TABLE OF CONTENTS

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 (Enterobacterales, anaerobes) and/or those organisms acquired through sexual contact.

Specimens included in this section:

Endometrial biopsies and currettings
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:

Media	Incubation
Chocolate Agar (CHOC)	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

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 CO_2.

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

Izenberg H.D.. 2003. Genital Cultures, in Clinical Microbiology Procedures Handbook, 2nd ed.

Vol.1 ASM Press, Washington, D.C.

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

Survey B-9412, Feb. 21, 1995. Microbiology Handling of Female Genital Specimens. A pattern of Practice Survey.

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:

Media	Incubation
Carrot Broth for Group B Strep (CAROT)	O_2, 35^oC x 24 hours

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 O_2 at 35^oC 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 O_2 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

Negative Report: "No Group B Streptococci isolated."

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."

Refer to "Isolate Notification and Freezing Table QPCMI16003" for calling all positive Group B Streptococcus results

VI. References

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

in the newborn. August, 1994. Canadian Pediatric Society and the Society of Obstetricians and Gynecologists of Canada. CIDC Newsletter.

Guidelines for prevention of Group B Streptococcus infection by chemoprophylaxis. Committee on Infectious Diseases and Committee on Fetus and Newborn. Pediatrics, 1992.

Bailey & Scott's Diagnostic Microbiology, Finegold & Baron: 7th Ed., C. V. Mosby Co., p. 290-291.

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.

ii. Gram stain: Examine for the presence of yeast, clue cells and organisms associated with bacterial vaginosis.

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

1. Observe for the presence of the following morphotypes:
   - Large gram-positive bacilli (*Lactobacillus* spp. morphotypes)

- Small gram-variable bacilli (*Gardnerella* spp. morphotypes)
- Curved gram-negative or gram-variable bacilli (*Mobiluncus* 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:

	***Lactobacilli* spp.**	***Gardnerella* spp.**	***Mobiluncus* spp.**
0	4+	0	0
1	3+	1+	1-2+
2	2+	2+	3-4+
3	1+	3+	
4	0	4+	

Total Nugent Score:

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

Examples:

		Score
1. *Gardnerella* spp.	4+	4
Lactobacilli spp.	2+	2
Mobiluncus spp.	2+	2
Total score =		8 (Report as Bacterial Vaginosis)
2. *Gardnerella* spp.	2+	2
Lactobacilli spp.	2+	2
Mobiluncus spp.	1-2+	1
Total score =		5 (Report as altered vaginal flora not consistent with bacterial vaginosis)
3. *Gardnerella* spp.	2+	2
Lactobacilli spp.	3+	1
Mobiluncus spp.	0	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".
Positive Report:	"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

1. Schreckenberger, Paul. Clinical Microbiology Newsletter, 1992 p. 126.

2. Spiegel, C., Amsel, R., Holmes, K. Journal of Clinical Microbiology, July, 1983 p. 170-177.

3. Cumitech 17A, 1993. "Lab. Diagnosis of Female Genital Tract Infections, ASM Press.

4. LPTP Survey B-9412, Feb. 21, 1995. Microbiology Handling of Female Genital Specimens. A Pattern of Practice Survey.

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:

Media	**Incubation**
Colistin Nalidixic Acid Agar (CNA)	CO_2, 35°C x 48 hours
Carrot Broth for Group B Strep (CAROT)	O_2, 35°C x 24 hours
If *N. gonorrhoeae* is requested, **add:**	
Chocolate Agar (CHOC)	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

If patient is <12 years old , **add:**	
MacConkey (MAC)	CO_2, 35^oC x 48 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 O_2 at 35^oC x 24 hours.
d) For the colourless broths, subculture a drop of the broth onto Colistin/Nalidixic Acid Agar (CNA) and incubate in O_2 at 35^oC 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*

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" andReportable Diseases to the Medical Officer of Health QPCMI17001.

VI. References

Schreckenberger, Paul. Clinical Microbiology Newsletter, 1992 p. 126.

Spiegel, C., Amsel, R., Holmes, K. Journal of Clinical Microbiology, July, 1983 p. 170-177.

Cumitech 17A, 1993. "Lab. Diagnosis of Female Genital Tract Infections, ASM Press.

QMP-LS Survey B-9412, Feb. 21, 1995. Microbiology Handling of Female Genital Specimens. A Pattern of Practice Survey.

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 theSend 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:

Media	Incubation
Chocolate Agar (CHOC)	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

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 toBacteria 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:	
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

1. Cumitech 4 "Lab. Diagnosis of Gonorrhoeae, ASM October, 1976.

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:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Colistin Nalidixic Acid Agar (CNA)	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)*	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML)*	CO_2, 35°C x 72 hours

*Occasionally, urethral swabs may be labelled as penile swabs. *Neiserria 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

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

Izenberg H.D.. 2003. Genital Cultures, in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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 $\geq 10^6$ 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:

Media	Incubation
Blood Agar (BA)*	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)*	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)[a,b]	CO_2, 35°C x 72 hours
Martin-Lewis Agar (ML)[a,b]	CO_2, 35°C x 72 hours

*Use a 10 µl disposable culture loop to inoculate media

[a]Dilute specimen 1:2 using sterile saline before inoculating CHOC and ML agar
[b]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.

No. of colonies on BA	**Colony Count**	**Work-up**
Seminal Tract Pathogens	Any amount	ID + sens
Seminal Tract Non-Pathogens (see list below)	Any amount	None
Possible Seminal Tract Pathogens (see list below):		
<10	$<10^6$ CFU/L	None
≥10	$\geq 10^6$ CFU/L	ID + sens

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

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

Seminal Tract Non-Pathogens
diphtheroids
coagulase-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 toBacteria Workup Manual.

C. Susceptibility testing:

Refer to.

V. Reporting Results

Negative Report:

No Growth @48hr	"No growth"
No Growth @72hr (update)	"No growth No *Neisseria gonorrhoeae* isolated."
Non-seminal tract pathogens and/or $<10^6$ CFU/L possible seminal tract pathogens and no *Neisseria gonorrhoeae* isolated @48hr	"No significant growth"
Non-seminal tract pathogens and/or $<10^6$ CFU/L possible seminal tract pathogens and no *Neisseria gonorrhoeae* isolated @72hr (update)	"No significant growth No *Neisseria gonorrhoeae* isolated."

Positive Report:

Preliminary report:

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

Report: Morphologic description of organisms with ISOLATE COMMENT:
≥10 x E6 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 E6 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.

Neisseria gonorrhoeae isolates in any amount:

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

Keck C, et al. 1998. Seminal tract infections: impact on male fertility and treatment options. Human Reproduction Update 4(6):891-903.

Jarvi K, et al. 1996. Polymerase chain reaction-based detection of bacteria in semen. Fertility and Sterility 66(3):463-467.

Cottell E. Fertility and Sterility 2000 74(3):465-470.

World Health Organization. 1992. Laboratory Manual for the Examination of Human Semen and Sperm – Cervical Mucus Interaction, 3rd ed. Cambridge University Press, Cambridge.

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.*

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:

Media		Incubation
Blood Agar (BA)	CO_2,	35°C x 48 hours
Chocolate Agar (CHOC)	CO_2,	35°C x 72 hours
Martin-Lewis Agar (ML)	CO_2,	35°C x 72 hours
MacConkey Agar (MAC)	O_2,	35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)*	AnO_2,	35°C x 48 hours
Kanamycin-Vancomycin Agar (KV)*	AnO_2,	35°C x 48 hours
Fastidious Anaerobic Broth (THIO)*	O_2,	35°C x 7 days

* 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 (CO_2 at 35°C x 24 hours) and BRUC (AnO_2 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 significant isolates with appropriate sensitivity results. Quantitate and report commensal flora if also present in culture. "*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.

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VI. References

Cumitech 17A, 1993. Lab. Diagnosis of Female Genital Tract Infections, ASM Press.

Bailey & Scott's Diagnostic Microbiology. Finegold & Baron: 7th Ed. C. V. Mosby Co., p. 59.

Abdulmassih, R., Makadia, J., Como, J., Paulson, M., Min, Z., & Bhanot, N. (2016, December). Propionibacterium acnes: Time-to-Positivity in Standard Bacterial Culture From Different Anatomical Sites. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27829959

Shannon, S., Mandrekar, J., Gustafson, D., Rucinski, S., Dailey, A., Segner, R., Patel, R. (2013, February). Anaerobic thioglycolate broth culture for recovery of Propionibacterium acnes from shoulder tissue and fluid specimens. Retrieved July 27, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3553932/

Schwotzer, N., Wahl, P., Fracheboud, D., Gautier, E., & Chuard, C. (2014, January). Optimal culture incubation time in orthopedic device-associated infections: A retrospective analysis of prolonged 14-day incubation. Retrieved July 27, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911454/

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 abd 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 QPCMI10001

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

Murray P.R., et al (Editors). 1999. Manual of Clinical Microbiology, 7th Ed., ASM Press.

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 QPCMI2001for *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

Gupta, P. K., Woodruff, J. D., l982. Actinomyces in Vaginal smears, JAMA 2247: 1175-76.

Valicente, J. F., Jr., et al. 1982. Detection and Prevalence of IUD-associated Actinomyces. Colonization and Related Morbidity. A prospective Study of 69,925 cervical smears. JAMA 247:1149-1152.

Record of Edited Revisions

Manual Section Name: Genital Tract Culture Manual

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

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	March 10,2015	Dr. T. Mazzulli
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	August 19, 2015	Dr. T. Mazzulli
Add Strep anginosus grp to non-seminal tract pathogens	September 29, 2015	Dr. T. Mazzulli
Annual Review Updated MSH logo in header Seminal fluid section added for final report: report with appropriate sensitivity results.	March 1st, 2016	Dr. T. Mazzulli
Annual Review	March, 1, 2017	Dr. T. Mazzulli
Updated negative vaginal Wet Mount phrase from: "The presence of *Trichomonas vaginalis* cannot be ruled out if there was a delay in transport and/or processing of this specimen" to: "(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.)"	August 11, 2017	Dr. T. Mazzulli
Annual Review	April 24, 2018	Dr. T. Mazzulli
Removed blank pages	June 21, 2018	Dr. T. Mazzulli
Annual Review	July 20, 2019	Dr. T. Mazzulli
Changed Fastidious Anaerobic Broth (THIO) incubation time to 7 days (UPPER GENITAL TRACT CULTURE)	July 26, 2020	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Multiple updates – see annual review hard copy	Jan 12, 2021	Dorna Zareianjahromi
Minor formatting change	April 11, 2021	Jessica Bourke
Nomenclature update – enterobacterales	April 19, 2021	Wayne Chiu

Page Number / Item	Date of Revision	Edited by:
Placenta reporting significant pathogen, mention commensal if present	July 13, 2021	Wayne Chiu
Updated "Telephone all positive Group B Streptococcus for patients admitted to a ward / case room" to "Refer to "Isolate Notification and Freezing Table QPCMI16003" for calling all positive Group B Streptococcus results. (pg8)	August 11, 2022	Qin Liu