

I. Introduction

Genital tract infections are caused by a variety of microbial agents, including some that can be detected by culture, wet mount, darkfield exam, or gram stain. Routine culture of the genital tract, particularly of the lower tract (vulva, vagina, cervix, and urethra), requires the selection and differentiation of potential pathogens from normal flora, especially in the female. Vaginal discharge from women who are older than puberty but pre-menopausal contains 10^8 - 10^9 CFU/mL of aerobic and anaerobic organisms. Factors such as pH, age, hormonal changes, hospitalization, instrumentation (IUD's, surgical manipulations, and childbirth), pregnancy, and immunosuppression affect the types of organisms present in the normal flora.

A. Specimen types

Specimen types of genital origin include, Bartholin cyst or abscess, cervix, IUD, ovarian abscess, pelvic abscess, penis, placenta, surgical wounds, tubo-ovarian abscess, urethra, uterine drainage, vagina, vulva.

B. Organisms

1. Normal flora

Alpha hemolytic streptococci	<i>Lactobacillus</i>
<i>Corynebacterium</i> species	<i>Staph</i> species not <i>aureus</i>

2. Potential Pathogens

Anaerobic bacteria (bring up on Rounds)	
<i>Enterococcus</i>	<i>Listeria monocytogenes</i>
Gram negative rods	<i>Neisseria gonorrhoeae</i>
Group A streptococcus	<i>Staph aureus</i>
Group B streptococcus	Yeast

II. Specimen Processing

A. GC Screen, including throat and rectal swabs

1. Chocolate/TM is incubated at 35°C in CO₂ for 48 h.
2. Gram stain – refer to Gram Stain Procedure.

B. Group B Beta strep screen

1. Strep B Carrot Broth is incubated for up to 24 h at 35°C in ambient air.
2. Sub-culture negative Carrot broth to GBS Detect, and incubate at 35°C in ambient air for 18-24 h.
3. No direct Gram stain is performed.

C. Routine Genital Culture

1. BAP and Chocolate/TM are incubated at 35°C in CO₂ for 48 h.
2. Add Strep B Carrot broth to cultures from women less than 50 years old.

3. Sub-culture negative Strep B Carrot Broth to GBS Detect plate, and incubate at 35°C in ambient air for 18-24 h.
4. Gram stain – refer to Gram Stain Procedure.
- D. Staph aureus (request to rule out Toxic Shock Syndrome)
 1. BAP and CNA are incubated at 35°C in ambient air for 48 h.
- E. Placenta
 1. BAP incubated at 35°C in CO₂ for 48 h.
 2. Strep B Carrot Broth is incubated for up to 24 h at 35°C in ambient air.
 3. Sub-culture negative Strep B Carrot Broth to GBS Detect, and incubate at 35°C in ambient air for 24 h.
 4. Gram stain – performed only on placentas obtained by C-section. Refer to Gram Stain Procedure for tissues.
- F. Wounds, Tissues, and Fluids of “Genital” Origin Culture Work-up
 1. BAP, CNA, chocolate, and MAC are incubated at 35°C in CO₂ for 48 h
 2. A BAP is incubated anaerobically at 35°C for 48 h for wounds and 5 days for tissues or sterile site fluids.
 3. Gram stain – refer to Gram Stain Procedure.
- G. Anaerobic Cultures on Genital Specimens
 1. An anaerobic BAP is included on wounds, tissues, and fluids from genital sites.

III. *Neisseria Gonorrhoeae* Culture Work-up

- A. At 24 h incubation, examine chocolate and TM for colonies resembling *N. gonorrhoeae* (small, gray, glistening colonies on chocolate and TM plate – some isolates fail to grow on TM).
 1. If no *N. gonorrhoeae* is present:
 - a. Report: **No growth to date.**
 - b. Re-incubate the plates.
 2. If potential *N. gonorrhoeae* is present:
 - a. Perform an oxidase on all suspicious colony types.
 - b. If the oxidase is positive, perform a Gram stain.
 - c. If the Gram stain shows gram-negative diplococci, perform the [CarboFerm Neisseria Test](#) and the [Superoxol Test](#) in a biological safety cabinet.
 - i. *N. gonorrhoeae* is glucose positive and is negative for maltose, sucrose, and lactose and produces a strong positive to the Superoxyl test.
 - ii. Notify Rounds before reporting *N. gonorrhoeae* isolated from non-genital sites or from children <15 years old.
 - d. If *Neisseria gonorrhoeae* is isolated, report: ***Neisseria gonorrhoeae***.
 - i. Notify Rounds.
 - ii. Notify the DOH.
- B. At 48 h incubation, examine chocolate and TM for colonies resembling *N. gonorrhoeae*.
 1. If no *N. gonorrhoeae* is present, report: **No *Neisseria gonorrhoeae* isolated.**
 2. If potential *N. gonorrhoeae* is present, refer to **III.A.2.** above.
 3. If yeast is present:
 - a. Perform a wet prep (unless feet are present).

- b. Report: **Yeast**
- c. Hold the plate for 7 days.

IV. Group B Streptococcus Culture Work-up

Screening cultures for group B strep should be performed on vaginal/rectal specimens. If the source description is not appropriate, report the following comment: **Culture screening for group B strep should include both vaginal and rectal specimens from all pregnant women at 35-37 weeks gestation. Vaginal/rectal sampling increases the recovery of group B strep by 40%, compared to vaginal sampling alone. [VAGR]**

A. Following incubation:

1. If Strep B Carrot Broth turns positive in 6-24 h, report: **Positive for Group B Strep. [GBP]** The following susceptibility comment is attached to positive results: **Group B Strep is predictably susceptible to penicillins and cephalosporins. Surveillance testing of regional isolates has shown 20% and 36% resistance to clindamycin and erythromycin, respectively. Please request testing of this isolate if these agents may be prescribed. [BSBS12]**
2. Subculture negative Strep B Carrot Broth to GBS Detect at 18-24 h.
3. Incubate the GBS Detect for 18-24 h at 35°C.

B. After 18-24 h, examine the GBS Detect plate for beta-hemolytic strep.

1. If no beta hemolytic strep is isolated, report: **Negative for group B strep.**
2. If beta-hemolytic strep is present, perform a group B [Strep Latex Agglutination Test](#).
 - a. If the latex test is negative, report: **Negative for group B strep.** Latex testing should also be performed to rule out group A strep.
 - b. If the latex test is positive, report: **Positive for Group B Strep. [GBP]** The following susceptibility comment is attached to positive results: **Group B Strep is predictably susceptible to penicillins and cephalosporins. Surveillance testing of regional isolates has shown 20% and 36% resistance to clindamycin and erythromycin, respectively. Please request testing of this isolate if these agents may be prescribed. [BSBS12]**
 - i. Report the following comment on positive screens from women 50 years of age or older and for all positive routine genital cultures on females of any age: **Vaginal colonization with group B strep frequently occurs and is not a significant finding in women who are not pregnant. Colonization is significant in pregnant women because it is a major risk factor for early onset group B strep disease in infants. [VAG50]**
 - ii. Hold the plate for 7 days.

V. Genital Culture

- A. Examine the CHOC and TM for *N. gonorrhoeae*. Refer to **III.A.-B** above.
- B. Examine the BAP, CHOC, and TM for yeast.
 1. At 24 h incubation:

- a. If yeast is present:
 - i. Perform a wet prep (unless feet are present).
 - ii. Hold the plates for 7 days.
 - iii. Report: **Yeast**.
- b. If no yeast is present:
 - i. Re-incubate the plates.
- 2. At 48 h incubation:
 - a. If yeast is present, refer to **V.B.1.a.** above.
 - b. If no yeast is present, report **No Yeast Isolated**.
- C. Examine Strep B Carrot broth for color change and subculture to GBS Detect plate if negative. Refer to **IV.A.-B.** above.
- D. Examine BAP for possible group A strep.
 - 1. At 18-24 h incubation examine plate for beta-hemolytic colonies with hard hemolysis suggestive of group A strep. Perform a group A [Strep Latex Agglutination Test](#) on suspect colonies.
 - a. If the latex test is negative, do not report the absence of beta-hemolytic strep.
 - b. If the latex test is positive, report: **Beta Streptococcus Group A. [BSGA]**
 - c. The following susceptibility comment is attached to positive results: **Group A Strep is predictably susceptible to penicillins and cephalosporins. Surveillance testing of regional isolates has shown 4% resistance to both clindamycin and erythromycin. [BSAS12]**
- E. Be sure the Gram stain for bacterial vaginosis has been reported.

VI. Staph aureus Work- up (for requests to rule out Toxic Shock Syndrome)

- A. At 24 h incubation:
 - 1. Examine the BAP and CNA for the presence or absence of *Staph aureus*.
 - a. If no *Staph aureus* is present, reincubate the plate and report: **Negative to date.**
 - b. If *Staph aureus* is present:
 - i. Identify presumptive *S. aureus*.
 - ii. Do not perform susceptibility testing.
- B. At 48 h incubation:
 - 1. If no *Staph aureus* is present, report: **No Staph aureus isolated.**
 - 2. If *Staph aureus* is present, see **VI.1.b.** above and report: **Staph aureus.**
 - a. Isolate must be sent out for toxin testing to Focus.
 - b. Order "Toxic-shock Syndrome Toxin Panel, MAID". This panel detects the toxins associated with toxic shock syndrome: TSST-1, enterotoxin B (SEB), and enterotoxin C (SEC).

VII. Placenta

- A. At 24 h incubation, examine the original BAP for the presence of group B strep and *Listeria* species. Examine Strep B Carrot Broth for color change.
 - 1. If no group B strep or *Listeria* species are present, re-incubate the plate and report: **No growth to date.**
 - 2. If group B strep or *Listeria* species are suspected:

- a. Identify and report the presumptive isolate.
 - b. Do not perform susceptibility testing.
 - c. Notify Rounds if *Listeria* is isolated.
 - d. Send the isolate to the DOH.
3. Transfer the negative Strep B Carrot Broth to a GBS Detect plate and incubate for 18-24 h at 35°C. Refer to **II.** above.
- B. At 48 h incubation:
1. Examine the original BAP for the presence of *Listeria* species.
 - a. If no *Listeria* species is present, discard original BAP and report: No *Listeria* species isolated
 - b. If *Listeria* species is suspected see **VII.A.2.**
 2. Examine the GBS Detect plate and the original BAP for the presence of group B strep see **IV.B.** above.
 3. Bring any placenta isolates up on Rounds.

VII. Wounds, Tissues, and Fluids of “Genital” Origin Culture Work-up

- A. Work-up and Reporting
1. See the **Wound and Tissue Culture** procedures for culture interpretation, workup and reporting.
 2. If normal vaginal flora is present, report: **Mixed flora.**
 3. If normal flora is present from a penile drainage and/or a mixture of potential pathogens is present, report: **Mixed flora including (list potential pathogens).**

VIII. Anaerobic Cultures on Genital Specimens

- A. Routinely anaerobic cultures are performed on pelvic abscesses, genital sites (especially if Actinomyces is suspected), amniotic fluid, endometrium, tubo-ovarian/ovarian abscesses and uterine drainage.
- B. All anaerobic cultures from “cervical” or “vaginal” sites as well as mixed anaerobic cultures should be brought up on Rounds

IV. References:

Murray et. al. 2003. Manual of Clinical Microbiology, American Society for Microbiology, 8th Edition.

Document Control

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05/27/2011 Updated for Carrot Broth and GBS Detect agar. 01/27/2012

Updated beta strep susceptibility comments. 11/29/2012 Updated for

reporting if no yeast isolated. Clarified culture for *S. aureus* for request to r/o TSS only.