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Management System\UHN_Mount Sinai Hospital Microbiology\Standard Operating Procedures\Bacteriology Procedures\
INTRODUCTION

I. Introduction

Organisms which are associated with infection or disease of the genital tract include *Neisseria gonorrhoeae* (GC), organisms associated with bacterial vaginosis (including *Gardnerella vaginalis, Mobiluncus* spp. and others), *Chlamydia trachomatis* (CT), *Haemophilus ducreyi*, yeasts, *Trichomonas vaginalis* and viruses such as Herpes simplex virus (HSV). Isolation or detection of other organisms such as Group A streptococcus, Group B streptococcus, *Staphylococcus aureus*, and others may be associated with certain specific clinical syndromes or risk of infection in the neonate (e.g. Group B streptococcus).

Proper handling, transport, processing and plating of specimens with selective, non-selective and enriched media, and incubating under specific environmental conditions will facilitate the recovery of fastidious genital tract pathogens such as *Neisseria gonorrhoeae*.

Requests for HSV or other viruses should be forwarded to the Virology section for processing.

Lower Genital Tract Infections

Infections of the lower genital tract (vulva, urethra, vagina and cervix) are generally caused by organisms acquired through sexual contact (GC, *Trichomonas vaginalis*, CT) or those which may be part of the normal vaginal flora (yeasts and those associated with bacterial vaginosis).

Specimens included in this section:

Bartholin’s abscess swab / aspirate see
- Cervical swabs
- Group B streptococcus screen
- Vaginal swabs for screen or culture
- Urethral swabs (Male or Female)
Upper Genital Tract Infections

Infection of the upper genital tract (uterus, fallopian tubes, and ovaries) may be caused by organisms that are part of the normal vaginal flora (Enterobacteriaceae, anaerobes) and/or those organisms acquired through sexual contact.

Specimens included in this section:

- Endometrial biopsies and curettings
- Cul de Sac/transvaginal aspirates
- Fallopian tube and Tubo-ovarian abscess
- Uterine swabs

Other Genital Tract Infections

Other genital tract infections include infections associated with Intra-uterine devices (IUDs), placentas, prostate glands and genital ulcers.
LOWER GENITAL TRACT

CERVICAL (ENDOCERVICAL) SWAB

I. Introduction

The recognized agents of cervicitis are *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT) and *Herpes simplex* virus (HSV). A Gram stain is not reliable for the presumptive diagnosis of GC cervicitis because of its low sensitivity and specificity.

For GC/CT and HSV tests, refer to the.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of specimens:

See Specimen Processing Procedure

a) Direct Examination: Not indicated.

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂  35°C x 72 hours</td>
</tr>
<tr>
<td>Martin-Lewis Agar (ML)</td>
<td>CO₂  35°C x 72 hours</td>
</tr>
</tbody>
</table>

If Group B streptococcus is requested, refer to the Group B streptococcus screen section.
B. Interpretation of culture:

   a) Examine CHOC and ML plates after 24, 48 and 72 hours incubation for colonies suspicious of GC. At 24 hours, if there is no visible growth observed, return plate quickly to the incubator to minimize loss of viability in the absence of CO₂.

   b) For GC work-up, refer to the Bacteria and Yeast Work-up Manual.

C. Susceptibility testing:

   Send to PHOL for susceptibility testing.

V. Reporting Results

Negative Report: “No Neisseria gonorrhoeae isolated”.

   If ML plate is overgrown by swarming Proteus or yeast, report ONLY as “Unable to rule out Neisseria gonorrhoeae due to bacterial/yeast overgrowth.”

Positive Report: “Neisseria gonorrhoeae” “isolated” (do not quantitate), “~susceptibilities from Public Health Laboratory to follow”

Telephone all positive GC cultures to floor/ordering Physician.
Refer to "Isolate Notification and Freezing Table QPCMI16003”

For all positive GC cultures, send a Communicable Disease Report to the Medical Officer of Health. Refer to Communicable Disease Results Reporting Process QPCMI17000 and Reportable Diseases to the Medical Officer of Health QPCMI17001.

VI. References


UNIVERSITY HEALTH NETWORK/MOUNT SINAi HOSPITAL, DEPARTMENT OF MICROBIOLOGY
Vol. 1 ASM Press, Washington, D.C.

Cumitech 17A, 1993. Laboratory Diagnosis of Female Genital Tract Infectious, ASM Press.

GROUP B STREPTOCOCCUS SCREEN

I. Introduction

Many women carry Group B streptococcus (Streptococcus agalactiae) in their vagina or large bowel. This organism may be transmitted to the neonate as it passes through the birth canal, resulting in potentially devastating systemic disease in the newborn.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure

   a) Direct Examination: Not indicated.

   b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot Broth for Group B Strep (CAROT)</td>
<td>O₂, 35°C x 24 hours</td>
</tr>
</tbody>
</table>

B. Interpretation of culture:

   a) Examine the CAROT broth after overnight incubation (10:30am each day) for orange or light orange colour.

   b) If broth is orange colour, set up Streptococcal grouping Latex Agglutination Test to identify Group B streptococcus. If agglutination test is negative for Group B, subculture broth to Colistin/Nalidixic Acid Agar (CNA) and incubate in O₂ at 35°C x 24 hours by 11:00am.

   c) For the colourless broths, bring them to the planting area for subculture by wasp.
i. A drop of the broth is put onto Colistin/Nalidixic Acid Agar (CNA) and incubated in O₂ at 35°C x 24 hours.

   d) In LIS “GBS New Worklist” mark each SUBCN plate and batch process those that were subcultured. Cancel label printing. Remark and Prelim all order marked. Incubate plate.

   Store colourless broths.

   e) After 24 hours incubation, set up Bile Esculin (BE) from colonies on the CNA which are suspicious of Group B (beta-haemolytic or non-haemolytic).

   f) Set up Streptococcal grouping Latex Agglutination Test on BE negative isolates to identify Group B streptococcus.

C. Susceptibility testing:

Refer to.

V. Reporting Results


Positive Report: “Group B Streptococci isolated.” do not quantitate; include ISOLATE COMMENT “This organism is intrinsically susceptible to penicillin. If treatment is required AND this patient cannot be treated with penicillin, please contact the Microbiology department within 48 hours to request sensitivity testing.”

Note: If GBS screen is requested on a cervical or vaginal swab, report the results with the following comment: “For optimal detection of Group B Streptococcus, a COMBINED recto-vaginal swab should be collected.”

Telephone all positive Group B Streptococcus for patients admitted to a ward / case room.
Refer to "Isolate Notification and Freezing Table QPCMI16003"

VI. References

National consensus statement on the prevention of early onset of Group B Streptococcal infection


VAGINITIS SCREEN

I. Introduction

The most common causes of adult vaginitis are Candida albicans, Trichomonas vaginalis, and bacterial vaginosis which can be diagnosed using a wet mount and gram stain. Routine cultures are not necessary. For pre-pubescent and post-menopausal patients, laboratory diagnosis of bacterial vaginosis has not been validated and interpretation of gram stain results needs to take this into account.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure

a) Direct Examination:

i. Wet mount: To be set up immediately. Gently press the swab into a drop of sterile saline on a slide. Place a cover slip on the slide and examine under the microscope using the 40X objective. Examine for the presence of Trichomonas vaginalis. Wearing of gloves is required while reading wet mounts.


Examine gram-stained slides under oil immersion (x1000).

I. Observe for the presence of the following morphotypes:

- Large gram-positive bacilli (Lactobacillus spp. morphotypes)
2. Quantitate each morphotype according to the following scale:
   - 0 = 0 cell in smear
   - 1+ = <1 cell per 1000x oil immersion field
   - 2+ = 1-4 cells per 1000x oil immersion field
   - 3+ = 5-30 cells per 1000x oil immersion field
   - 4+ = >30 cells per 1000x oil immersion field

3. Calculate a total numerical score by summing the scores for the three components as indicated in the following table and examples:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td>2</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>3</td>
<td>1+</td>
<td>3+</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>4+</td>
</tr>
</tbody>
</table>

**Total Nugent Score:**

- 0-3 = Normal
- 4-6 = gram stains shows altered vaginal flora not consistent with bacterial vaginosis.
- 7-10 = Bacterial vaginosis

**Examples:**

1. *Gardnerella* spp. 4+  
   *Lactobacilli* spp. 2+  
   *Mobiluncus* spp. 2+  
   Total score = 8 (Report as Bacterial Vaginosis)

2. *Gardnerella* spp. 2+  
   *Lactobacilli* spp. 2+  
   *Mobiluncus* spp. 1-2+  
   Total score = 5 (Report as altered vaginal flora not consistent with bacterial vaginosis)

3. *Gardnerella* spp. 2+  
   *Lactobacilli* spp. 3+  
   *Mobiluncus* spp. 0  
   Total score = 3 (Report as No bacterial vaginosis)
Note: The presence or absence of clue cells is not part of the Nugent score and not required for diagnosis.

V. Reporting Results

Wet Mount:

Negative Report: “No *Trichomonas vaginalis* seen.”
The following message will automatically be added to ALL negative reports: “(A negative result should NOT be used to rule out Trichomonas vaginalis given the poor sensitivity of this assay. A delay in transport and/or processing of this specimen further decreases this assay’s sensitivity.)”

Positive Report: “*Trichomonas vaginalis* seen.”

Gram Stain (Yeast results):

Negative Report: “No yeast seen”.
Positive Report: “Yeast seen.”

Gram Stain (Bacterial vaginosis results):

Negative Report: “No evidence of bacterial vaginosis seen”.

or
“Altered vaginal flora not consistent with bacterial vaginosis seen.”

For patients <12 and >60 years, add TEST COMMENT “Laboratory diagnosis of bacterial vaginosis has not been validated for pre-pubescent and post-menopausal patients; interpretation of such results needs to take this into account.” (Pick TEST COMMENT <12V or >60V from the keypad.)
VI. References


5. Mandell 5th Editional Principels and Practice of Infectious Diseases
VAGINAL CULTURE

I. Introduction

Vaginal infections are occasionally caused by *Staphylococcus aureus* and beta-hemolytic streptococci (not *S. anginosus (milleri)* group), and in children, *Salmonella* and *Shigella*. Vaginal culture can be used for diagnosis. *Neisseria gonorrhoeae* and (GC) and *Chlamydia trachomatis* (CT) will also cause vaginal infections but vaginal swabs are not the optimal specimen to detect these agents.

Toxic-shock syndrome may be associated with vaginitis or vaginal colonization due to *S. aureus* and beta-hemolytic streptococci (not *S. anginosus (milleri)* group). Vaginal culture may be helpful; positive cultures should be tested to determine if they are toxin-producing strains.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure

a) Direct Examination: Not required

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin Nalidixic Acid Agar (CNA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Carrot Broth for Group B Strep (CAROT)</td>
<td>O₂, 35°C x 24 hours</td>
</tr>
</tbody>
</table>

If *N. gonorrhoeae* is requested, add:

| Chocolate Agar (CHOC)                      | CO₂, 35°C x 72 hours |
| Martin-Lewis Agar (ML)                     | CO₂, 35°C x 72 hours |
B. Interpretation of culture:

a) Examine the CNA plate at 24 and 48 hours incubation for colonies suspicious of *S. aureus*, beta-hemolytic streptococci (not *S. anginosus* (milleri) group) (Refer to Bacteria Workup Manual for identification). If toxin testing is requested on *S. aureus* isolates, consult medical microbiologist before contacting PHOL for approval and freeze all toxin-producing strain.

b) Examine the CAROT broth after overnight incubation for orange colour.

c) If broth is orange colour, set up Streptococcal grouping Latex Agglutination Test to identify Group B streptococcus. If agglutination test is negative for Group B, subculture broth to Colistin/Nalidixic Acid Agar (CNA) and incubate in O2 at 35°C x 24 hours.

d) For the colourless broths, subculture a drop of the broth onto Colistin/Nalidixic Acid Agar (CNA) and incubate in O2 at 35°C x 24 hours.

e) Examine CHOC and ML plates at 24, 48 and 72 hours. For GC work-up, refer to Bacteria Workup Manual.

f) Examine MAC at 24 and 48 hours. Work-up oxidase-negative non-lactose-fermenters as per Bacteria Workup Manual.

C. Susceptibility testing

Refer to.

V. Reporting Results

Culture:

Negative Report: If toxic shock syndrome requested:

“No *Staphylococcus aureus* or beta-hemolytic streptococci isolated.”

If CHOC and ML are set up:

“No *Neisseria gonorrhoeae* isolated”.

If vaginal swab is received for GC culture on adults, report with comment: “The recommended specimen for *Neisseria*
A gonorrhoeae culture is an endocervical swab.”

If MAC is set up:
Report “No Salmonella or Shigella isolated.”
Positive Report: If toxic shock syndrome requested:
Report all significant isolates with appropriate susceptibilities (do not quantitate).

If CHOC and ML are set up:
“Neisseria gonorrhoeae isolated” (do not quantitate)

If MAC is set up:
Report all significant isolates with appropriate susceptibilities (do not quantitate).

Telephone all positive GC cultures to floor/ordering Physician. Refer to
"Isolate Notification and Freezing Table QPCMI16003"

For all positive GC cultures, send a Communicable Disease Report to the Medical Officer
of Health. Refer to "Communicable Disease Results Reporting Process QPCMI17000"
and Reportable Diseases to the Medical Officer of Health QPCMI17001.

VI. References


URETHRAL SWAB

I. Introduction

Urethritis is usually caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. Gonococcal urethritis can be diagnosed with excellent specificity by Gram stain of the urethral exudate.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

For *Chlamydia trachomatis*, refer to the PHOL courier section of the Send Out Manual.

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure

a) Direct Examination: Gram stain

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
<tr>
<td>Martin-Lewis Agar (ML)</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
</tbody>
</table>

B. Interpretation of culture:

a) Examine CHOC and ML plates after 24, 48 and 72 hours incubation for colonies suspicious of GC.
b) For GC work up, refer to Bacteria Workup Manual.

C. Susceptibility Testing:
   Refer to.

V. Reporting Results

Gram stain: Quantitate and report the presence or absence of pus cells and gram negative diplococci. Do not mention presence or absence of other bacterial morphologies

Culture:


If ML plate is overgrown by swarming Proteus or yeast, report ONLY as “Unable to rule out Neisseria gonorrhoeae due to bacterial/yeast overgrowth.”

Positive Report: “Neisseria gonorrhoeae” “isolated” (do not quantitate), “~susceptibilities from Public Health Laboratory to follow”

Telephone all positive GC cultures to floor/ordering Physician. Refer to: "Isolate Notification and Freezing Table QPCMI16003"

For all positive GC cultures, send a Communicable Disease Report to the Medical Officer of Health. Refer to "Communicable Disease Results Reporting Process QPCMI17000" and Reportable Diseases to the Medical Officer of Health QPCMI17001.

VI. References

PENIS SWAB

I. Introduction

Irritation and cellulitis of the penis can be caused by organisms that cause typical wound infections.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure

   a. Direct Examination: Gram stain.

   b. Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Colistin Nalidixic Acid Agar (CNA)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)*</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
<tr>
<td>Martin–Lewis Agar (ML)*</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
</tbody>
</table>

*Occasionally, urethral swabs may be labelled as penile swabs. Neisseria gonorrhoeae culture is set up for this reason.
The ML should be inoculated by rotating the swab in a Z streak manner. The inoculum is then streaked by the ISOplater to obtain discrete colonies.

B. Interpretation of Cultures:

Work up as per.

Examine CHOC and ML plate after 24, 48 and 72 hours incubation.

For GC work-up refer to Bacteria Workup Manual

C. Sensitivity Testing:

Refer to.

V. Reporting

Gram stain: Report with quantitation presence of pus cells and organisms.

Culture:

Negative Report: Report as per Superficial Wound Specimens. (DO NOT report "No GC isolated").

Positive Report: "Neisseria gonorrhoeae" “isolated” (do not quantitate), “~susceptibilities from Public Health Laboratory to follow”

Quantitate all other significant isolates with appropriate sensitivities as per Superficial Wound Specimens.

Telephone all positive GC cultures to floor/ordering Physician. Refer to Isolate Notification and Freezing Table QPCMI16003

For all positive GC cultures, send a Communicable Disease Report to the Medical Officer of Health. Refer to "Communicable Disease Results Reporting Process QPCMI17000"
and

Reportable Diseases to the Medical Officer of Health QPCMI17001.

VI. References


Cumitech 17A, 1993. Laboratory Diagnosis of Female Genital Tract Infectious, ASM Press.
SEMINAL FLUID

I. Introduction

Bacterial infections of the seminal tract have been postulated to potentially play a role in male infertility. Pathogens include Neisseria gonorrhoeae (GC) and Chlamydia trachomatis (CT). Other possible pathogens include Enterococci, S. aureus, Klebsiella species, Escherichia coli and other gram negative bacilli. Possible pathogens in seminal fluid at concentrations >10^6 CFU/L has been defined as “significant bacteriospermia” which may be associated with infertility. However, bacteria in these concentrations may also represent contamination given the circumstances of sample collection and colonization of the peri-urethral orifice.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure

a) Direct Examination: Not Required

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)*</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)*</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)(a,b)</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
<tr>
<td>Martin-Lewis Agar (ML)(a,b)</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
</tbody>
</table>

*Use a 10 μl disposable culture loop to inoculate media
Dilute specimen 1:2 using sterile saline before inoculating CHOC and ML agar
Use a sterile pipette apply one drop to media and streak

B. Interpretation of cultures:

1. Perform colony counts on all morphotypes of **Seminal Tract Possible Pathogens** isolated on BA.

2. Work up organisms other than GC as per the table below.

<table>
<thead>
<tr>
<th>No. of colonies on BA</th>
<th>Colony Count</th>
<th>Work-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminal Tract Pathogens</td>
<td>Any amount</td>
<td>ID + sens</td>
</tr>
<tr>
<td>Seminal Tract Non-Pathogens (see list below)</td>
<td>Any amount</td>
<td>None</td>
</tr>
<tr>
<td>Possible Seminal Tract Pathogens (see list below):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>&lt;10^6 CFU/L</td>
<td>None</td>
</tr>
<tr>
<td>≥10</td>
<td>≥10^6 CFU/L</td>
<td>ID + sens</td>
</tr>
</tbody>
</table>

**Seminal Tract Pathogens**
Neisseria gonorrhoeae
Chlamydia trachomatis (not detected by routine culture)

**Seminal Tract Possible Pathogens**
Enterobacteriaceae
*Pseudomonas aeruginosa*
Other gram negative bacilli
*Enterococcus* species
*Staphylococcus aureus*
Beta-haemolytic streptococci

**Seminal Tract Non-Pathogens**
diphtheroids
coaagulase-negative staphylococci
*Bacillus* species
viridans streptococci
*Streptococcus anginosus* grp
*Lactobacillus* species
Yeasts

3. Examine the CHOC and ML plates at 24, 48 and 72 hours for colonies suspicious for GC. For GC work up, refer to Bacteria Workup Manual.

C. Susceptibility testing:

Refer to.

V. Reporting Results

Negative Report:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Report/Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Growth @48hr</td>
<td>“No growth”</td>
</tr>
<tr>
<td>No Growth @72hr (update)</td>
<td>“No growth No Neisseria gonorrhoeae isolated.”</td>
</tr>
<tr>
<td>Non-seminal tract pathogens and/or &lt;10⁶ CFU/L possible seminal tract pathogens and no Neisseria gonorrhoeae isolated @48hr</td>
<td>“No significant growth”</td>
</tr>
<tr>
<td>Non-seminal tract pathogens and/or &lt;10⁶ CFU/L possible seminal tract pathogens and no Neisseria gonorrhoeae isolated @72hr (update)</td>
<td>“No significant growth No Neisseria gonorrhoeae isolated.”</td>
</tr>
</tbody>
</table>

Positive Report:

Preliminary report:

≥10 colonies possible seminal tract pathogens AND no other growth:

Report: Morphologic description of organisms with ISOLATE COMMENT:

≥10 x 10⁶ cfu/L; “Significance of this result is unclear and may represent contamination.”

≥10 colonies possible seminal tract pathogens AND with other non-significant growth:

Report: Morphologic description of organisms with ISOLATE COMMENT:

≥10 x 10⁶ cfu/L; “Significance of this result is unclear and may represent contamination.”; “Other insignificant growth noted.”

Final report:
≥10 colonies possible seminal tract pathogens AND no other growth:
   Report: Organism Name with ISOLATE COMMENT: ≥10 x E6 cfu/L;
   “Significance of this result is unclear and may represent contamination.” Report with appropriate sensitivity results.

≥10 colonies possible seminal tract pathogens AND with other non-significant growth:
   Report: Organism Name with ISOLATE COMMENT: ≥10 x E6 cfu/L;
   “Significance of this result is unclear and may represent contamination.”; “Other non-significant growth noted. Report with appropriate sensitivity results.

*N. gonorrhoeae* isolates in any amount:
   Report: “*N. gonorrhoeae*” “isolated” (do not quantitate),
   “~susceptibilities from Public Health Laboratory to follow”

Telephone all positive GC cultures to floor/ordering Physician. Refer to
Isolate Notification and Freezing Table QPCMI16003

For all positive GC cultures, send a Communicable Disease Report to the Medical Officer of Health. Refer to "Communicable Disease Results Reporting Process QPCMI17000" and
Reportable Diseases to the Medical Officer of Health QPCMI17001.

VI. References


UPPER GENITAL TRACT CULTURE

Endometrial Swabs, Biopsies and Curettings, Placenta Swab/Tissue, Products of Conception, Endometrial/Uterine, Cul de sac/Transvaginal, Fallopian Tube, Tubo-Ovarian Swabs or Aspirates

I. Introduction

The microbiologic diagnosis of endometritis is difficult. Anaerobes play an important role in this infection. However, most cases of endometritis follow childbirth, and it has been demonstrated that in the postpartum period, whether or not there is endometrial infection, significant numbers of anaerobes and other organisms from the cervical and vaginal flora may be found in the uterine cavity.

Although any organism may cause infection of the placenta, the most common organisms associated with this syndrome include *S. aureus*, beta-hemolytic streptococci, *Listeria monocytogenes* and *E. coli*.

Upper genital tract specimens include endometrial/uterine, cul de sac/transvaginal, fallopian tube, tubo-ovarian swabs or aspirates. Organisms typically associated with infections of these sites include *Staphylococcus aureus*, beta-hemolytic streptococci, GC and CT.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

B. Processing of Specimens:

See Specimen Processing Procedure

a) Direct examination: Gram stain
b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
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<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
<tr>
<td>Martin-Lewis Agar (ML)</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Fastidious Anaerobic Agar (BRUC)*</td>
<td>AnO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Kanamycin-Vancomycin Agar (KV)*</td>
<td>AnO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Fastidious Anaerobic Broth (THIO)*</td>
<td>O₂, 35°C x 7 days</td>
</tr>
</tbody>
</table>

* If tissue/aspirate or anaerobic swab is received, add BRUC, KV and THIO.

For tissues and biopsies, freeze remaining tissue in the -70°C freezer for minimum of 3 months.

A. Interpretation of cultures:

Examine the BA and MAC plates after 24 and 48 hours incubation and the CHOC and ML plates after 24, 48 and 72 hours incubation. Work up:

- any growth of *S. aureus*, beta hemolytic streptococci, *Listeria* and GC.
- any pure or predominant growth (heavier than that of vaginal flora) of other organisms
- any specific organism(s) that is requested.

For GC work up, see Bacteria Workup Manual.

Examine the BRUC and KV plates after 48 hours incubation. Workup ≤ 2 types of anaerobes. See Bacteria Workup Manual.

a) If no growth is visible on the culture plates but organism seen in gram smear, subculture the THIO (if turbid) onto CHOC (CO₂ at 35°C x 24 hours) and BRUC (AnO₂ at 35°C x 48 hours).

B. Susceptibility testing:

Refer to.
V. Reporting Results

Gram stain: Report with quantitation the presence of the pus cells and organisms.

Culture:

Negative Report:  “No significant growth” or “No growth.”
“No Neisseria gonorrhoeae isolated.”
“Commensal Flora”

If ML plate is overgrown by swarming Proteus or yeast, report ONLY as “Unable to rule out Neisseria gonorrhoeae due to bacterial/yeast overgrowth.”

Positive Report: Quantitate and report all other significant isolates with appropriate sensitivity results.

“Neisseria gonorrhoeae” “isolated” (do not quantitate),
“~susceptibilities from Public Health Laboratory to follow”

Telephone all positive GC cultures to floor/ordering Physician. Refer to Isolate Notification and Freezing Table QPCMI16003

For all positive GC cultures, send a Communicable Disease Report to the Medical Officer of Health. Refer to "Communicable Disease Results Reporting Process QPCMI17000" and Reportable Diseases to the Medical Officer of Health QPCMI17001.

VI. References


OTHER GENITAL SPECIMENS

GENITAL ULCER SWAB

I. Introduction

The most common bacterial causes of genital ulcers are syphilis (*Treponema pallidum*). Other sexually transmitted diseases with ulcerative lesions of the genitalia are relatively uncommon and include *Chlamydia trachomatis* (lymphogranuloma venereum LGV serotypes), granuloma inguinale (*Calymmatobacterium granulomatis*) and chancroid (*Haemophilus ducreyi*).

II. Specimen Collection and Transport

See [PHOL Specimen Testing Guide](#).

III. Reagent / Materials / Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List](#).

IV. Procedure

A. Processing of Specimens:

Genital ulcer specimens are sent to PHOL for testing.

Refer to [Send-out Information Manual](#) for send out procedures.

V. Reference

INTRA-UTERINE DEVICE (IUD)

I. Introduction

Genital colonization by actinomycetes has been associated with the use of (IUDs). Actinomyces may be seen in smears from secretions around the IUD, but has rarely been isolated in culture. Therefore there is no value in culturing these specimens.

II. Specimen Collection and Transport

See Pre-Analytical – Specimen Collection QPCMI2001 for Intra-Uterine Device

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure

   a) Direct Examination: Gram stain of secretions. Examine for the presence of branching gram positive bacilli suggestive of Actinomyces species.

   b) Culture: Not indicated.

V. Reporting Results

Gram Stain:

   Negative Report: “No organisms resembling Actinomyces seen on Gram stain.”

   Positive Report: “Organisms resembling Actinomyces seen on Gram stain”.

VI. References


### Record of Edited Revisions

**Manual Section Name:** Genital Tract Culture Manual

<table>
<thead>
<tr>
<th>Page Number / Item</th>
<th>Date of Revision</th>
<th>Signature of Approval</th>
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<tbody>
<tr>
<td>Annual Review</td>
<td>March 30, 2001</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review</td>
<td>May 10, 2002</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Pg.25-27 Changed entire section of Prostatic/Seminal Fluid</td>
<td>January 22, 2003</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 12, 2003</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>May 26, 2004</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 12, 2005</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Separated vaginal swabs into GBS screen, Vaginal screen and Vaginal culture sections.</td>
<td>April 10, 2006</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>GMV reporting phrase for &lt;12 and &gt;60 years added</td>
<td>April 10, 2006</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Moved Bartholin’s abscess to Wounds/Tissues/Aspirates Manual</td>
<td>April 10, 2006</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Separated Prostatic Fluid from Seminal Fluid. Prostatic Fluid moved to Urine section.</td>
<td>April 10, 2006</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Revised Seminal Fluid Workup and report</td>
<td>April 10, 2006</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Vaginal screen reporting – 0 cell was missing – added</td>
<td>July 12, 2006</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>July 12, 2006</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>April 11, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>April 10, 2008</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>August 10, 2009</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added CHOC to all GC cultures</td>
<td>August 10, 2009</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>October 15, 2010</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>GBS screen changed to Carrot Broth</td>
<td>October 15, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>October 15, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>September 9, 2012</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 31, 2013</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Send to PHOL for GC susceptibility testing.</td>
<td>January 9, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Seminal Fluid change to use pipette instead of swab to culture</td>
<td>March 28, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added proper header/footer</td>
<td>September 19, 2014</td>
<td>Dr. T. Mazzulli</td>
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### Annual Review

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<thead>
<tr>
<th>Event Description</th>
<th>Date</th>
<th>Reviewer</th>
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<tbody>
<tr>
<td>GBS section: changed subculture to “by WASP” added with step ‘d’-In LIS, GBS New worklist, mark each SUBCN plate and batch process those that were subcultured. Cancel label printing. Remark. Prelim all orders marked. Incubate plates</td>
<td>August 19, 2015</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Add Strep anginosus grp to non-seminal tract pathogens</td>
<td>September 29, 2015</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review Updated MSH logo in header Seminal fluid section added for final report: report with appropriate sensitivity results.</td>
<td>March 1(^{st}), 2016</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Updated negative vaginal Wet Mount phrase from: &quot;The presence of \textit{Trichomonas vaginalis} cannot be ruled out if there was a delay in transport and/or processing of this specimen&quot;. to &quot;(A negative result should NOT be used to rule out \textit{Trichomonas vaginalis} given the poor sensitivity of this assay. A delay in transport and/or processing of this specimen further decreases this assay’s sensitivity.)&quot;</td>
<td>August 11, 2017</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review Removed blank pages</td>
<td>June 21, 2018</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Changed Fastidious Anaerobic Broth (THIO) incubation time to 7 days (UPPER GENITAL TRACT CULTURE)</td>
<td>July 26, 2020</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Multiple updates – see annual review hard copy</td>
<td>Jan 12, 2021</td>
<td>Dr. T. Mazzulli</td>
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