TML\MSH Microbiology Department Policy & Procedure Manual	**Policy #MI/GEN/v04** Page 1 of 1
Section: **Genital Tract Culture Manual**	Subject Title: **Table of Contents**
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GENITAL TRACT CULTURE MANUAL

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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 (eg. 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.

All reagents, kits and media **MUST** be quality controlled before use. Tests are run with appropriate controls when used (Refer to Quality Control Manual).

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
Cervical swabs
Group B streptococcus screen
Post-partum / post-operative / post therapeutic abortion vaginal swabs
Urethral swabs (Male or Female)
Vaginal swabs

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 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.

BARTHOLIN'S ABSCESS SWAB / ASPIRATE

I. Introduction

Bartholin's glands are small mucus-producing glands located on each side of the vaginal opening close to the base of the labia minora.

Bartholinitis may be caused by *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), or organisms normally present in the vagina resulting in a polymicrobial infection.

II. Specimen Collection and Transport

Specimens for culture are collected using a syringe or swab placed in Amies transport medium.

For detection of CT, refer to the Virology Manual.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

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 48 hours
Martin –Lewis Agar (ML)	CO_2, 35°C x 72 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours

B. Interpretation of cultures:

a) Examine the BA, CHOC, and MAC plates after 24 and 48 hours incubation and the ML plate after 48 and 72 hours incubation. Quantitate the bacterial growth according to the criteria in Appendix II.
b) All potential pathogens should be identified.
c) For GC work-up, refer to Appendix VII.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

D. Procedure Notes:

1. If a specific organism is requested, it will be looked for and its presence or absence reported. If anaerobic culture is requested, discuss with the microbiologist or supervisor.

V. Reporting Results

Gram Stain:	Report with quantitation the presence of pus cells and organisms.
Culture:	
Negative Report:	"No significant growth" or " No growth" "No *Neisseria gonorrhoeae* isolated".
Positive Report:	"*Neisseria gonorrhoeae* isolated (do not quantitate), beta lactamase negative or positive" (enter beta lactamase result under "Breakpoint Panel" in LIS Isolate Screen). Quantitate and report all other significant isolates with appropriate sensitivity results.

Telephone all positive GC cultures to floor/ordering Physician.

For Centenary Health Centre (CHC) in-patients, inform CHC infection control of all positive GC isolates.

For all positive GC cultures, a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

VI. References

1. Isenberg, Henry D. Clinical Microbiology Procedures Handbook, Vol. 1, 199l: p. 1.11.1-1.ll.7.

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

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

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 HSV and CT, refer to the Virology Manual.

II. Specimen Collection and Transport

Specimens for GC are collected from the endocervical canal using a clean, sterile swab and transported in Amies transport medium.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of specimens:

a) Direct Examination: Not indicated.

b) Culture:

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

B. Interpretation of culture:

a) Examine ML plate after 48 and 72 hours incubation for suspect GC colonies.
b) For GC work-up, refer to Appendix VII.

C. Procedure Notes:

1. If Group B streptococcus is requested, refer to the Group B streptococcus screen section.

IV. 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), beta lactamase negative or positive (enter beta lactamase result under "Breakpoint Panel" in LIS Isolate Screen).

Telephone all positive GC cultures to floor/ordering Physician.

For CHC in-patients, inform CHC infection control of all positive GC isolates.

For all positive GC cultures, a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

V. References

1. Isenberg, Henry D. Clinical Microbiology Procedures Handbook, Vol. 1, 1991: pp. 1.11.2-1.11.9

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

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

ENDOMETRIAL BIOPSIES AND CURETTINGS

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.

Currettings may also be submitted specifically for *Mycobacterium tuberculosis* (TB) examination. These should be sent to the Public Health Laboratory (PHL) for processing.

II. Specimen Collection and Transport

Scrapings and small tissue samples of the endometrium should be collected aseptically, avoiding lower genital tract contamination, and transported in sterile saline and an anaerobic container.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Preparation of specimen for culture (Refer to Planting Manual).

1. Aseptically macerate the tissue with the use of a tissue grinder or stomacher.
2. Prepare 2 smears: one for Gram stain and one to be held in reserve. If STAT TB is requested and approved by the Microbiologist, prepare a slide for Acid Fast Bacilli (AFB) stain. A portion of the specimen should be forwarded to the Public Health Laboratory (PHL) for processing.
3. If TB culture is requested, send half of the specimen to PHL.

b) Direct examination: Gram stain.

c) Culture:

Media		**Incubation**
Blood Agar (BA)	CO_2,	35°C x 48 hours
Chocolate Agar (CHOC)	CO_2,	35°C x 48 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 48 hours

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

B. Interpretation of cultures:

a) Examine the BA, CHOC, and MAC plates after 24 and 48 hours incubation and the ML plate after 48 and 72 hours incubation.
b) All potential pathogens should be identified. In particular, examine for any growth of *S. aureus*, beta hemolytic streptococci and GC.
c) For GC work up, refer to Appendix VII.
d) Examine the BRUC and KV plates after 48 hours incubation. Identify anaerobes (Refer to Identification Manual).
e) If no growth is visible on the culture plates, subculture the THIO (if turbid) onto BA (O_2 at 35^0C x 24 hours) and BRUC (AnO_2 at 35^0C x 48 hours).

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

D. Procedure Notes:

1. A heavy growth of any organism(s), including anaerobes, that correlates with the predominant organism(s) seen on the Gram stain is considered significant if there is >1+ pus cells.
2. If a specific organism is requested, it will be looked for and its presence or absence reported.

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."

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), beta lactamase negative or positive (enter the beta lactamase result under "Breakpoint Panel" in LIS Isolate Screen).

For TB reports, Refer to TB Manual.

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

Telephone all positive GC cultures to floor/ordering Physician.

For CHC in-patients, inform CHC infection control of all positive GC isolates.

For all positive GC cultures, a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

VI. References

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

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

GENITAL ULCER SWAB

I. Introduction

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

II. Procedure

Treponema pallidum

The laboratory will not perform direct darkfield examination for *Treponema pallidum.* Requests should be referred to the Public Health Laboratory (PHL) @ 235-5952.

Herpes simplex virus

Isolation requires viral transport medium. Refer to the Virology Manual.

Chlamydia trachomatis (LGV serotypes),Calymmatobacterium granulomatis,
Haemophilus ducreyi (chancroid)

Requests should be referred to the PHL @ 235-5952.

III. Reference

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

GROUP B STREPTOCCUS 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

A swab obtained from the combined introital (vaginal) and anorectal areas should be collected in Amies transport medium. Cervical and vaginal swabs are not recommended for this type of culture but will be processed if received in the laboratory.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Not indicated.

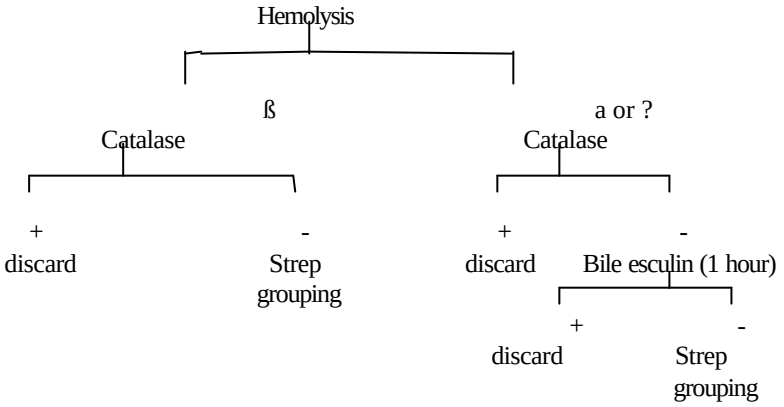
b) Culture:

Media	Incubation
Colistin Nalidixic Acid Agar (CNA)	0_2, 35^0C x 48 hours
Group B Strep Broth (GBS)	0_2, 35^0C x 24 hours

B. Interpretation of culture:

1. Examine the CNA plate after 24 hours incubation for colonies suspicious of Group B streptococcus. Work-up colonies according to the flow chart below.
2. If the original CNA plate has no suspect colonies, reincubate and examine the following day.
3. After 24 hours incubation, if the original CNA plate is negative for Group B streptococcus, subculture a drop of GBS broth onto CNA and incubate in 0_2 at 35^0C x 24 hours.

Identification tests for Group B streptococcus



C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

D. Procedure Notes:

1. Not all Group B streptococci are ß- hemolytic.

V. Reporting Results

Negative Report: "No Group B streptococci isolated."

Positive Report: "Group B streptococci isolated." (Do not quantitate).

Note: If GBS screen is requested on a cervical or vaginal swab, report the results with the following comment: "The recommended specimen for Group B streptococcus screen is a combined introital (vaginal) /anorectal swab."

Telephone all positive reports on in-patients to floor/ordering Physician.

VI. References

1. 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.

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

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

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

The IUD should be collected and transported in a dry, sterile container.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

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

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

Positive Report: "Organisms resembling Actinomyces seen on Gram stain".

VI. References

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

2. 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.

PENIS SWAB

I. Specimen Collection and Transport

Penile swabs should be transported in Amies transport medium.

II. Processing of Specimens

Direct Examination: Gram stain.

Culture:

Media	Incubation
BA	CO_2, 35°C, X 48 hours
MAC	O_2, 35°C, X 48 hours
CAN	O_2, 35°C, X 48 hours
ML	CO_2, 35°C, X 72 hours

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. Examine ML plates at 48 and 72 hours.

III. Isolation and Identification

Any growth of *S. aureus, P. aeruginosa* and Gp. A Strep is significant.

For GC work-up refer to Appendix VII.

IV. Sensitivity Testing

Refer to Susceptibility Manual.

V. Reporting

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

Culture:

Negative Report: "Commensal flora" (DO NOT report "No GC isolated").

Positive Report: "*Neisseria gonorrhoeae*" and beta-lactamase - or + (enter under "Breakpoint

Quantitate all other significant isolates with appropriate sensitivities.

Telephone all positive GC smears and reports to the ward/attending physician. Inform CHC infection control on CHC in-patients.

For all positive GC cultures a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

PLACENTA SWAB / TISSUE AND PRODUCTS OF CONCEPTION

I. Introduction

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*.

II. Specimen Collection and Transport

Swabs should be collected aseptically and transported in Amies transport medium. Tissues should be placed in a clean, sterile container.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Preparation of specimen for culture (Refer to Planting Manual).

1. If tissue is received, aseptically macerate the tissue with the use of a tissue grinder or stomacher.
2. Prepare 2 smears: one for Gram stain and one to be held in reserve. If STAT TB is requested and approved by the Microbiologist, prepare a slide for AFB stain. Forward a portion of the specimen to Public Health Laboratory (PHL) for processing.
3. If TB culture is requested, send half of the specimen to PHL.

b) Direct Examination: Gram stain.

c) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_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 48 hours
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours

B. Interpretation of cultures:

a) Examine the BA, CHOC, CNA and MAC plates after 24 and 48 hours incubation and the ML plate after 48 and 72 hours incubation.
b) All potential pathogens should be identified.
c) For GC work up, refer to Appendix VII.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

Culture:

Negative Report: "No significant growth" or "No growth".
"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), beta lactamase negative or positive". (enter the beta lactamase result under "Breakpoint Panel" in LIS Isolate Screen).

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

Telephone all positive GC cultures to floor/ordering Physician.

For CHC in-patients, inform CHC infection control of all GC isolates.

For all positive GC cultures, a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

VI. References

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

2. Bailey & Scott's Diagnostic Microbiology. Finegold & Baron; 7th. Ed., C.V. Mosby Co. p. 301.

POST-PARTUM, POST-OPERATIVE, POST-THERAPEUTIC ABORTION VAGINAL SPECIMENS

I. Introduction

Infection of these sites may be due *Staphylococcus aureus*, Group A streptococcus and Group B streptococcus.

II. Specimen Collection and Transport

Vaginal discharge should be collected with a clean, sterile swab and transported in Amies transport medium.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Colistin Nalidixic Acid Agar (CNA)	O_2, 35°C x 48 hours
Group B Strep Broth (GBS)	O_2, 35°C x 24 hours

B. Interpretation of cultures:

a) Examine the CNA plate after 24 hours incubation for colonies suspicious of *Staphylococcus aureus* , Group A Streptococcus, and Group B streptococcus (Refer to Group B streptococcus Screen for identification).
b) If the original CNA plate has no suspect colonies, reincubate and examine the following day.

c) After 24 hours incubation, if the original CNA plate is negative, subculture a drop of GBS broth onto CNA and incubate in O_2 at 35^0C x 24 hours.

C. Susceptibility testing

Refer to Susceptibility Testing Manual.

V. Reporting Results

Negative Report:	"No *Staphylococcus aureus* or beta hemolytic streptococci isolated."
Positive Report:	Report all significant isolates with appropriate sensitivity results. Do not quantitate.

VI. References

1. Schereckenberger, Paul: Clinical Microbiology Newsletter, 1992 p. 126.
2. Spiegel, C., Amsel, R., Holmes, K.: Journal of Clinical Microbiology, July 1983 p. 170-177.
3. LPTP Survey B-9412, Feb. 21, 1995. Microbiology Handling of Female Genital Specimens. A pattern of Practice Survey.

PROSTATIC / SEMINAL FLUID

I. Introduction

Bacterial infections of the genital tract may be important etiological factors for male infertility. Potential pathogens include *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), Ureaplasma, Enterococci, *S. aureus*, *Klebsiella* species, *Escherichia coli* and other gram negative bacilli. World Health Organization guidelines (WHO, 1992) define a seminal tract infection as a pure growth of $\geq 10^6$ bacteria or colony forming units (CFU) /L of ejaculate. Other references use $\geq 10^7$ bacteria/L. Mixed bacterial growth is common and is of questionable significance, often containing mixed commensal flora.

II. Specimen Collection and Transport

Ejaculate should be collected aseptically into a clean, sterile container. If a delay in transport or processing is anticipated, keep the specimen refrigerated until processing.
All specimens will be set up for culture and sensitivity testing as outlined below. After inoculating the culture media below, forward the remainder of the specimen to the Virology lab for molecular detection of GC and CT.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain.

b) Culture:

Media	Incubation
Blood Agar (BA)*	$C0_2$, 35^0C x 48 hours
Martin-Lewis Agar (ML)[a]	$C0_2$, 35^0C x 72 hours
MacConkey Agar (MAC)*	0_2, 35^0C x 48 hours

*Use a 10 µl disposable culture loop to inoculate media
[a]Use a swab to inoculate media
[a]Dilute specimen 1:2 using sterile saline before inoculating ML agar

B. Interpretation of cultures:

1. Perform a total colony count (regardless of the different organism morphotypes) on the BA and MAC.

No. of colonies on BA or MAC	**Work-up**
<100 bacteria, not *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	None
≥100 bacteria, not *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	None
<10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	None
≥10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	ID and Sensitivities

2. Examine the ML plate for colonies suspicious for GC. For GC work up, refer to Appendix VII.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

No. of colonies on BA or MAC	**Report as:**
No growth <100 bacteria, not *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	"No growth" LIS TEST window: <10 E7 CFU/L (mixed) bacteria
<100 bacteria with <10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	LIS TEST window: <10 E7 CFU/L (mixed) bacteria
<100 bacteria with ≥10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	LIS TEST window: <10 E7 CFU/L (mixed) bacteria; LIS ISOLATE window: "Isolate Name " ≥10 E6 CFU/L with sensitivities as appropriate
>100 bacteria, not *Enterobacteriaceae*	LIS TEST window:

No. of colonies on BA or MAC	Report as:
or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	≥10 E7 CFU/L (mixed) bacteria

No. of colonies on BA or MAC	**Report as:**
≥100 bacteria with <10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	LIS TEST window: >10 E7 CFU/L (mixed) bacteria
>100 bacteria with >10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	LIS TEST window: ≥10 E7 CFU/L (mixed) bacteria LIS ISOLATE window: "Isolate Name " ≥10 E6 CFU/L with sensitivities as appropriate.
<10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	<10 E7 CFU/L (mixed) bacteria
≥10 *Enterobacteriaceae* or *Enterococcus* or *Ps. aeruginosa* or *S. aureus*	LIS ISOLATE window: "Isolate Name " ≥10 E6 CFU/L with sensitivities as appropriate.

For GC culture results from ML plates:

Negative Report: LIS TEST window:
"No *Neisseria gonorrhoeae* isolated."

Positive Report: LIS ISOLATE window:
"*Neisseria gonorrhoeae*" (do not quantitate) with beta-lactamase result.

VI. References

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

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

UPPER GENITAL TRACT SWABS AND ASPIRATES

I. Introduction

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

Swabs should be collected aseptically, avoiding lower genital tract contamination, and transported in Amies transport medium. If anaerobes are requested, a separate specimen must be collected in an anaerobic transport container. Aspirates are transported in an anaerobic transport container.

If CT is requested, refer to the Virology Manual.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:
   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 48 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 48 hours

*If aspirate or anaerobic swab received, add THIO, KV and THIO.

B. Interpretation of cultures:

a) Examine the BA, CHOC and MAC plates after 24 and 48 hours incubation and the ML plate after 48 and 72 hours incubation.
b) All potential pathogens should be identified.
c) For GC work up, refer to Appendix VII.
d) Examine AnO_2 culture media after 48 hours incubation.
e) If no growth is visible on the culture plates, subculture the THIO (if turbid) onto BA (O_2 at 35^0C x 24 hours) and BRUC (AnO_2 at 35^0C x 48 hours).

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

D. Procedure Notes:

1. A heavy growth of any organism(s), including anaerobes, that correlates with the predominant organism(s) seen on the Gram stain is considered significant if there is >1+ pus cells.
2. If a specific organism is requested, it will be looked for and its presence or absence reported.

V. Reporting Results

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

Culture:

Negative Report: "No significant growth" or "No growth."
"No *Neisseria gonorrhoeae* isolated."

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

Positive Report: "*Neisseria gonorrhoeae* isolated" (do not quantitate), beta lactamase negative or positive" (enter under "Breakpoint Panel" in LIS Isolate Screen).

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

Telephone all positive GC cultures to floor / ordering Physician.

For CHC in-patients, inform CHC infection control of all positive GC isolates.

For all positive GC cultures, a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

VI. References

1. Cumitech 17A, 1993. "Laboratory Diagnosis of Female Genital Tract Infections", ASM Press .
2. Murray, P. R., et al (Editors). Manual of Clinical Microbiology, 7th Ed, ASM Press.

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

Exudate from the urethra should be collected using a clean, sterile swab and transported in Amies transport medium.

For *Chlamydia trachomatis*, refer to the Virology Manual.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain - Quantitate the presence of pus cells and intracellular gram negative diplococci. (Refer to Appendix VI).

b) Culture:

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

B. Interpretation of culture:

a) Examine ML plate after 48 and 72 hours incubation for colonies suspicious of GC.
b) For GC work up, refer to Appendix VII.

C. Procedure Notes:

1. Correlate Gram stain results with culture.

V. Reporting Results

Gram stain: Quantitate and report the presence or absence of pus cells and Gram negative diplococci. (Refer to Appendix VI).

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), beta lactamase negative or positive" (enter beta lactamase result under "Breakpoint Panel" in LIS Isolate Screen).

Telephone all positive GC cultures to the floor/ordering Physician.

For CHC in-patients, inform CHC infection control of all positive GC isolates.

For all positive GC cultures, a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

VI. References

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

VAGINAL SWAB

I. Introduction

Vaginal infections may be caused by yeast, *Trichomonas vaginalis*, and bacterial vaginosis.

II. Specimen Collection and Transport

Swabs from the posterior vaginal vault or vaginal orifice are collected and transported in Amies transport medium. Specimen should be transported to the laboratory as soon as possible. The yield of Wet Prop for *Trichomonas vaginalis* is significantly diminished if slides are not examined within 15 minutes of collection.

Vaginal swabs are not recommended for GC culture on adults. However, if specifically requested, cultures will be set up.

III. Reagents and Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination:

i. Wet prep: 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 40 X objective. Examine for the presence of *Trichomonas vaginalis*..

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

- If clue cells are present, this is interpreted as bacterial vaginosis.

- In the absence of clue cells, grade and score the bacterial findings as follows:

Grading:

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

Scoring:

Score	Lactobacilli	Gardnerella	Mobiluncus
0	4+	0	0
1	3+	1+	1-2+
2	2+	2+	3-4+
3	1+	3+	
4	0	4+	

Total score: ≥ 6 = Bacterial vaginosis
0-5 = Normal

				Score	
Examples:	1.	Gardnerella	4+	4	
		Lactobacilli	2+	2	
			Total score =	6	(Report as Bacterial Vaginosis)
	2.	Gardnerella	2+	2	
		Lactobacilli	2+	2	
		Mobiluncus	3-4+	2	
			Total score =	6	(Report as Bacterial Vaginosis)
	3.	Gardnerella	2+	2	
		Lactobacilli	3+	1	
		Mobiluncus	3-4+	2	
			Total score =	5	(Report as No Bacterial Vaginosis)

b) Culture: For all PMH patients, MSH out-patients, TTH out-patients and referred-in patients (Baycrest, QE, QMH, CHC routine), culture is not routinely performed unless Group B strep. is requested.

In cases of suspected toxic shock syndrome or for MSH & TTH in-patients, specimens are to be cultured for *S. aureus*, Group A streptococcus and Group B streptococcus.

Media	**Incubation**
Colistin Nalidixic Acid Agar (CNA)	O_2, 35°C x 48 hours
Group B Strep Broth (GBS)	O_2, 35°C x 24 hours

Vaginal swab specimens from children under 14 years of age are to be cultured for GC.

Media	**Incubation**
Martin-Lewis Agar (ML)	$C0_2$, 35°C x 72 hours

B. Interpretation of culture:

a) Examine the CNA plate after 24 hours incubation for colonies suspicious of *S. aureus*, Group A streptococcus and Group B streptococcus (Refer to Group B streptococcus Screen for identification).
b) If the original CNA plate has no suspicious colonies re-incubate and examine the next day.
c) After 24 hours incubation, if the original CNA plate is negative for Group B streptococcus, subculture a drop of GBS broth onto CNA and incubate in O_2 at 35°C x 24 hours.
d) For GC work-up, refer to Appendix VII.

C. Susceptibility testing

Refer to Susceptibility Testing Manual.

D. Procedure Note:

1. Send *S. aureus* isolates to PHL for toxin testing and freeze all toxin-producing strains.

V. Reporting Results

Wet Prep:

Negative Report:	"No *Trichomonas vaginalis* seen." The following message will automatically be added to **ALL** negative reports: "The presence of *Trichomonas vaginalis* cannot be ruled out if there was a delay in transport and/or processing of this specimen".
Positive Report:	"*Trichomonas vaginalis* seen."

Gram Stain:

Negative Report:	"No yeast or evidence of bacterial vaginosis seen".
Positive Report:	"Yeast present. No evidence of bacterial vaginosis." or "Evidence of bacterial vaginosis seen. No yeast present." or "Yeast and bacterial vaginosis seen."

Culture:

Negative Report:	If toxic shock syndrome requested: "No *Staphylococcus aureus* or beta hemolytic streptococcus isolated." If ML is 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."

Positive Report:	If toxic shock syndrome requested: Report all significant isolates with appropriate susceptibilities. Do not quantitate except *S. aureus*. If ML is set up: "*Neisseria gonorrhoeae* isolated" (do not quantitate), beta lactamase negative or positive (enter under "Breakpoint Panel" in LIS Isolate screen).

Telephone all positive GC cultures to floor / ordering Physician.

For CHC in-patients, inform CHC infection control of all positive GC isolates.

For all positive GC cultures, a Communicable Disease Report is sent to the Medical Officer of Health by the microbiologist or supervisor.

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.

Comment:

APPENDIX I
(REAGENTS / MATERIALS / MEDIA)

Reagents/Materials/Media

Catalase (3% H_2O_2) -Ingram & Bell
Gonogen - Medicorp
Gram stain - Refer to Media Manual for preparation
Oxidase - (Tetramethyl-p-phenylenediamine dihydrochloride) Refer to Media Manual for preparation
Strep. Latex Agglutination - Pro-Lab Diagnostics
Quadferm - biomerieux
Staph. Latex Agglutination - Sanofi
Sterile saline - Refer to Media Manual for preparation
Vitek susceptibility cards - biomerieux
Amies charcoal transport - NCS Diagnostics
Blood Agar (BA) - PML
Chocolate Agar (CHOC) - Oxoid
Colistin Nalidixic Agar (CNA) - Medprep
Deoxyribonucleic Acid Agar (DNA) - Oxoid
Fastidious Anaerobic Agar (BRUC) - Medprep
Fastidious Anaerobic Broth (THIO) - Medprep
Kanamycin-Vancomycin Agar (KV) -Medprep
MacConkey Agar (MAC) - PML
Martin-Lewis Agar (ML) - Biomed
Mueller Hinton Agar, plain (MH) - Oxoid
Group B Strep Broth (GBS) (Todd-Hewitt with Gent/NA) - PML

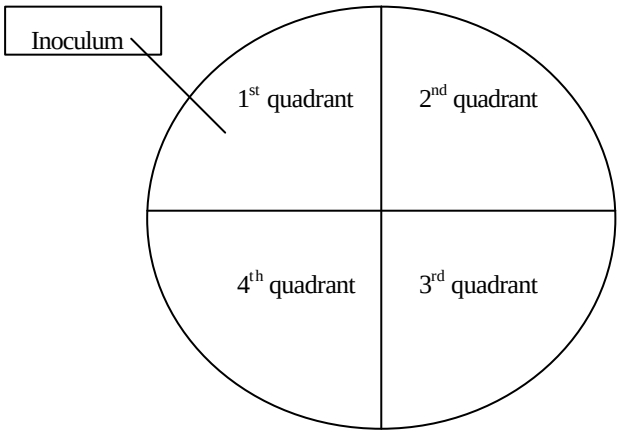
APPENDIX II
(QUANTITATION OF CULTURES)

Quantitation of Cultures:

1+ = growth on the first and second quadrant

2+ = growth up to the third quadrant

3+ = growth up to the fourth quadrant



APPENDIX III
(GONO GEN)

Principle of the Procedure

The **GonoGen** test for *N. gonorrhoeae* is comprised of a specific anti-gonococcal anti-serum and several control reagents. The specific anti-gonococcal anti-serum is composed of a pool of murine monoclonal antibodies (IgG) that have been prepared against a purified outer membrane protein, Protein I, of N. gonorrhoeae. Protein I is a major outer membrane molecule that is exposed on the surface of the gonococcus and its antigens are largely responsible for serotype specific reactions of the gonococcus. By including monoclonal antibodies to the various serotypes of *N. gonorrhoeae*, maximum specificity and sensitivity are achieved. These antibodies are absorbed to the Cowan I strain of *Staphylococcus aureus* which has been chemically fixed and heat-killed. When a sample containing *N. gonorrhoeae* is mixed with the GonoGen Reagent, agglutination occurs due to recognition of the Protein I antigens on the gonococcus by the antibodies bound to the *S. aureus*.

Performance of Test

The testing area, reagents, and test components should be at room temperature when used. Use only the glass slide recommended for these kits. Before use of the glass slide, thoroughly clean with a lint-free tissue.

1. Remove presumptively identified Neisseria colonies from the culture plate with a wire loop or cotton swab and suspend in approximately 0.5 ml of distilled water in a test tube to create a suspension equivalent to a McFarland No. 3 turbidity standard or 10^9 organisms per ml.

2. Heat the suspension prepared in step 1 at 100°C for 10 min using a heating block or water bath. Allow the heated suspension to cool to room temperature before testing. After heating, the suspension is stable and suitable for testing for up to 48 h if stored at 2 to 8°C. The suspension should be vortexed or vigorously shaken prior to testing to insure the suspension is smooth and free of clumps or aggregates which may be mistaken for a reaction with the **GonoGen** Reagent.

3. For every patient to be tested, place one drop of **Reagent G** on separate circles on the glass slide. Using a transfer pipette, add one drop of a well mixed, heated suspension of organisms to one circle containing the **Reagent G**. Using the clean paddle end of the transfer pipette, mix and spread the mixture to cover most of the compartment area. NOTE: For use of **Reagent S** and Quality Control see below.

4. Gently rock the slide in a rotary motion for 2 min, by hand or by mechanical rotator. Following rotation, view the slide under a lamp set at an oblique angle to the slide against a dark background. The negative control reaction serves as an index for identifying weak positive reactions.

5. A positive coagglutination reaction consists of clumping of the reagents accompanied by partial or total clearing of the milky white suspension within the 2 min. period. Strong positive reactions are reproduced by the Positive **Control**. Weak positive reactions will show more clumping than the negative control.

Caution: When the slide is rotated for more than 2 min, weak clumping may occur non-specifically. Strong positive reactions can still be reported as positive, but weak reactions should be repeated.

Staph Control Reagent: All positive samples may be tested with **GonoGen** Staph Control Reagent to verify the specificity of the reaction. One drop of the organism suspension is added to **Reagent S** and tested as above in Steps 3,4, and 5. Agglutination with this reagent within 2 min is indicative of a possible reaction to the staph cells and not the **GonoGen** test. The patient should not be reported positive by the **GonoGen** test if this occurs. Rather, carbohydrate utilization may then be used as an alternative method of culture confirmation. The occurrence of Staph Control Reagent positive organisms have been only rarely observed.

User Quality Control: Quality Control should be performed on the kit each day of use, using the Positive **Control** and Negative **Control**. One drop of **Reagent G** is placed in each of two areas of the test slide. To one circle, add one drop of Positive **Control,** and to the other, add one drop of Negative **Control.** The test is then reacted as outlined above in Steps 3,4, 5, and the results read. The Positive **Control** should yield a strong agglutination relative to the Negative **Control,** which should be judged as having zero reactivity. If significant agglutination is seen using the Negative **Control,** or if the Positive **Control** fails to react with **GonoGen** Reagents, do not use the kit.

Limitations of the Procedure

No single diagnostic test result should be considered conclusive. The overall clinical and laboratory findings should be taken into consideration before a physician renders a diagnosis. The **GonoGen** test is comprised of a pool of specific monoclonal antibodies to the outer membrane protein, Protein I, of *N. gonorrhoeae*. Depending upon exposed antigenic sites and antigenic composition, some gonococci may not be identifiable with the **GonoGen** Reagent and others may vary in the strength of the agglutination reaction. In the rare case of extremely weak reaction of an organism with **GonoGen** Reagent, not clearly differentiated from the reaction of the Negative **Control** with

GonoGen Reagent, confirmation by carbohydrate utilization may be necessary.

Reference

GonoGen Product Information.

APPENDIX IV

API NH - IDENTIFICATION OF *NEISSERIA* AND *HAEMOPHILUS*

Principle

The API NH strip consists of 10 microtubes containing dehydrated substrates, which enable the performance of 12 identification tests (enzymatic reactions or sugar fermentations), as well as the detection of a penicillinase (particular interest in *Haemophilus influenzae, Haemophilus parainfluenzae, Branhamella catarrhalis (Moraxella catarrhalis)* and *Neisseria gonorrhoeae).*

The reactions produced during incubation result in spontaneous color changes or are revealed by the addition of reagents.

After a 2-hour incubation period at a temperature of 35-37°C, the reading of the reactions is performed visually and identification is obtained by consulting the profile list.

Reagents

API NH strips
NaCl 0.85% Medium (2 ml)
JAMES reagent
ZYM B reagent
Swab
Incubation box
Result sheet
1 package insert
McFarland Standard, point 4 on the scale
Mineral oil
Pipettes
Ampule rack
Ampule protector

Procedure

1. Specimen Processing

   The microorganisms to be identified must first be isolated as separate colonies by streaking the specimen onto Blood agar, Chocolate agar or Martin-Lewis agar according to standard

microbial techniques.

2. Preparation of Strip

Each strip is composed of 10 cupules. Each cupule has an open and closed area (cupule and tube). An incubation tray is supplied for each strip. It serves as a support and individual chamber while both protecting the strip from contaminants in the air and assuring the humid atmosphere necessary to avoid dehydration during incubation.

- Remove the strip from its individual packaging
- Place the strip in the incubation box
- Discard the desiccant sachet

Record the specimen number on the flat portion of the tray (do not record the number on the lid as it may be misplaced during handling).

3. Preparation of the Inoculum

- Open an ampule of NaCl 0.85% Medium (2 ml) with the ampule protector.
- Using a swab, pick up a few well-isolated colonies and prepare a suspension with a turbidity equivalent to **4 McFarland, ensuring it is well mixed**
- The suspension should be used immediately after preparation.

4. Inoculation of the Strip

- Distribute the prepared bacterial suspension into the cupules, avoiding the formation of bubbles (tilt the strip slightly forwards and place the tip of the pipette or PSIpette against the side of the cupule):
  - Only fill the tube part of the first 7 microtubes (PEN to URE): about 50 µl.
  - Fill tube and cupule of the last 3 microtubes LIP/ProA, PAL/GGT, βGAL/IND: about 150 µl, avoiding the formation of a convex meniscus.
- Cover the first 7 tests (PEN to URE) with mineral oil (underlined tests).

NOTE: The quality of the filling is very important: tubes which are insufficiently or excessively full may cause false positive or false negative results.

- Close the incubation box.
- Incubate for 2 hours at 35-37°C **in aerobic conditions.**

5. Incubation

Incubate for 2 hours at 35-37°C in aerobic conditions.

6. Reading the Strip

Refer to the Reactions Table for a description of how to read the reactions.

Note all spontaneous reactions (PEN to βGAL) and record them as + or -.

- Add 1 drop of ZYM B reagent to microtubes 8 and 9: LIP/ProA and PAL/GGT.
- Add 1 drop of JAMES reagent to microtube 10: βGAL/IND.
- **Wait 2 minutes** then read the reactions by referring to the Reading Table in this package insert and record them on the result sheet.
  - If the LIP reaction is positive (blue pigment), interpret the ProA reaction as **negative**, whether the ZYM B reagent has been added or not.
  - If, after a 2-hour incubation period, several reactions (fermentation, penicillinase) are doubtful, re-incubate the strip for another 2 hours and read the reactions again (the enzymatic tests should not be re-read in this case).

Reactions Table

TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS	
				NEGATIVE	POSITIVE
1) PEN	PENicillinase	Penicillin G	1.36	Blue (penicillinase absent)	Yellow Yellow -green Yellow -blue (penicillinase present
2) GLU 3) FRU 4) MAL 5) SAC	GLUcose (Acidification) FRUctose (Acidification) MALtose (Acidification) SACcharose/Sucrose (Acidification)	Glucose Fructose Maltose Sucrose	0.5 0.1 0.1 0.5	Red Red-orange	Yellow Orange
6) ODC	Ornithine DeCarboxylase	Ornithine	0.55	Yellow-green Grey-green	Blue
7) URE	UREase	Urea	0.41	Yellow	Pink-violet
8a) LIP	LIPase	5-bromo-3-indoxyl-caprate	0.033	Colorless Pale grey	Blue (+precipitate)
9a) PAL	Alkaline Phosphatase	Para-Nitrophenyl-phosphate 2CHA	0.038	Colorless Pale yellow	Yellow

10a) βGAL	Beta GALactosidaase	Para-Nitrophenyl-BD galactopyranoside	0.04	Colorless	Yellow

Reactions Table (Cont'd)

TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS NEGATIVE	RESULTS POSITIVE
				ZYM B / 3 min	
8b) ProA	Proline Arylamidase If LIP is +. ProA is always -	Proline-4-methoxy- β naphthylamide	0.056	Yellow Pale orange (brown if LIP +)	Orange
				ZYM B / 3 min	
9b) GGT	Gamma Glutamyl Transferase	Gamma glutamyl 4-methoxy- β naphthylamide	0.049	Yellow Pale orange (yellow -orange if PAL +)	Orange
				JAMES / 3 min	
10b) IND	INDole	Tryptophane	0.036	Colorless	Pink

Quality Control

To be performed on receipt of every new lot of strip by the Q.C bench technologist.

Reference

QC organisms to be used:

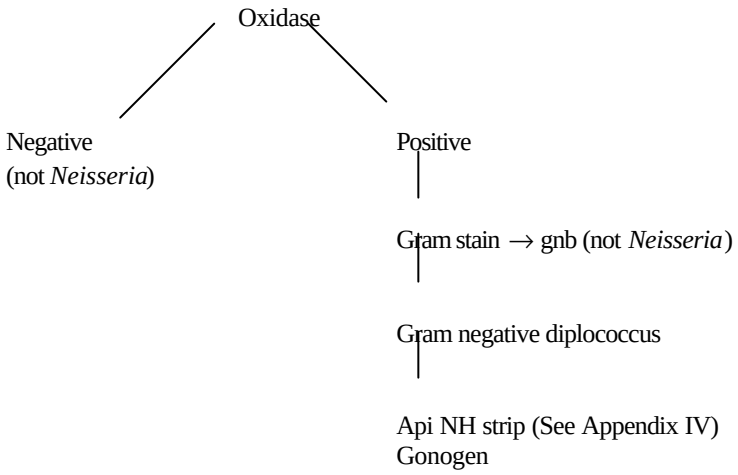
Neisseria gonorrhoea	ATCC 31426
Haemophilus influenzae	ATCC 10211
Branhamella catarrhalis	ATCC 23246
Haemophilus paraphrophilus	ATCC 49917

Reference Package Insert - api NH system for the identification of *Neisseria* and *Haemophilus* bioMerieux Inc., Missouri USA.

APPENDIX V

(IDENTIFICATION OF *NEISSERIA GONORRHOEAE)*

Identification of *Neisseria gonorrhoeae*



APPENDIX VI
(READING OF GRAM STAIN)

Reading of Gram Stain

1. Examine stained smear microscopically by first focusing under low power.

2. Pick the best area for white cells, bacteria, and other structures and quantitate as below:

N.B. PUS CELLS/WBC will be reported as PMN

< 1 cell per 1000 x oil immersion field = ±
1-4 cells per 1000 x oil immersion field = +
5-10 cells per 1000 x oil immersion field = + +
> 10 cells per 1000 x oil immersion field = + + +

Reference

Cumitech 4 - Laboratory Diagnosis of Gonorrhoeae, Oct. 1976

APPENDIX VII
(GC WORK-UP)

Work-up of Suspected *Neisseria gonorrhoeae*

1. Examine ML plate after 48 and 72 hours incubation.

2. Perform oxidase test and Gram stain on suspected GC. If oxidase positive Gram negative diplococci:
   - (i) Perform a GonoGen GC coagglutination test from the primary plate if there is sufficient growth (Refer to Appendix III).
   - (ii) Make two CHOC purity plates on suspected GC and incubate in CO_2 at 35^0C x 24 hours.
   - (iii) After 24 hours incubation perform the following from the purity plates:
     - (a) api NH strip (refer to Appendix IV)
     - (b) GonoGen GC coagglutination test if not performed from the primary plate (Refer to Appendix III).

   GC is identified by the reactions listed in Appendix V.

3. After 72 hours incubation of the original (primary) ML plate, if the bacterial growth is not typical of GC, flood the plate with oxidase reagent. If oxidase positive colonies are present, perform a Gram stain and proceed as in step 2.

Record of Edited Revisions

Manual Section Name: Genital

Page Number / Item	Date of Revision	Signature of Approval
Pg.25-27 Changed entire section of Prostatic/Seminal Fluid	January 22, 2003	
