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

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. Dry swabs. 4. Do not accept vaginal swabs from women >12 years of age for genital culture unless significant clinical information is provided. Refer to MIC30300-Genital Culture-Bacterial Vaginosis. 5. Do not process vaginal swabs for yeast culture unless significant clinical information is provided. Refer to MIC30300-Genital Culture-Bacterial Vaginosis.

NOTE:

- **Genital culture is performed on vaginal specimens if patient is < 12 yrs. 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
- Biosafety cabinet
- 35° ambient air and 37° CO_2 incubators
- 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 lower genital tract culture	
1	In the biosafety cabinet, inoculate Blood agar, Chocolate agar, Thayer Martin agar and MacConkey agar from the swab. Make gram stain. Inoculate Sabouraud Dextrose agar if yeast culture is requested on vaginal swabs and significant clinical information is provided.
2	Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
3	Place MAC plate in the O_2 incubator. Place BA, CHO and TM plates in the CO_2 incubator. If applicable, incubate SAB plate at room temperature for 48 hours. Write on plate the date of the 48 hour read.
4	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 BA plate for an additional 24 hours. Re-incubate CHO and TM plates for an additional 48 hours. Discard O_2 plate.
7	If applicable, after 48 hours, examine SAB plate for white, creamy colonies resembling yeast.
8	Re-incubate and re-examine CHO and TM plates for a total of 3 days. 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.

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* • *Streptococcus pneumoniae* • *Neisseria meningitidis* • *Pseudomonas* spp. and other non-glucose fermenting Gram-negative bacilli

NOTE: Do not examine vaginal specimens for other Enterobacteriaceae, as these microorganisms are normally found in the female genital tract.

INTERPRETATION OF RESULTS:

Step	Action
1	Confirm gram stain has 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	Observe plates at 24 hours and 48 hours for growth.
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: o Growth is heavy o Growth is predominant
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 RESULTS:

IF	REPORT
No growth after 2 days on BA and no growth on CHO and TM after 3 days	• Report: **"No Growth 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"**
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** to state: **"GenProbe or Aptima nucleic acid amplification test is the optimum test for detection of N.gonorrhoeae, as well as Chlamydia trachomatis"**
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** to state: **"GenProbe or Aptima nucleic acid amplification test is the optimum test for detection of N.gonorrhoeae, as well as Chlamydia trachomatis"**
Growth of pathogen(s)	• Report organism(s) identification under the isolates tab. • List quantitation. • Report susceptibility results as per ASTM. • Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1. • Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control. • Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location.

Listeria monocytogenes isolated	• Add organism: **"Listeria monocytogenes"** • List quantitation. • Report susceptibility results as per ASTM. • In Order Entry; copy report to Chief Medical Officer of Health (HPU1). • Freeze isolate and log into stored isolates binder.
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.**	• 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. 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.

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		Initial Release	L. Steven