ○ Multiple cultures are positive for the same organism
6	• If organism is *Gardnerella vaginalis,* report: ➢ When present in quantities less than other normal microbiota, *Gardnerella vaginalis* should be included as part of normal vaginal flora. However, for females <12 years of age, *Gardnerella vaginalis* should be reported regardless of quantity present ➢ If *Gardnerella vaginalis* is the predominant organism from vaginal specimens and is isolated in moderate to heavy growth, report regardless of patient's age ➢ Do not perform susceptibility testing

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 3 days	• Report: "**No Growth after 3 days"** • Report: "**No Neisseria gonorrhoeae isolated"** • If only swab received add culture comment **{GENP**
Mix of commensal genital flora	• Report: "**Mixed commensal genital flora"** • List quantitation • Report: "**No Neisseria gonorrhoeae isolated"** • If only swab received add culture comment **{GENP**
Mix of enteric Gram-negative bacilli	• Report: **"Mixture of coliform organisms"** • List quantitation • Report: "**No Neisseria gonorrhoeae isolated"** • If only swab received add culture comment **{GENP**
Growth of pathogen(s)	• Report organisms(s) identification • List quantitation • Report susceptibility as per interpretation of results • Report: "**No Neisseria gonorrhoeae isolated"**

Listeria monocytogenes isolated	• Add organism: "**Listeria monocytogenes"** • List quantitation • Report susceptibility results as per ASTM • Freeze isolate(s) and log into stored isolates log
Neisseria gonorrhoeae isolated **NOTE:** If *Neisseria gonorrhoeae* is isolated on a child <12 years of age, these results need to be phoned to the ordering location **Note:** If growth of yeast is present, add culture comment **{GCY**	• 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 MIC36200-Referral of Category A Specimens to APL for sending category A isolates to APL
- Refer to MIC36300-Referral of Category B Specimens to APL for sending isolates to APL
- Refer to MIC36400-Referral of Category B Specimens to DL for sending isolates to *Dyna*LIFE

LIMITATIONS:

1. A negative genital specimen culture does not eliminate the possibility of a genital tract infection. Organisms such as viruses, Mycoplasmas and Chlamydia are not detected by routine culture. Inadequate specimen collection, improper specimen handling and low organism levels in the specimen may yield a false negative result.
2. 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 CHO if culture is positive for yeast species.

CROSS-REFERENCES:

- LQM70620-Laboratory Critical Results List-Microbiology
- MIC20115-Gram Stain Procedure
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36200-Referral of Category A Specimens to APL
- MIC36300-Referral of Category B Specimens to APL
- MIC36400-Referral of Category B Specimens to DL

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	L. Steven
3.0	11 Jan 21	Procedure reviewed and added to NTHSSA policy template	L. Steven