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

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 DynaLIFE.**

REAGENTS and/or MEDIA:

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

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Biosafety cabinet
- Anaerobic jar and pouch
- 35° ambient air and 35° 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 upper genital tract culture	
1	In the biosafety cabinet, inoculate Blood agar, Chocolate agar, Thayer Martin agar, MacConkey Agar, Brucella agar and Brucella Laked Blood agar with Kanamycin and Vancomycin from the specimen. Make gram stain. Inoculate Sabouraud Dextrose agar if yeast culture is requested.
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	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation.
5	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
6	Examine aerobic plates after 24 hour incubation. Record observations in the LIS.
7	Re-incubate CO_2 plates for an additional 48 hours. Re-incubate O_2 plate for an additional 24 hours.
8	If applicable, after 48 hours, examine SAB plate for white, creamy colonies resembling yeast.
9	At 48 hours, examine plates and record observations in the LIS.
10	At 72 hours, examine plates and record observations in the LIS.
11	Examine anaerobic plates after 48 hours incubation and record observations in the LIS. Re-incubate BRU anaerobically for an additional 72 hours. After 5 days, examine plate and record observations in the LIS.

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 aerobic plates at 24 hours, 48 hours and 72 hours for growth.
3	Observe anaerobic plate at 48 hours. Examine under stereoscope.
4	Thayer Martin needs to be set up on these specimens for the detection of *Neisseria gonorrhoeae*.
5	Due to the invasive nature of these specimens, they are processed as fluids, deep wounds or tissue culture. Refer to: • MIC33100 – Deep Wound Culture • MIC34100 – Body Fluid Culture • Tissue Culture – Refer specimen to DynaLIFE
6	All organisms grown from sterile specimens should be reported. Some of the common pathogens from these sites include: • *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 Gram-negative bacilli for *Shigella* spp. or other enteric pathogens, especially from pediatric patients.
7	Refer to Deep Wound Culture and Body Fluid Culture for susceptibility reporting.

REPORTING RESULTS:

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: **"Mixture of commensal genital flora"** • List quantitation. • Report: **"No Neisseria gonorrhoeae isolated"**
Mix of enteric Gram-negative bacilli	• Report: **"Mixture of coliform organisms"** • List quantitation. • Report: **"No Neisseria gonorrhoeae isolated"**
Mix of anaerobic organisms	• Report: **"Mixture of anaerobic organisms"** • List quantitation. • Report: **"No Neisseria gonorrhoeae isolated"**
Growth of pathogen(s)	• Report organism 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. • Freeze isolate and log into stored isolates binder.

Listeria monocytogenes isolated	• Add organism: **"Listeria monocytogenes"** • List quantitation. • Report susceptibility results as per ASTM. • Go to 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 chocolate agar 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