I. GENERAL PRINCIPLE

Accurate diagnosis of genital infections depends on the separation of pathogens from normal flora. The female genital tract is colonized by a wide array of organisms. However, many infections arise from this normal flora upon activation by patient condition and history, and other organisms; and in some instances, it is just as important to identify the carrier state, regardless of patient symptoms. For example, Group B streptococcus isolated from the vagina during late pregnancy is considered a risk factor for the successful outcome of the newborn. In contrast, the male urethra usually contains just a few organisms normally found on the skin. Non-culture methods of detecting some genital infections or syndromes are available and, in some instances, are actually the best choice for diagnosis. Recovery of specific pathogenic organisms depends on culture of the proper specimen, with special care taken to exclude normal flora.

II. SPECIMEN COLLECTION

A. GENERAL INSTRUCTIONS FOR CULTURE

1. Use specimen collection/transport system appropriate for suspected pathogen.
   - Bacteria and fungus -- Aerobic culturette. Squeeze sponge in the bottom of the culturette to moisten the swab after sampling.
   - *Neisseria gonorrhoeae* -- Sterile swab. For optimal recovery of this pathogen, media warmed to room temperature should be inoculated at the bedside. Notify laboratory before collection to obtain media.
   - Anaerobes -- Anaerobic culturette. Vaginal specimens are not acceptable for anaerobic culture and any specimen collected from higher in the female genital tract should be collected through a catheter to avoid contamination with vaginal flora.
   - Chlamydia and/or viral -- Sterile swab placed in a viral transport media at the bedside. Contact laboratory before collection procedure to obtain the transport vial.

2. Every genital culture should be accompanied by a separate swab or smear for gram stain.

3. Indicate any specific organism suspected, as well as any pertinent clinical history, such as presence of an IUD; pregnant or postpartum female; sexual partner positive for STD; suspected sexual abuse; etc.

4. Any growth from a specimen collected with a syringe will be considered significant.

5. Specimens collected for GROUP B STREPTOCOCCUS SCREENING MUST BE SPECIFICALLY IDENTIFIED AS SUCH. These should not be ordered as vaginal cultures.
B. GENERAL INSTRUCTIONS FOR SCREENING TESTS

1. Microscopy
   a. Some infections can be diagnosed immediately by simple microscopy.
   b. Syphilis -- Fluid expressed from a genital surface lesion can be examined by darkfield microscopy for the presence of *Treponema pallidum*. This lab currently does not have equipment to perform darkfield microscopy, so diagnosis of syphilis must be by other means.
   c. *Neisseria gonorrhoeae* -- For males only, the observation of intracellular diplococci on a gram stained smear of urethral discharge is considered diagnostic. Sample the urethral discharge with a sterile swab and prepare a smear at the bedside by rolling the swab over the surface of a clean glass slide. Do not smear the sample over the slide and do not coverslip.
   d. *Trichomonas vaginalis* AND/OR Yeast -- A wet prep can be examined microscopically for the presence of budding yeast and/or a motile flagellate. Vaginal discharge is usually collected on a swab and placed directly into a clean tube containing a small amount of sterile saline, mixed well, and delivered to the laboratory. Urethral discharge from a male is not an acceptable specimen for wet prep. For a male, urine is the better specimen for identifying *Trichomonas* sp. and a yeast infection in a male is uncommon.

2. Group B Strep Screen
   a. CDC guidelines suggest specimen collection at 35-37 weeks gestation. The later in pregnancy that cultures are performed, the closer the correlation with intra-partum conditions.
   b. The optimal method for GBS screening is the collection of two separate swabs from the distal vagina and the anorectum. These specimens should be accompanied by one request only and include weeks gestation and penicillin allergy history.
   c. **CERVICAL CULTURES ARE NOT ACCEPTABLE.**
   d. Insert culturette swab into the rectum, hold in place for a few seconds to absorb fluid, withdraw, remove plastic cap and place swab in the plastic sleeve. Squeeze sponge to moisten swab.
   e. The vaginal swab should be collected without the use of a speculum. Insert culturette swab is into the lower vagina, hold in place for a few seconds to absorb fluid, withdraw, remove plastic cap and place swab in the plastic sleeve. Squeeze sponge to moisten swab.
C. GENERAL INSTRUCTIONS FOR GEN-PROBE TEST

1. GEN-PROBE collection device is unisex for female endocervical and male urethral specimens. A separate GEN-PROBE collection device is available for Chlamydia / Gonorrhoea detection from a urine specimen.

2. Unisex collection device contains transport media and a swab. Use enclosed swabs only. Transport and store at 2-30°C. Testing must occur within 60 days of specimen collection.

3. Females – endocervical collection
   a. Use white shaft swab labeled “cleaning swab – not for specimen collection” to remove excess mucus from cervix and discard.
   b. Insert second swab (blue shaft) from collection kit about 1 to 1.5 cm into endocervical canal.
   c. Rotate swab clockwise for 10-30 seconds to ensure adequate sample.
   d. Withdraw swab carefully to avoid contact with vaginal mucosa.
   e. Insert swab into transport tube and snap off shaft at the score line.
   f. Recap tightly, label with patient name and DOB, and transport specimen to lab.

4. Males – urethral collection
   a. Patient should not have urinated for at least one hour prior to sample collection.
   b. Insert the swab (blue shaft) provided in the kit 2 to 4 cm into the urethra.
   c. Rotate clockwise for 2 to 3 seconds to ensure contact with all mucosal surfaces.
   d. Withdraw swab carefully to avoid contact with vaginal mucosa.
   e. Insert swab into transport tube and snap off shaft at the score line.
   f. Recap tightly, label with patient name and DOB, and transport specimen to lab.

5. Urine collection
   a. Patient should not have urinated for at least one hour prior to specimen collection. Female patients should NOT cleanse the labia prior to specimen collection.
   b. Instruct patient to collect only the first 20-30 ml of initial urine stream into a urine collection cup. Large volume collections may result in dilution and reduce test sensitivity.
   c. Transfer 2 ml of urine into the urine specimen transport tube using the disposable pipette provided in the package. The correct volume has been added when the fluid level is between the black lines on the tube label.
   d. Alternatively, urine in the original specimen collection container may be transported to the lab either refrigerated or at room temperature. Transfer to the GEN-PROBE transport tube must be within 24 hrs of specimen collection. Processed urine (urine in transport tube) must be tested within 30 days of collection.

D. COLLECTION INSTRUCTIONS for CULTURE BY SITE -- FEMALE

1. Vagina -- Use a speculum without lubricant. Collect mucosal secretions from high in the vaginal canal with a sterile pipette or swab.
2. Cervix -- Do not use lubricant. Wipe the cervix clean of vaginal secretions and mucus. Rotate a sterile swab to obtain exudate from the endocervical glands; or if no exudate is present, rotate swab in the endocervical canal.

3. Bartholin gland -- Decontaminate the skin and aspirate material from the duct.

4. Endometrium -- Collect by transcervical aspiration through a telescoping catheter.

5. Fallopian tube -- Swab or preferably an aspirate obtained during surgery.

6. Amniotic fluid -- Aspirate by catheter, amniocentesis, or at C-section.

7. Vulvar lesion -- Clean lesion with saline and remove crust, if present. Scrape lesion until serous fluid emerges, then wipe away fluid and debris. Press base of lesion until fluid is expressed. Aspirate with a fine gauge needle, OR unroof the vesicle and collect fluid with a sterile swab for viral culture, OR scrape the base of an open vesicle with a sterile blade and then rub vigorously with sterile swab.

8. Urethra -- Wait at least 1 hour after urination to collect specimen. Collect discharge with a sterile swab by gentle massage of urethra against the pubic symphysis through the vagina. If no discharge is obtained, cleanse external urethra with betadine soap and rinse with water. Insert a female urogenital swab 2-4 cm into the urethra, gently rotate and leave in place for 1-2 seconds before withdrawing.

E. COLLECTION INSTRUCTIONS for CULTURE BY SITE -- MALE

1. Urethra -- Wait at least 2 hours after urination to collect specimen. Cleanse head of penis and then collect discharge with sterile swab by gently squeezing penis with a milking motion. Or if no discharge is present, insert a male urogenital swab 2-4 cm into the urethra, gently rotate, leave in place 1-2 seconds, and withdraw.

2. Prostate fluid -- Perform digital massage through rectum and collect fluid in a sterile tube or swab; OR specimen may be collected during surgery.

3. Epididymis -- Needle and syringe aspirate.

4. Penile lesion -- Cleanse lesion surface with saline, and remove crust if present. Scrape lesion until serous fluid is obtained, then wipe away fluid and debris. Press lesion base until fluid is expressed. Aspirate with a fine gauge needle; OR unroof the vesicle and sample with a sterile swab for viral culture; OR scrape base of open vesicle with a sterile blade, then rub vigorously with sterile swab.

III. TRANSPORT

A. DO NOT REFRIGERATE ANY GENITAL SPECIMENS FOR ROUTINE CULTURE. Many of the pathogens are sensitive to cold and recovery of organisms will be severely compromised by exposure to refrigeration or a cold environment.

B. TRANSPORT ALL GENITAL SPECIMENS TO THE LAB AS RAPIDLY AS POSSIBLE. The same pathogens are also fastidious in their nutritional and atmospheric requirements and are
susceptible to the effects of drying. They live on mucous membranes and transport media is not a good substitute.

C. Specimens in saline for wet prep must arrive in the lab in less than 1 hour after collection, as organisms lose their motility. Do not refrigerate.

D. GEN-PROBE specimens should be transported refrigerate or at room temperature and tested within 30 days of collection for urine and within 60 days for genital specimens.

E. As always, transport specimens to the lab in a biohazard container or bag.

See original policy in the Laboratory for all documented biennial reviews.

REFERENCES

Clinical Microbiology Procedures Handbook; 1992; Isenberg; American Society for Microbiology.


Package inserts.