Proc.# **2013.005**

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| Procedure | Gential Tract Cultures |

**Principle**:

Specimens from genital sites are sent to the lab for detection of microoganisms from females presenting with clinical syndromes such as cervicitis, vulvovaginits, urethritis, bacterial vaginosis, salpingitis, endometritis or genital ulcers, and from males exhibiting urethritis, epididymitis, prostatitis, or genital ulcers.

Accurate diagnosis depends on the separation of microbial pathogens form the normal genital microbiota. Proper collection of specimens is also needed to ensure recovery of pathogenic organisms. See references for a listing of pathogens from specific sites.

**Specimen**:

1. ***Patient Preparation***:
2. ***Type of Specimen***:
3. ***Specimen Handling***:

**Equipment and Materials**:

1. ***Equipment***: 35°C incubator with a candle jar or other mechanism of generating a CO2 atmosphere.
2. ***Materials***:      Specimens are setup for Grp B Strep screens with LIM broth.
3. ***Materials*** ***Preparation***:
4. ***Performance Parameters***:
5. ***Storage Requirements***:

**Calibration**: Not applicable

**Quality Control**: See media Quality Control procedure.

**Procedure**:

1. **Specimens and sites of collection**

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| **Site** | **Specimen** |
| Amniotic fluid........................ | Catheter aspirate, amniocentesis fluid, swab from C-section |
| Bartholin gland..................... | Duct aspirate in syringe or on a swab |
| Cervix.................................. | Swab of endocervical canal |
| Endometrium....................... | Transcervical aspirate obtained via telescoping catheter |
| Fallopian tubes................... | Abscess aspirate, culdocentesate swab obtained during pelvic surgery |
| Genital ulcer........................ | Dry or moistened swab of ulcer base, bubo aspirate |
| IUD...................................... | IUD, associated secretions and pus |
| Vagina................................. | Discharge on swab, swab from posterior vaginal vault or vaginal orifice |
| Urethra, female................... | Discharge on swab, Ca alginate swab in absence of discharge |
| Urethra, male...................... | Expressed exudate on swab or Ca alginate swab from distal urethra |
| Vesicle ................................ | Vesicular material in syringe or swab |
| Epididymis........................... | Calginate swab of urethra |
| Penile discharge.................. | Discharge on swab |
| Prostate............................... | Expressed prostatic secretions on swab or in cup |
| Testicle................................ | Syringe containing (abscess) aspirate, aspirate on swab |

1. **Media setup**:
   1. Set up media according to the chart in the Media Setup procedure (1008.100). If a specimen is specifically collected for anaerobes, set up anaerobe media also.
   2. Specimens must be inoculated onto media warmed to room temperature to enhance the growth of gonococcus.
   3. On the MTM media, use a “Z” pattern to inoculate and then cross streak closely (1008.103).
   4. Incubate all plates at 35 –36°C in a CO2 atmosphere. Hold plates for 3 days before issuing final report. If actinomycete is suspected, plates should be held up to 14 days before issuing final report.
2. **Direct Smear**
   1. Prepare a smear by rolling (rather than rubbing) a swab on a clean slide. This distributes pus cells in a monolayer, permitting accurate observation of intracellular organisms.
   2. If KOH prep or wet mount is ordered, smear the swab on a second slide and proceed as in 1008.117.
   3. Gram stain smear and observe for gram negative intracellular diplococci, WBC, epithelial cells and other microbial flora. Note the absence or presence of both epithelial & WBC. Gram neg diplococci should be reported (when present) as “intra(extra)cellular gram negative diplococci resembling gonococci found.”
   4. Also observe vaginal/cervical smears for yeast and clue cells and make note of these on report.
   5. Direct smears are done on all genital specimens when material submitted is adequate except for marketing specimens. On marketing specimens direct smears are to be made and filed in the gram stain box for use if the bacti tech determines a need for direct smear results when interpreting the culture.
3. **Examination of culture media**
   1. Correlate varieties and quantities of colonial morphotypes with direct smear gram stain results. Consider the presence of epithelial cells and/or WBC to determine the significance of growth.
   2. All suspicious colonies for gonococcus should be checked by oxidase and gram stain. If organism is oxidase positive and gram stain shows gram negative diplococci, setup up an identification for Neisseria.
   3. Follow the guidelines in “Interpretation of genital cultures by specimen source” when reporting culture results.
4. **Interpretation of Genital Cultures by Specimen Source**
   1. ***Amniotic fluid:*** Consider any organism present in any quantity in pure culture from a properly collected specimen as a significant pathogen. Organisms most commonly noted include *S. agalactiae, S. pyogenes, L. monocytogenes, N. gonorrhoeae*, and others.
   2. ***Bartholin gland, duct, abscess:*** 
      1. Presence of mixed organisms associated with normal vaginal microbiota, and the presence of epithelial cells with the absence of WBC on direct smear suggests a poorly collected specimen.
      2. Identify the organisms, and report the presence and quantity of any of the following aerobic bacteria in pure culture: *S. aureus*, streptococci, *E. coli, G. vaginalis, H. influenzae, P. mirabilis, N. gonorrhoeae*.
   3. ***Cervix, endocervix, endocervical canal:*** 
      1. The presence of mixed vaginal microbiota and epithelial cells with absence of WBC suggest vaginal tract contamination. Consider selectively for presence of the following: *S. agalactiae, N. gonorrhoeae*.
      2. Identify, semiquantitate, and report the presence of any organism in pure or predominant culture when the direct smear correlates with the culture and includes WBC.
   4. ***Endometrium:***
      1. The presence of mixed vaginal microbiota and epithelial cells with absence of WBC suggest vaginal tract contamination. Identify and report the presence of *N. gonorrhoeae* in any amount.
      2. Identify, quantitate, and report the presence of a single aerobic isolate including the following: *Enterococcus* sp., *S. agalactiae, S. pyogenes, L. monocytogenes*, Enterobacteriaceae, *G. vaginalis, Haemophilus* sp., *N. gonorrhoeae*.
      3. See anaerobe procedures for workup if requested.
   5. ***Epididymis, abscess***
      1. If direct smear shows urethral microbiota, epithelial cells, and absence of WBC, report descriptively, and semiquantitate. Any *N. gonorrhoeae* is significant.
      2. Identify, semiquantitate, and report as significant growth when predominant or in pure culture: *N. gonorrhoeae*, Enterobacteriaceae or *Pseudomonas* sp., other miscellaneous isolates in pure culture when WBC are observed in direct smear.
   6. ***Genital Ulcer:***
      1. Presence of skin, vaginal, or urethral normal microbiota in a specimen suggests contamination. However, selectively observe for presence of *Candida* sp., *N. gonorrhoeae*, beta hemolytic streptococci, and/or *H. ducreyi*.
      2. Presence of large amounts of organisms that correlate with direct smear should be reported.
   7. ***Orchitis, testicle:***
      1. Presence of mixed skin or bowel microbiota suggests contamination. Report descriptively, and quantitate.
      2. Presence of pure or predominant culture of gram negative rods, staphylococci, or streptococci requires identification, semiquantitation, and reporting.
   8. ***Pelvic infection associated with IUD:***
      1. The presence of mixed vaginal microbiota and epithelial cells with absence of WBC suggest vaginal tract contamination. Report descriptively.
      2. Presence of isolate in pure or predominant culture, with or without WBC in direct smear, is treated as significant.
      3. If actinomycete infection is suspected, see comment section.
   9. ***Prostate, prostatic massage secretions, prostatic fluid:***
      1. The presence of urethral and/or skin microbiota and epithelial cells with absence of WBC suggest contamination. Report descriptively.
      2. *N. gonorrhoeae* in any amount is significant.
      3. Presence of a single aerobic isolate, especially when correlated with the direct smear, is significant. Organisms commonly encountered include *Enterococcus* sp., Enterobacteriaceae, *Pseudomonas* sp, *S. aureus*.
   10. ***Salpingitis, fallopian tubes, PID***
       1. Identify, quantitate, and report as significant pathogens, endogenous aerobic agents recovered in pure or predominant culture.
       2. *N. gonorrhoeae* in any amt. is significant.
       3. Anaerobic organisms considered significant include *Bacteroides* spp. and *P. bivia*. See comment section for actinomycetes.
   11. ***Uretha, female & male***
       1. The presence of a mixture of aerobic organisms considered skin, fecal or vaginal microbiota in the absence of WBC on the direct smear suggests contamination. Report descriptively. *N. gonorrhoeae* in any amt. is significant.
       2. Identify, quantitate, and report as significant the presence of predominant organisms when a direct smear shows WBC.
   12. ***Vagina, vulva, vulvovagina***
       1. Interpretation of growth in vaginal cultures is guided by direct smear results.
       2. The presence of mixed bacterial morphotypes, including *G. vaginalis*, and squamous epithelial cells, and relative absence of WBC, suggest normal vaginal microbiota and should be reported as such.
       3. *Note*: presence of *G. vaginalis* (even in mixed culture) in children may indicate sexual abuse.
       4. The presence of clue cells and a mixture of short and curved gram negative bacilli and gram variable coccobacilli with reduced numbers of lactobacilli suggest bacterial vaginosis.
       5. Presence of yeast in any number is semiquantitated and reported.
       6. Presence of *N. gonorrhoeae* and/or *L. monocytogenes*  is significant.
       7. *S. aureus* may be considered part of the normal microbiota. Do not specifically identify except in following cases:
          1. If *S. aureus* is identified in a mixed culture in association with labial abscess or wound exudate, and if WBC are noted on direct smear, quantitate and report.
          2. *S. aureus* in mixed culture in females with TSS or vaginal ulceration may be significant.
       8. The presence of a single aerobic isolate, especially with correlation of microscopic morphology and presence of WBC on direct smear is considered significant.

**Calculations**: Not applicable

**Reporting Results**: The microbiology DMS and/or LIS system is used to report results.

**Medical Alert Values**: None defined at PLH. Use prudent judgment when calling results. Any isolates of *N. gonorhoeae* are reported to the SDSHL by the Infection Control department.

**Notes**:

1. ***Reference Ranges***: Reports must be correlated with clinical picture by physician
2. ***Abnormal Results***: See above
3. ***Reporting Format***: See reporting results section

**Limitations**:

1. If an actinomycete infection is suspected, this should be indicated by physician on request. These plates are held up to 14 days before issuing a final report. Any IUD submitted for culture should be processed for actinomycetes irregardless of physician request. See references for colony morphology etc. Suspected organisms will need to be submitted to reference lab for identification.
2. If requests are made for *C. trachomatis*, specimens are referred to SDSHL for direct antigen assay or to CLM for culture if indicated. All requests for *C. trachomatis* on suspected sexual abuse need to be referred for culture in stead of or in addition to the Chlamydiazyme test.
3. If viral agents are suspected, refer to CLM handbook for acceptable specimens for viral detection.
4. *Mycobacterium* cultures are referred to SDSHL.
5. *Gardnerella vaginalis* will grow on BAP and CNA in 24-48 hr and appears as tiny pinpoint colonies. This organism may also be detected by the presence of clue cells on a wet mount prep or gram stain. Presumptive identification of *G. vaginalis* can be made on the basis of a gram stain (small gram neg to gram variable rods) and beta hemolysis on V agar.
6. Any concerns or questions concerning cultures should be referred to section technologist/patholgist. Be sure and refer to the references for additional information.

**References**:

1. Isenberg, Henry D. Editor in Chief*, Clinical Microbiology Procedures Handbook*, ASM, Washington, DC, 1992.
2. Baron, Ellen Jo, L. R. Peterson, S. M. Finegold, *Diagnostic Microbiology*, 9th ed., CV Mosby, St. Louis, 1994.
3. Murray, Patrick, et al, Ed., *Manual of Clinical Microbiology*, ASM, Washington, DC. 1995.

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