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:	Next Review Date:
Issuing Authority: Director, Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
The microbiologic diagnosis of endometritis is difficult. Anaerobes play an important role in this infection. Although any organism may cause infection of the placenta, the most common organisms associated with this syndrome include *Staphylococcus aureus*, β-hemolytic Streptococci and *Listeria monocytogenes*. Organisms typically associated with infections of the upper genital tract include *Staphylococcus aureus*, β-hemolytic Streptococci, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

PURPOSE/RATIONALE:
This standard operating procedure describes how to determine the significance of growth in upper genital tract specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for upper 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, 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. 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), MacConkey agar (MAC) and Brucella agar (BRU)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- 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 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 specimens for upper genital tract culture	
1	In the biosafety cabinet: • Inoculate BA, CHO, TM, MAC and BRU with the specimen • Ensure all surfaces of the 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 • 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.

Probable Pathogens	
• *Actinomyces* spp. • *Arcanobacterium* • *Aeromonas* • *Bacillus anthracis**+ • β-hemolytic *Streptococci* • *Brucella**+ • *Campylobacter* • *Candida* spp. • *Capnocytophaga* spp. • *Chromobacterium* • *Erysipelothrix* • *Francisella**+ • *Haemophilus influenzae* • *Helicobacter* • *Kingella kingae* • *Listeria* spp.	• Molds • *Moraxella catarrhalis* • *Neisseria gonorrhoeae* • *Neisseria meningitidis* *+ • *Nocardia* spp. • *Pasteurella multocida* • *Pseudomonas aeruginosa* • *Salmonella* spp. • *Shigella* spp. • *Sphingobacterium* • *Staphylococcus aureus* • *Streptococcus anginosis* grp. • *Streptococcus pneumoniae* • *Vibrio* spp. • *Yersinia* spp.

Potential Pathogens	Commensal Flora
• Anaerobes not listed above • Enteric Gram-negative bacilli not listed above • *Enterococcus* spp. • *Staphylococcus intermedius* • *Staphylococcus lugdunensis* • Yeasts not listed above	• *Bacillus* spp. not listed above • Coagulase-negative *Staphylococci* • *Corynebacterium* spp. • *Micrococcus* spp. • Non-pathogenic *Neisseria* spp. • viridans *Streptococcus* grp.

* Risk group 3 organism. If suspected, refer to Policy B-0160: "Specimens Containing Suspected Risk Group 3 Pathogens" for Primary Specimen Handling Flow Chart

+ All work-up should be performed in the BSC

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of aerobic growth in upper genital specimens	
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
3	**Single morphology growing on plates:** • <u>If organism is a probable pathogen</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing as per ASTM • <u>If organism is a potential pathogen or commensal flora</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing if ANY of the following are true: ○ 3 to 4+WBC in the gram stain ○ Clinical diagnosis is infection ○ Patient is immunocompromised ○ Multiple cultures are positive for the same organism • <u>If organism is an anaerobe</u>: ➢ Refer to "Interpretation of anaerobic growth for deep wound specimens" portion of this procedure
4	**Multiple morphologies growing on plates:** • <u>If organism is a probable pathogen</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing as per ASTM • <u>If organism is a potential pathogen</u>: ➢ Perform minimal identification and list if ANY of the following are true:

	○ Moderate to numerous epithelial cells in the gram stain ○ No WBC in the gram stain ○ No clinical history that indicates infection was provided ○ ≥3 organisms growing, excluding probable pathogens **NOTE:** Mixed enteric Gram-negative rods should be reported as mixture of coliform organisms, not reported individually ➢ <u>If none of the above are true</u>: ○ Perform full identification and report 1 or 2 potential pathogens ○ Perform susceptibility testing as per ASTM and report if ANY of the following are true: ❖ 3 to 4+WBC in the gram stain ❖ Clinical diagnosis is infection ❖ Patient is immunocompromised ❖ Multiple cultures are positive for the same organism • <u>If organism is commensal flora</u>: ➢ Perform minimal identification and report as commensal flora **NOTE:** Mixed commensal flora should be reported as commensal flora, not reported individually and not reported as mixed
5	Perform a flood oxidase test on both chocolate and TM agar on day 3. Sub any oxidase positive organisms to chocolate agar immediately.

Step	Action
Interpretation of anaerobic growth in upper genital specimens	
1	• Observe BRU at 48 hours and 5 days and KV at 48 hours • 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
2	**Single morphology growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU for anaerobic growth • <u>If growth does not resemble growth on aerobic plates:</u> ➢ Perform identification • <u>If organism is a probable pathogen</u>: ➢ Report full identification ➢ Refer to *Dyna*LIFE for susceptibility testing • <u>If organism is a potential pathogen or commensal flora</u>: ➢ Report full identification ➢ Refer to *Dyna*LIFE for susceptibility testing if ANY of the following are true: ○ 3 to 4+WBC in the gram stain ○ Clinical diagnosis is infection ○ Patient is immunocompromised ○ Multiple cultures are positive for the same organism
3	**Multiple morphologies growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU for anaerobic growth

	• <u>If 2 anaerobes are isolated with or without aerobic growth</u>: ➢ List organisms based on gram stain identification • <u>If >2 anaerobes are isolated with or without aerobic growth:</u> ➢ Report anaerobes as "Mixture of anaerobes"

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth after 1 Day"** • Report: **"Further report to follow"**
No aerobic growth after 3 days and no anaerobic growth	**INTERIM:** • Report: **"No aerobic growth at 3 days"** • Report: **"@Anaerobic culture to follow"**
Aerobic growth at 2 or 3 days and no anaerobic growth	**INTERIM:** • Report aerobic growth as per procedure • Report: **"@Anaerobic culture to follow"**
No growth on anaerobic media after 5 days	**FINAL:** • Report: "**No anaerobes isolated after 5 days"**
Growth of probable pathogen, NOT *Neisseria gonorrhoeae*	• Report organism identification • List quantitation • Report susceptibility results as per ASTM • Report: "**No Neisseria gonorrhoeae isolated"**
Neisseria gonorrhoeae isolated **NOTE:** *N.gonorrhoeae* is a critical result if isolated on a child ≤13	• Add organism: "**Neisseria gonorrhoeae"** • List quantification as: "**Isolated"** • Report susceptibility results as per ASTM • Add isolate comment **&REF6** • Refer isolate to APL for susceptibility testing • Freeze isolate and log into stored isolates log
Growth of potential pathogen or commensal flora where full identification is required	• Report organism identification • List quantitation • If indicated by procedure, perform and report susceptibility testing as per ASTM • Report: "**No Neisseria gonorrhoeae isolated"**
Growth of potential pathogens where minimal identification and listing is required	• Report the minimal identification (i.e., Gram Negative Bacilli - Lactose Fermenter) • List quantitation • Report: "**No Neisseria gonorrhoeae isolated"**
Growth of commensal flora where minimal identification and listing is required	• Report: **"Commensal flora"** • List quantitation • Report: "**No Neisseria gonorrhoeae isolated"**

NOTE: If *Neisseria gonorrhoeae* is NOT isolated on upper genital tract specimens:
- If only a C&S swab is received, add culture comment **{GENP**
- If growth of yeast is present, add culture comment **{GCY**

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.
2. Inadequate specimen collection, improper specimen handling and low organism levels in the specimen may yield a false negative result.
3. 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
- 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	22 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	27 Feb 23	Procedure reviewed	L. Steven