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): 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:
Organisms which are associated with infection or disease of the genital tract include *N.gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) and Yeasts. Isolation of other organisms such as *S.pyogenes*, *S.agalactiae*, *S.aureus*, and others may be associated with certain specific clinical syndromes or risk of infection in the neonate.

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

SCOPE/APPLICABILITY:
This standard operating 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	• Labia • Penis • Vagina • Vulva
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 >13 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 from patients ≤13 years of age

REAGENTS and/or MEDIA:

- Blood agar (BA) Chocolate agar (CHO), Columbia Naladixic Acid agar (CNA), Thayer Martin agar (TM) 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 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 lower genital tract culture	
1	In the biosafety cabinet: • Inoculate BA, CHO, CNA, TM, and MAC with the swab • Ensure all surfaces of the 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, CNA 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.
4	This procedure is divided into 2 sections that include the 4 sources of lower genital tract culture specimens: • Vaginal and vulva culture • Labia and penis culture

1. Vaginal Culture and Vulva Culture:

Probable Pathogens^	
GNB Aerobic: • *Salmonella* spp.(≤13 yrs. old) • *Shigella* spp. (≤13 yrs. old)	GNDC Aerobic: • *Neisseria gonorrhoeae* GPB Aerobic: • *Listeria monocytogenes*
GPC Aerobic: • *Staphylococcus aureus* • *Streptococcus agalactiae* • *Streptococcus pyogenes*	Yeast: • *Candida* spp.
Potential Pathogens^	
• *Gardnerella vaginalis* • Gram negative bacilli other than Enterobacteriaceae • *Haemophilus influenzae*	• *Neisseria meningitidis* • *Pseudomonas* spp. • *Streptococcus pneumoniae*
Commensal Flora	
• Anaerobes • Coagulase-negative *Staphylococci* • *Corynebacterium* spp. • Enteric Gram-negative bacilli not listed above	• *Enterococcus* spp. • *Lactobacillus* spp. • Non-pathogenic *Neisseria* spp. • viridans *Streptococcus* grp.

^ For organisms not listed, consult the Microbiology Technical Supervisor or refer to the *Manual of Clinical Microbiology*

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, CNA and TM plates at 24 hrs, 48 hrs, and 72 hrs • Observe MAC plate at 24 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</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing as per ASTM if ANY of the following are true: o 3 to 4+WBC in the gram stain o Clinical diagnosis is infection o Patient is immunocompromised o Multiple cultures are positive for the same organism • <u>If organism is commensal flora</u>: ➢ Perform minimal identification and report as commensal flora
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 and report full identification if BOTH are true: o Growth is heavy and growth is predominant ➢ Perform and report susceptibility testing as per ASTM if ANY of the following are true: o 3 to 4+WBC in the gram stain o Clinical diagnosis is infection o Patient is immunocompromised o Multiple cultures are positive for the same organism ➢ If none of the above are true: o Perform minimal identification and report as commensal flora **NOTE:** Mixed enteric Gram-negative rods should be reported as mixture of coliform organisms, not reported as commensal flora • <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.

REPORTING INSTRUCTIONS FOR VAGINAL AND VULVA CULTURE:

IF	REPORT
No growth after 3 days	• Report: "**No growth after 3 days"**
Growth of probable pathogen, NOT *Neisseria gonorrhoeae*	• Report organism full identification • List quantitation • Report susceptibility results as per ASTM
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 where full identification is required	• Report organism full identification • List quantitation • If indicated by procedure, perform and report susceptibility testing as per ASTM
Growth of potential pathogen where minimal identification is required	• Report **"Commensal flora"** • List quantitation
Growth of commensal flora	• Report: **"Commensal flora"** • List quantitation
Mix of enteric Gram-negative bacilli	• Report: **"Mixture of coliform organisms"** • List quantitation

NOTE:
If clinical history states query STI, sexual assault or requests *Neisseria gonorrhoeae* screen and *Neisseria gonorrhoeae* is NOT isolated:

- Report **"No Neisseria gonorrhoeae isolated"**
- Add culture comment **{GENP**
- If growth of yeast is present, add culture comment **{GCY**

2. Labia Culture and Penis Culture:

Probable Pathogens^		
GNB Aerobic: • *Aeromonas* spp. • *Brucella* spp.*+ • *Chromobacterium* spp. • *Eikenella corrodens* • *Pasteurella multocida* • *Pseudomonas aeruginosa* • *Salmonella* spp. • *Shigella* spp. • *Sphingobacterium* spp. • *Vibrio* spp. • *Yersinia* spp. GNB Anaerobic: • *Bacteroides fragilis* • *Capnocytophaga* spp.	GNC/CB Aerobic: • *Francisella tularensis*+ • *Haemophilus influenzae* • *Kingella kingae* • *Moraxella catarrhalis* • *Neisseria gonorrhoeae* • *Neisseria meningitidis* GPC Aerobic: • β-hemolytic *Streptococci* • *Staphylococcus aureus* • *Streptococcus anginosis* grp. • *Streptococcus pneumoniae*	GPB Aerobic: • *Bacillus anthracis*+ • *Bacillus cereus* • *Erysipelothrix* spp. • *Listeria* spp. • *Nocardia* spp. GPB Anaerobic: • *Actinomyces* spp. • *Arcanobacterium* spp. • *Clostridium perfringens* Others: • *Candida* spp. • Molds
Potential Pathogens^	**Commensal Flora**	
• Anaerobes not listed above • Enteric GNB not listed above • Non-enteric GNB 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 MIC40100-Suspect High Risk Organism Workup
+ All work-up should be performed in the BSC
^ For organisms not listed, consult the Microbiology Technical Supervisor or refer to the *Manual of Clinical Microbiology*

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, CNA and TM plates at 24 hrs, 48 hrs, and 72 hrs • Observe MAC plate at 24 hours

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

REPORTING INSTRUCTIONS FOR LABIA AND PENIS CULTURE:

IF	REPORT
No growth after 3 days	• Report: "**No Growth after 3 days"**
Growth of probable pathogen, NOT *Neisseria gonorrhoeae*	• Report organism full identification • List quantitation • Report susceptibility results as per ASTM
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 full identification • List quantitation • If indicated by procedure, perform and report susceptibility testing as per ASTM
Growth of potential pathogens where minimal identification and listing is required	• Report the minimal identification (i.e., Gram Negative Bacilli - Lactose Fermenter) • List quantitation
Growth of commensal flora where minimal identification and listing is required	• Report: **"Commensal flora"** • List quantitation
Mix of enteric Gram-negative bacilli	• Report: **"Mixture of coliform organisms"** • List quantitation

NOTE:
If clinical history states query STI, sexual assault or requests *Neisseria gonorrhoeae* screen and *Neisseria gonorrhoeae* is NOT isolated:

- Report **"No Neisseria gonorrhoeae isolated"**
- Add culture comment **{GENP**
- If growth of yeast is present, add culture comment **{GCY**

NOTE:

- Refer to Reportable Diseases Act-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 category B isolates to APL

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.
4. In prepubescent females, diptheroids and coagulase-negative staphylococci are predominant.
5. In the adult female, lactobacilli are predominant.
6. In postmenopausal women, fewer lactobacilli are present and a greater number of Enterobacteriaceae are predominant.

CROSS-REFERENCES:

- 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
- MIC40100-Suspect High Risk Organism Workup
- LQM70620-Laboratory Critical Results List-Microbiology

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4thed.) 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