PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC33300 – Genital Culture-Upper Genital Tract	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Effective Date:
Issuing Authority: Director, Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

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 preferred Aspirates/tissue • Clean, sterile container
Source	• Endometrial swabs, biopsies and curettings • Placenta swabs and tissues

	• Products of conception, endometrial/uterine, Cul de Sac/transvaginal, fallopian tube, tubo-ovarian swabs, or aspirates
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. Dry swabs 4. Improperly collected, labeled, transported, or handled irretrievable specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse

NOTE:

- Refer to MIC34100-Body Fluid Culture for amniotic fluid
- Refer tissue or biopsy specimens for culture to *Dyna*LIFE

REAGENTS and/or MEDIA:

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

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Anaerobic jar and pouch
- Wooden sticks

EQUIPMENT:

- Biosafety cabinet
- 35° ambient air and 35° CO2 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 upper genital tract culture	
1	In the biosafety cabinet: • Inoculate BA, CHO, TM, MAC and BRU with the specimen • Inoculate SAB if yeast is required • Ensure all surfaces of specimen 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 • If applicable, incubate SAB at room temperature with 48 hour read date • Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with day 2 date and place in the O_2 incubator **NOTE:** Anaerobes should not be exposed to air for 42 to 48 hours
3	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain Procedure.

Common Pathogens	
• *Neisseria gonorrhoeae* • *Streptococcus agalactiae* • *Streptococcus pyogenes* • *Listeria monocytogenes* • *Candida* spp. • *Staphylococcus aureus* • *Streptococcus pneumoniae* • *Neisseria meningitides* • *Gardnerella vaginalis* • *Haemophilus influenzae*	• Gram-negative bacilli: ➢ Enteric Gram-negative bacilli ➢ *Pseudomonas* spp. and other non-glucose fermenting Gram-negative bacilli ➢ *Capnocytophaga* spp. ➢ Examine non-lactose fermenting from pediatric patients.

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 and 48 hours • If applicable, observe SAB plate at 48 hours
3	• Observe BRU plate at 48 hours and 5 days • If anaerobic growth is suspected, perform gram stain. If gram stain resembles growth on aerobic plates, further workup is not indicated. If growth does not resemble growth on aerobic plates, perform aerotolerance test. Refer to MIC53700-Aerotolerance Test

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: "**No Growth after 1 Day. Further report to follow"**
No growth on aerobic media after 3 days	**INTERIM:** • Report: "**No growth aerobically after 3 days"** • Report: "**No Neisseria gonorrhoeae isolated"** • Report: **"@Anaerobic culture to follow"**
No growth on anaerobic media after 5 days	**FINAL:** • Report: "**No anaerobes isolated after 5 days"**
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**
Mix of anaerobic organisms	• Report: "**Mixture of anaerobic organisms"** • List quantitation • Report: "**No Neisseria gonorrhoeae isolated"**
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
- MIC34100-Body Fluid Culture
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36300-Referral of Category A Specimens to APL
- MIC36400-Referral of Category B Specimens to *Dyna*LIFE and APL
- MIC53700-Aerotolerance Test

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	20 Oct 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