WOUNDS / TISSUES / ASPIRATES / MISCELLANEOUS CULTURE MANUAL

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SUPERFICIAL SWABS AND DRAINAGE

I. Introduction

This section includes specimens from decubitus ulcers, episiotomies, non-intravenous or non-central line exit sites, chest tube drainage, abdominal drainage, and tracheal swabs. Many different bacterial species can cause infection of these sites. The presence of squamous epithelial cells may indicate that the specimen is superficial and therefore the organism isolated may not reflect the true etiology of the infection.

II. Specimen Collection and Transport

Specimens should be collected using a clean, sterile swab and sent in Amies transport medium. Drainage material should be collected into a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain - Quantitate the presence of pus cells, squamous epithelial cells, and organisms. (Refer to Appendix II).
- Not required for exit site swabs.

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
For chest tube drainage and tracheal swabs, add:		
Haemophilus Isolation Medium (HI)	CO_2,	35°C x 48 hours

B. Interpretation of Cultures:

Examine the plates after 24 and 48 hours incubation.

Any growth of *S. aureus*, group B streptococcus from neonates, beta-hemolytic streptococcus groups A, C and G and *Pseudomonas aeruginosa* is significant. For chest tube drainage and tracheal swabs, any growth of *H. influenzae* and *S. pneumoniae* is also significant. A heavy, pure growth of other organisms that correlates with the predominant organism seen in the Gram stain is significant if there is ≥1+ pus cells (not for exit sites). If a specific organism is requested, then it will be looked for and its presence or absence reported. Growth of ≥ 3 types of coliforms or other Gram negative bacilli will be reported as a negative report stating commensal flora including mixed Gram negative bacilli.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. <u>Reporting Results</u>

a) Gram stain: Report with quantitation the presence of pus cells, squamous epithelial cells and organisms.

b) Culture:

Negative report:	"No growth" or "Commensal flora" "Commensal flora including mixed Gram negative bacilli".
Positive report:	Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

WOUND SWABS

I. Introduction

Wound infections may be due to a variety of organisms, but are most commonly associated with *S. aureus*, beta-hemolytic streptococci, *S. milleri* group, *P. aeruginosa* and enteric Gram negative bacilli. The presence of squamous epithelial cells may indicate that the specimen is superficial and therefore the organisms isolated may not reflect the true etiology of the infection.

II. Specimen Collection and Transport

Specimens should be collected using a clean, sterile swab and sent in Amies transport medium. If anaerobic culture is requested, an anaerobic swab placed in an anaerobic transport tube must be collected. If a delay in transport or processing is anticipated, the aerobic swab should be kept at 4^oC. If both an aerobic and anaerobic swab are received, both swabs should be kept at room temperature until processed.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain – Quantitate the presence of pus cells, squamous epithelial cells and organisms. (Refer to Appendix II).

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35^oC x 48 hours
MacConkey Agar (MAC)	O_2, 35^oC x 48 hours
Colistin Nalidixic Acid Agar (CNA)	O_2, 35^oC x 48 hours
If anaerobic culture is requested **and** anaerobic swab received, add:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35^0C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35^0C x 48 hours

B. Interpretation of Cultures:

Examine the aerobic plates after 24 and 48 hours incubation and the anaerobic plates after 48 hours incubation.
Any growth of *S. aureus*, beta-hemolytic streptococci groups A, B, C and G and *Pseudomonas aeruginosa* is significant. For sternal wound specimens, isolation of coagulase negative staphylococci is significant and appropriate antimicrobial susceptibility testing should be performed and reported. A growth of 1 or 2 types of organisms other than skin commensals is significant if there was ≥1+ pus cells seen on the original Gram stain. Growth of ≥ 3 types of coliforms or other Gram negative bacilli will be reported as a negative report stating "commensal flora including mixed Gram negative bacilli".

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

a) Gram stain: Report with quantitation the presence of pus cells, squamous epithelial cells and organisms.

b) Culture:

Negative report: "No growth" or "Commensal flora"
"Commensal flora including mixed Gram negative bacilli".

Positive report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

"The __________________ seen in the Gram stain failed to grow in aerobic culture".

NB: If anaerobic culture requested and no anaerobic swab received, report the following phrase with <u>both</u> the negative and positive reports:

"No anaerobic swab received; anaerobic culture not done". Enter under test field in LIS.

BITE WOUND SWABS

I. Introduction

Bite wounds may become infected with many different organisms but most commonly include *S. aureus, Pasteurella* spp., *S. milleri* group and beta-hemolytic streptococci. The presence of squamous epithelial cells may indicate that the specimen is superficial and therefore the organisms isolated may not reflect the true etiology of the infection.

II. Specimen Collection and Transport

Specimens should be collected using a clean, sterile swab and sent in Amies transport medium. If anaerobic culture is requested, an anaerobic swab placed in an anaerobic transport tube must be collected. If a delay in transport or processing is anticipated, the aerobic swab should be kept at 4^oC and the anaerobic swab at room temperature.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Processing of Specimens

A. Processing of Specimens:

a) Direct Examination: Gram stain – Quantitate the presence of pus cells, squamous epithelial cells, and organisms. (Refer to Appendix II)

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35^oC x 48 hours
MacConkey Agar (MAC)	O_2, 35^oC x 48 hours
Chocolate Agar (CHOC)	CO_2, 35^oC x 48 hours
If anaerobic culture is requested **and** anaerobic swab received, add:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35^oC x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35^oC x 48 hours

B. Interpretation of Cultures:

Examine aerobic plates after 24 and 48 hours incubation and anaerobic plates after 48 hours incubation.

Any growth of *S. aureus*, *Pasteurella* spp., *S. milleri* group, beta-haemolytic streptococci and *Pseudomonas aeruginosa* is significant. For other organisms such as Enterobacteriaceae and other Gram negative bacilli, a significant result is determined by the isolation of a moderate to heavy predominant growth, or if growth correlates with the predominent organism seen on Gram stain.

For suspected anaerobes, minimal identification is performed.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

a) Gram stain: Report with quantitation the presence of pus cells, squamous epithelial cells and organisms.

b) Culture:

Negative Report: "No growth" or "Commensal flora"

Positive Report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

NB: If anaerobic culture requested and no anaerobic swab received, report the following phrase with both the negative and positive reports:

"No anaerobic swab received; anaerobic culture not done". Enter under test field in LIS.

SKIN BIOPSIES

I. Introduction

A variety of organisms may be associated with skin lesions and thus any growth of organisms other than skin commensals should be considered significant.

II. Specimen Collection and Transport

Skin biopsy specimens should be placed in a sterile container with sterile saline. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials /Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimen:

1. Macerate the entire specimen with a small volume of sterile saline from the original container using a grinder (small tissue sample) or a scalpel and stomacher (large tissue sample).
2. Prepare 3 slides from the ground material: one for Gram stain, one for Calcofluor white stain and one unstained (stored in the "extra smear" slide box).
3. If TB has been requested, forward half the specimen to the Public Health Laboratory (PHL) for processing. If viral isolation is requested, a portion of the ground material should be given to the Virology section for processing. If parasitology is requested, forward a portion of the specimen (before macerating) to the Parasitology section for processing.

a) Direct Examination: Gram stain: Quantitate the presence of pus cells and organisms. (Refer to Appendix II).
Calcofluor white 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
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 3 weeks
Esculin Base Medium (EBM)*	O_2, 30°C x 3 weeks

* Forward the fungus culture media to the Mycology section for incubation and work-up.

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 hours incubation. Any growth of organisms other than skin commensals should be considered significant.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

b) Culture:

Negative Report: "No growth" or "Commensal flora"

Positive Report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

TML\MSH Microbiology Department Policy & Procedure Manual	**Policy # MI\WND\02\v01**	Page 1 of 2
Section: **Wounds / Tissues / Aspirates Culture Manual**	Subject Title: **Pus & Abscess Material (Other than liver and rectal)**	
Issued by: **LABORATORY MANAGER**	Original Date: September 22, 1999	
Approved by: Laboratory Director	Revision Date: July 26, 2000	

PUS & ABSCESS MATERIAL (OTHER THAN LIVER AND RECTAL)

I. Introduction

Abscesses are usually due to a mixture of different aerobic and anaerobic bacteria depending on the location of the abscess.

II. Specimen Collection and Transport

Pus from an abscess should be sent in a clean, sterile container and an anaerobic transport container. An aspirate of pus or abscess material may be collected using a syringe and sent to the laboratory with the needle removed. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).
Modified acid fast stain - If Actinomyces or Nocardia is requested or suggested on Gram stain.
Calcofluor white stain - If fungus is requested. (Refer to Mycology Manual).

b) Culture:

Media	Incubation
Blood Agar (BA)[1]	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
Chocolate Agar (CHOC)[1]	CO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)[2]	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)[2]	AnO_2, 35°C x 48 hours

If Nocardia is requested, add: Na Pyruvate Agar (NPA)* **AND** fungus media below	O_2,	35^0C x 4 weeks
If fungus culture is requested, add: Inhibitory Mold Agar (IMA)*	O_2,	30^oC x 3 weeks
Esculin Base Medium (EBM)*	O_2,	30^oC x 3 weeks

*Forward the fungus culture media and NPA to the Mycology section for incubation and work-up.

NOTE:

1. If Nocardia is requested, send the BA and CHOC plates to mycology after 48 hours incubation. The plates will be incubated in mycology for 4 weeks.
2. If Actinomyces is requested, anaerobic media are to be incubated for 7 days.
3. If Nocardia or Actinomyces is suggested on Gram stain, send BA and CHOC plates to Mycology after 48 hours incubation and incubate the anaerobic media for 7 days.

B. Interpretation of Cultures:

Examine the aerobic culture plates after 24 and 48 hours incubation and the anaerobic plates after 48 hours and 7 days (if Actinomyces requested or suggested on Gram stain) incubation. All isolates should be identified. For anaerobic growth, perform minimal work-up.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

b) Culture:

Negative report: "No growth"

If Actinomyces is requested, report: "No Actinomyces isolated after 7 days incubation"

Positive report: Quantitate significant isolates with appropriate sensitivities.

RECTAL ABSCESS

I. Introduction

Rectal abscesses may contain a variety of organisms usually from the gastrointestinal flora. Both aerobic and anaerobic bacteria may be present.

II. Specimen Collection and Transport

Rectal abscess swabs should be sent in Amies transport medium. Pus from a rectal abscess should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4^0C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).

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

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 hours incubation.
Any growth of *S. aureus*, beta-haemolytic Streptococci, *S. milleri* or *Pseudomonas aeruginosa* will be identified. Ignore organisms that are usually part of the faecal flora (i.e. Gram negative bacilli).

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

b) Culture:

Negative Report: "No growth" or "Mixed faecal flora"

Positive Report: Quantitate all significant isolates with appropriate sensitivities. Report "Mixed faecal flora" if also present.

LIVER & BRAIN ASPIRATES (PUS / CYST FLUID OR ABSCESS MATERIAL)

I. Introduction

Liver and brain abscesses may be due to bacteria, fungi (e.g. hepatic candidiasis) and parasites (e.g. *Echinococcus*). Both aerobic and anaerobic bacteria may be present.

II. Specimen Collection and Transport

Pus from an abscess should be sent in a clean, sterile container and an anaerobic transport container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimen:

1. If >1 ml of thin fluid is received, centrifuge specimen at 3500 rpm for 20 minutes. For purulent and thick specimens or if <1 ml is received, centrifugation is not required.

2. If parasitology is requested, send a portion of the fresh specimen to Parasitology section (Mondays to Fridays). On weekends and holidays, mix an equal volume of specimen with SAF and send to Parasitology section. Note on the specimen label that it has been mixed with SAF.

   If TB culture is requested, send a portion of the specimen to the Public Health Laboratory (PHL) for processing.

   a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).
   Calcofluor white stain - Refer to Mycology Manual.

   Prepare an extra smear and store in the "extra smear" slide box.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours[1]
MacConkey Agar (MAC)	O_2, 35°C x 48 hours[1]
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours[1]
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 72 hours
Kanamycin/Vancomycin Agar (KV)	AnO_2, 35°C x 72 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days[1]
Inhibitory Mold Agar (IMA)[2]	O_2, 30°C x 3 weeks
Esculin Base Medium (EBM)[2]	O_2, 30°C x 3 weeks

[1] If organisms were seen in direct gram stain and cultures yields no growth, check original gram stain and reincubate all aerobic plates and broth for 7 days.

[2] Forward the fungus culture media to the Mycology section for incubation and processing.

B. Interpretation of Cultures:

Examine the aerobic culture plates after 24 and 48 hours incubation and the anaerobic plates after 72 hours incubation. Examine the THIO daily for evidence of growth. If no growth on culture plates but evidence of growth in THIO, then perform Gram stain and subculture THIO onto BA, MAC, CHOC and BRUC (as appropriate) and incubate and process as above.

All isolates should be identified.

D. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

b) Culture:

Negative Report: "No growth"

Positive Report: Report all isolates with appropriate sensitivities.

Telephone results of the Gram stain and positive isolates to the ward / ordering physician.

SURGICAL BIOPSY / TISSUE

I. Introduction

Surgical biopsies should be considered sterile specimens and therefore the isolation of any organism(s) should be considered significant.

II. Specimen Collection and Transport

Tissue should be collected in a clean, sterile container with a small amount of sterile saline. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimen:

1. Macerate the tissue using a grinder (small tissue sample) or a scalpel and stomacher (large tissue sample). Bone should be inoculated directly into Fastidious Anaerobic Broth and is not macerated.
2. Prepare 3 slides from the macerated material: one for Gram stain, one for Calcofluor white stain and one unstained (Stored in the "extra smear" slide box).
3. Fungus culture is **NOT** set up for wound debridement tissue, joint capsules, gas gangrene tissue and necrotizing fasciitis tissue unless specifically requested.
4. Send a portion of **ALL** macerated specimens or tissues to the Public Health Laboratory (PHL) for TB **except** for wound debridement tissue, joint capsules, gas gangrene tissue, and necrotizing fasciitis tissue unless specifically requested. If viral isolation is requested, send a portion of the specimen to the Virology section for processing.
5. Inoculate the following media with the remaining sample:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours[1]
MacConkey Agar (MAC)	O_2, 35°C x 48 hours[1]
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours[1]
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[1]

Inhibitory Mold Agar (IMA)[2]	O_2,	30°C x 4 weeks
Esculin Base Medium (EBM)[2]	O_2,	30°C x 4 weeks
Blood Egg Albumin Agar (BEAA)	O_2,	30°C x 4 weeks

[1] If organisms were seen on direct gram stain and cultures yields no growth, check original gram stain and reincubate all aerobic plates and broth for 7 days.
[2] Forward the fungal culture media to the Mycology section for incubation and work-up.

B. Interpretation of Smears:

a) Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).
b) Calcofluor white stain – Refer to Mycology Manual.

C. Interpretation of Cultures:

Examine the aerobic culture plates after 24 and 48 hours incubation and the anaerobic plates after 48 hours incubation. Examine the THIO daily for evidence of growth. If no growth on culture plates but evidence of growth in THIO, then perform Gram stain and subculture THIO onto BA, MAC, CHOC and BRUC (as appropriate) and incubate and process as above. After 48 hours incubation, keep the THIO at room temperature for a total of 5 days before discarding. All isolates are to be identified as appropriate.

D. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

a) Gram stain: Report with quantitation the presence of pus cells and organisms.
b) Culture:

Negative Report: "No growth"

Positive Report: Report all isolates with appropriate sensitivities.

AUTOPSY

I. Introduction

Specimens collected at autopsy are often contaminated with faecal or skin flora. Interpretation of cultures must take into account the presence of commensal flora from different body sites.

II. Specimen Collection and Transport

This specimen should be received in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

1. Macerate the tissue using a grinder (small tissue sample) or a scalpel and stomacher (large tissue sample). Bone should be inoculated into Fastidious Anaerobic Broth and not macerated.
2. Prepare 2 slides from the macerated material: one for a Gram stain and a second unstained. (Stored in the "extra smear" slide box).
3. A portion of all autopsy lung tissue (except newborns) is to be sent to the Public Health Lab (PHL) for *Legionella* detection and TB culture.
4. Inoculate the following media with the remaining sample:

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

For all lung tissue or if fungal culture is requested, add:

Inhibitory Mold Agar (IMA)*	O_2,	30°C x 4 weeks
Esculin Base Medium (EBM)*	O_2,	30°C x 4 weeks

* Forward the fungus culture media to the Mycology section for incubation and work-up.

B. Interpretation of Smears:

a) Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).

C. Interpretation of Cultures:

Examine plates after 24 and 48 hours incubation. Identify all pure growth Gram negatives and all significant pathogens.

D. Susceptibility Testing:

Not Required.

V. Reporting Results

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

b) Culture:

Negative Report: "No growth" or "Mixed flora suggesting contamination"

Positive Report: Report all significant isolates **without** sensitivities.

INTRAVASCULAR CATHETER TIPS

I. Introduction

Intravascular catheters may include central, CVP, Hickman, Broviac, peripheral, arterial, umbilical, hyperalimentation, hemodialysis, port-a-cath and Swan-Ganz catheters.

II. Specimen Collection and Transport

These specimens should be sent in a clean, sterile container.
If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials/ Media

Refer to Appendix I.

IV. Processing of Specimens

A. Processing of Specimens:

a) Direct Examination: Not indicated.

b) Culture:

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

Roll the segment back and forth 4 times across the surface of the BA using sterile forceps.

B. Interpretation of Culture:

Examine the BA plate after 24 and 48 hours incubation.
Any growth of *S. aureus*, β-haemolytic streptococci, *Pseudomonas* spp., other Gram negative bacilli and yeasts are significant; quantitate and identify. Other organisms will be quantitated and identified only if $\geq$15 colonies of that organism are present and there are no more than 3 different bacterial types. Otherwise simply list the morphotypes.

C. Susceptibility Testing:

Sensitivity testing is only performed on significant isolates. Refer to Susceptibility Testing Manual.

V. Reporting Results

Negative Report: "No growth"

Positive Report: **For non-significant organisms:**
"<15 colonies of (list morphotypes of non-significant organisms)". No sensitivities required.

">15 colonies of (list morphotypes of mixed non-significant organisms)". No sensitivities required.

For significant organisms:
"<15 colonies of (organism name)" or "≥15 colonies of (organism name)". Report appropriate sensitivities.

For UHN, if *S. aureus*, Gram negative bacilli, or yeast isolated, telephone result to ward / ordering physician.

PERITONEAL DIALYSIS CATHETER/CANULA & PROSTHETIC DEVICES

I. Introduction

Peritoneal dialysis catheters or canula (PD Canula) and any other prosthetic devices removed from patients may be sent for sterility testing.

II. Specimen Collection and Transport

These specimens should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Processing of Specimens

A. Processing of Specimens

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days

Examine THIO daily for up to 5-days. If there is evidence of growth, perform Gram stain and sub-culture THIO onto BA, CHOC and other media as appropriate.

B. Interpretation of Culture:

S. aureus, β-haemolytic streptococci, *Pseudomonas* spp., other Gram negative bacilli and yeasts are significant; worked up. Other organisms will be worked up only if there are no more than 3 different bacterial types. Otherwise simply list the morphotypes.

C. Susceptibility Testing:

Sensitivity testing is only performed on significant isolates. Refer to Susceptibility Testing Manual.

V. Reporting Results

Negative Report: "No growth" or "No significant growth including (list of non-significant organisms)"

Positive Report: Report all significant isolates with appropriate sensitivities.

BILE

I. Introduction

Bile is a normally sterile fluid. However, it may become contaminated when collected from a post-op drain. Bile may also be collected at the time of percutaneous cholangiography (PTC).

II. Specimen Collection and Transport

Bile may be aspirated with a syringe during surgery or collected in a sterile container from a post-op drain. If a delay in transport or processing is anticipated, the specimen should be kept at 4^oC.

III. Reagents / Material / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).

b) Culture:

Media	Incubation	
Blood Agar (BA)	CO_2,	35^oC x 48 hours
MacConkey Agar (MAC)	O_2,	35^oC x 48 hours
If anaerobic culture is requested or bile is collected by PTC, add:		
Fastidious Anaerobic Agar (BRUC)	AnO_2,	35^oC x 48 hours
Kanamycin/Vancomycin Agar (KV)	AnO_2,	35^oC x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2,	35^oC x 48 hours

B. Interpretation of Cultures:

Examine the aerobic culture plates after 24 and 48 hours incubation and the anaerobic plates after 48 hours incubation. Any growth of *Salmonella* species, *S. aureus,* and *Ps. aeruginosa* are significant. For other organisms, a significant result is determined by the isolation of ≤ 3 organisms. For non-lactose fermenters (NLF), screen for *Salmonella* species.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. <u>Reporting Results</u>

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

b) Culture:

Negative Report: "No growth" or "Mixed faecal flora"

Positive Report: Quantitate all significant isolates with appropriate sensitivities. If faecal flora is also present, report without quantitation.

BONE BANK, BONE GRAFT & CADAVER FASCIA / TISSUE/ SWAB SPECIMENS

I. Introduction

Bone specimens for the bone bank are collected for use in transplantation. Specimens are usually collected ante-mortem or just prior to transplantation and should normally be sterile. Occasionally, fascia may be used for transplantation in which case a swab or tissue sample may be collected for sterility testing.

II. Specimen Collection and Transport

Swabs from the donor bones or fascia should be collected using a clean, sterile swab and sent in Amies transport medium. If anaerobic culture is requested, an anaerobic swab sent in anaerobic transport medium should be collected. Bone or fascia tissue should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the aerobic swab and bone/fascia tissue should be kept at 4^oC and the anaerobic swab at room temperature.

III. Reagents / Material / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Not indicated

b) Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)*	O_2, 35^oC x 7 days

* A separate THIO should be inoculated for each specimen / swab received.

B. Interpretation of Culture:

Examine the THIO daily for 7 days. If evidence of growth, perform Gram stain and subculture THIO onto Blood agar (BA), MacConkey (MAC), Chocolate (CHOC) and (BRUC) as appropriate based on Gram stain.
All isolates are to be identified. Freeze all isolates at -70°C.

C. Susceptibility Testing:

Not required for isolates from bone collected ante-mortem.

For isolates from bone or fascia cultured just prior to transplantation, perform susceptibility testing on all isolates. Refer to Susceptibility Testing Manual.

V. <u>Reporting Results</u>

Negative Report: "No growth after 7 days of incubation".

Positive Report: Report all isolates with sensitivities as appropriate.

BREAST MILK

I. Introduction

Breast milk may become infected with a variety of organisms and all species should be identified except skin commensals.

II. Specimen Collection and Transport

Breast milk should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

III. Reagents / Materials/ Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

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

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 incubation
Any growth of organisms other than skin commensals should be considered significant.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

Negative Report: "No growth" or "Commensal flora"

Positive Report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

TML\MSH Microbiology Department Policy & Procedure Manual	**Policy # MI\WND\11\v01**	Page 1 of 2
Section: **Wounds / Tissues / Aspirates Culture Manual**	Subject Title: **Total Parental Nutrition (TPN)**	
Issued by: **LABORATORY MANAGER**	Original Date: September 22, 1999	
Approved by: Laboratory Director	Revision Date: July 26, 2000	

TOTAL PARENTAL NUTRITION (TPN)

I. Introduction

Total parenteral nutrition fluids are normally sterile.

II. Specimen Collection and Transport

A TPN set disconnected from patients with fever consists of a TPN bag, tubing and a lipid bottle. Distinguish these from Pharmacy TPN samples for sterility testing which are sent in small vials. Inform Infection Control Nurse when TPN set is received.

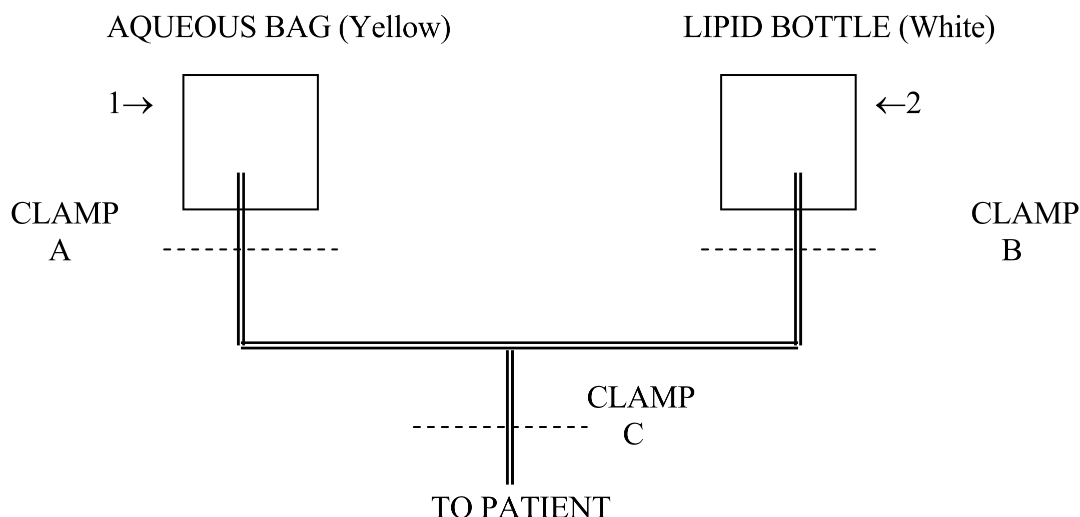
III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimen:
1. Determine if tubings are clipped at positions A, B & C at bedside (See diagram below). If not, clip at these positions and note on work card.
2. Using aseptic technique, remove ~ 1 mL each from **1** & **2** and culture as outlined below.

Save the entire TPN set up for Infection Control.



b) Culture:

Media	**Incubation**	
Blood Agar (BA)	CO_2,	35°C x 2 days
Fastidious Anaerobic Broth (THIO)	O_2,	35°C x 5 days
Inhibitory Mold Agar (IMA)*	O_2,	30°C x 3 weeks
IMA with sterile olive oil overlay (olive oil is stored in media room)*	O_2,	30°C x 1 week

*Forward these plates to the Mycology section for incubation and work-up.

B. Interpretation of Cultures:

Examine the BA plate after 24 and 48 hours incubation and the THIO daily for up to 5 days.
Any growth should be considered significant.
Freeze all isolates at -70°C and put into "Sterile Sites" box.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Result

Culture:

Negative Report: "No growth"

Positive Report: Report all organisms with appropriate sensitivities. Do not quantitate.

INTRAVENOUS & CENTRAL LINE CATHETER EXIT SITE SWABS

I. Introduction

The intravenous or central line catheter exit site may become infected with a variety of organisms which may lead to tunnel infections or bacteraemia.

II. Specimen Collection and Transport

Specimens should be collected using a clean, sterile swab and sent in Amies transport medium. If a delay in transport or processing is anticipated, keep the specimen at 4^0C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation	
Blood Agar (BA)	CO_2,	35°C x 48 hours
MacConkey Agar (MAC)	O_2,	35°C x 48 hours

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 hours incubation.
Quantitate and identify any growth of *S. aureus*, *Pseudomonas* species, yeast and beta-haemolytic streptococci. Quantitate and identify any pure or predominant growth of other Gram negative bacilli and enterococci. A heavy, pure growth of any other organism is significant.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

Negative report: "No growth" or "Commensal flora"

Positive report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

EAR SPECIMENS - EAR SWABS

I. Introduction

Ear swabs are collected for the diagnosis of otitis externa; they are not useful in the diagnosis of otitis media. Otitis externa is a bacterial infection of the external auditory canal usually caused by *P. aeruginosa, S. aureus, S. pneumoniae,* Group A streptococcus or fungus / yeast.

II. Specimen Collection and Transport

The ear swab should be collected using a clean, sterile swab and sent in Amies transport medium. If a delay in transport or processing is anticipated, the specimen should be kept at 4^0C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).
Calcofluor white stain (If fungus is requested). - Refer to Mycology Manual.

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
If fungus culture is requested, add:		
Inhibitory Mold Agar (IMA)*	O_2,	30°C x 3 weeks
Esculin Base Medium (EBM)*	O_2,	30°C x 3 weeks

* Forward the fungal culture media to the Mycology section for incubation and work-up.

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 hours incubation.
Any growth of *S. aureus*, *P. aeruginosa, S. pneumoniae,* Group A streptococcus or yeast is significant. For specimens from neonates only, identify and report Group B streptococcus. For other organisms, a significant result is determined by the presence of a moderate to heavy growth of an organism which correlates with the predominant organism on the Gram stain. The Gram stain should also show ≥1+ pus cells. Full identification is required for all significant organisms except yeast.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

b) Culture:

Negative Report: "Commensal flora" or "No growth".

Positive Report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

TYMPANOCENTESIS FLUID

I. Introduction

Tympanocentesis fluid is obtained for the diagnosis of otitis media. These specimens are handled as sterile fluids. (Refer to Sterile Fluids Culture Manual)

EYE / CONJUNCTIVAL / LID SWABS

I. Introduction

Eye / conjunctival / lid swabs are collected for the diagnosis of conjunctivitis.

II. Specimen Collection and Transport

It is preferable that both eyes be swabbed, even if the infection is unilateral. Swabs should be collected prior to the instillation of topical anaesthetics or antibiotics, and sent in Amies transport medium. Viral isolation requires special transport media. If a delay in transport or processing is anticipated, the specimen should be kept at 4^0C.

Occasionally, specimens collected by an ophthalmologist will be inoculated directly onto culture plates at the bedside. The ophthalmologist will inoculate the plates in a short spiral line. If lid swabs are also collected, these will be inoculated onto the same culture plates next to the conjunctival inoculation. Lid swabs will be inoculated in the shape of an "L" or "R" indicating left or right, respectively. These plates should be kept in the incubator (35^0C) until processed.

III. Reagents / Materials / Media

A. Processing of Specimens:

NB: If pre-inoculated culture plates are received, these should be incubated as listed below. No Gram stain will be performed.

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
For all neonates < 1 week of age, or if *N. gonorrhoeae* is requested, add:	
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

B. Interpretation of Cultures:

Examine the BA and CHOC plates after 24 and 48 hours incubation and the ML plate after 48 and 72 hours incubation. Any growth of *S. aureus, H. influenzae, M. catarrhalis*, *N. gonorrheae*, Gp.A Strep, *S. pneumoniae*, *Moraxella* species, and *P. aeruginosa* is potentially significant. For other organisms, a significant result is determined by the isolation of a moderate or heavy growth of a potential pathogen correlated with the predominant organism on the Gram stain. There should be ≥1+ pus cells on the Gram stain. Full identification is required for all significant organisms.

For work-up and identification of *N. gonorrhaeae*, refer to the Genital Tract Manual.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. <u>Reporting Results</u>

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

b) Culture:

Negative Report: "Commensal flora" or "No growth".

Positive Report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

EYE / CORNEAL SCRAPINGS, CONTACT LENSES & SOLUTIONS

I. Introduction

Eye / corneal scrapings are collected for the diagnosis of keratitis. Contact lenses and solutions may be submitted to the microbiology laboratory for detection of contamination including the presence of acanthamoeba.

II. Specimen Collection and Transport

The physician usually prepares two or three slides and inoculates the appropriate media at the time of specimen collection. The following media is to be supplied to the physician for each eye: BA, CHOC, IMA and THIO. The physician will inoculate the plates in rows of "C" - shaped marks, with each row representing a separate sample. If a delay in transport or processing is anticipated, the specimen should be kept in the incubator (35^0C) in Specimen Management area. Virus and chlamydia detection require special transport media. (See Virology Manual). If acanthamoeba is requested, forward specimen to Parasitology section for processing. If *E. coli* overlay plate is received and parasitology section is closed, incubate plate at 35^0C in O_2 until processed. All other specimens received for acanthamoeba should be kept at room temperature until processed.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

NB: If previously inoculated plates received and no specimen or swab received, then direct examination is not performed.

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).
Calcofluor white stain. (If two smears are provided).
An extra smear is held in reserve for special stains (eg, Giemsa stain if requested).

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 4 days
Chocolate Agar (CHOC)	CO_2, 35°C x 4 days
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 3 weeks

*Forward the fungal culture media to the Mycology section for incubation and workup.

B. Interpretation of Cultures:

Examine the culture plates daily. If no growth on culture plates but growth in THIO, perform Gram stain and sub-culture THIO onto BA, and CHOC and incubate x 48 hours.
For Conjunctival scrapings see Eye swabs.
For Corneal scrapings all organisms should be identified.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

For conjunctival scrapings, see Eye swabs.

For corneal scrapings:

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

b) Culture:

Negative report: "No growth."

Positive report: Quantitate all isolates with appropriate sensitivities.

INTRAOCULAR ASPIRATES

I. Introduction

Aspirates of intraocular fluids are submitted for the diagnosis of uveitis and endophthalmitis. These specimens are handled as sterile fluids. (Refer to the Sterile Fluids Culture Manual)

Any requests for specialized procedures should be discussed with a medical microbiologist or the chief technologist before proceeding.

LACRIMAL (TEAR DUCT) STONE / SECRETIONS

I. Introduction

Stones may form in the lacrimal duct resulting in obstruction and secondary infection of the lacrimal gland.

II. Specimen Collection and Transport

Specimens are to be collected and transported in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4^0C.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct examination: Crush specimen on glass slide to obtain a thin smear for Gram stain. Examine for pus cells and organisms especially branching gram positive bacilli resembling *Actinomyces* species.

b) Culture: Crush specimen using a sterile wood applicator stick or urine loop before plating onto the following media:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 hours incubation. Examine the THIO daily for evidence of growth. If no growth on culture plates but evidence of growth in THIO, then perform Gram stain and subculture THIO onto BA, CHOC and BRUC (as appropriate) and incubate and process as above. Identify all significant isolates.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. <u>Reporting Results</u>

a) Gram stain: Report presence of organisms.
"Organisms resembling Actinomyces seen in Gram stain".

b) Culture:

Negative Report: "Commensal flora" or "No growth".

Positive Report: Quantitate all significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

FACIAL SPECIMENS

I. Introduction

Infections of the facial structures may be due to a variety of aerobic and anaerobic bacteria usually from the oral cavity. Actinomyces is a particularly important pathogen.

II. Specimen Collection and Transport

These specimens should be transported in either an anaerobic transport container or Amies transport medium. If a delay in transport or processing is anticipated, the specimen should be kept at 4^0C.

III. Reagents / Materials/ Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms. (Refer to Appendix II).
Calcofluor white stain (If fungus is requested).
Modified acid fast stain - If *Actinomyces* is requested or suggested on 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
MacConkey Agar (MAC)	O_2, 35°C x 48 hours

If Actinomyces is requested or an anaerobic swab collected or thick pus is received, add:

Media	Incubation
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin (KV)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 5 days

If fungus culture is requested, add:

Medium	Atmosphere	Incubation
Inhibitory Mold Agar (IMA)*	O_2,	30°C x 3 weeks
Esculin Base Medium (EBM)*	O_2,	30°C x 3 weeks

*Forward the fungal culture media to the Mycology section for incubation and work-up.

NOTE: 1. If *Actinomyces* is requested and appropriate specimen is received or organism is suggested on Gram stain, anaerobic media are to be incubated for 7 days.

B. Interpretation of Cultures:

Examine the aerobic culture plates after 24 and 48 hours incubation and the anaerobic plates after 48 hours and 7 days incubation (If Actinomyces requested or suggested on Gram stain). In general, these specimens are handled as wound swabs, except that some specimens may be contaminated with oral flora.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

b) Culture:

Negative Report: "Commensal flora" or "No growth".

Positive Report: Quantitate significant isolates with appropriate sensitivities. If commensal flora is also present, report with quantitation.

IN VITRO TISSUE CULTURE SPECIMENS

I. Introduction

Human cells may be collected from patients for in vitro culture to expand certain cell populations. This is usually done in the Cytogenetics Laboratory. As with any cell culture, these may become contaminated with bacteria or other organisms. Because this is a normally sterile procedure, the isolation of any organism should be considered significant.

II. Specimen Collection and Transport

A minimum of 1 ml of the cell culture media should be collected into a clean sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4^0C.

III. Reagents / Material / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

a) Direct Examination: Gram stain: Note the presence of organisms.

c) Culture:

Media	Incubation	
Blood Agar (BA)	CO_2,	35^0C x 48 hours
Chocolate Agar (CHOC)	CO_2,	35^0C x 48 hours
MacConkey Agar (MAC)	O_2,	35^0C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2,	35^0C x 5 days
If fungus culture is requested, add:		
Inhibitory Mold Agar (IMA)*	O_2,	35^0C x 3 weeks
Esculin Base Medium (EBM)*	O_2,	35^0C x 3 weeks

*Forward the fungal culture media to the Mycology section for incubation and work-up.

B. Interpretation of Cultures:

Examine the plates after 24 and 48 hours incubation. Identify all isolates.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

a) Gram Stain: Report the presence of organisms.

b) Culture:

Negative Report: "No growth".

Positive Report: Report all isolates with appropriate sensitivities.

If requested STAT, telephone Gram stain and positive culture results to ordering laboratory.

APPENDIX I

REAGENTS / MATERIALS / MEDIA

Acid-fast stain – VWR
Calcofluor white – Difco
Catalase (3% H_2O_2) – Ingram & Bell
Gram Stain – Refer to Media Manual for preparation
Strep. Latex agglutination – Pro Lab Diagnostics
Staph. Latex agglutination – Sanofi
Tube coagulase - VWR
Amies transport median – NCS Diagnostics / Quelab
Blood Agar (BA) – MedPrep
Blood Egg Albumin Agar (BEAA) – Biomedia
Chocolate Agar (CHOC) – Oxoid Unipath
Colistin Nalidixic Acid Agar (CNA) – MedPrep
Esculin Base Medium (EBM)
Fastidious Anaerobic Agar (BRUC) - MedPrep
Fastidious Anaerobic Broth (THIO) - MedPrep
Haemophilus Isolation Agar (HI) - MedPrep
Inhibitory Mold Agar (IMA) – MedPrep
Kanamycin / Vancomycin Agar (KV) - MedPrep
MacConkey Agar (MAC) – MedPrep
Mannitol Salt Agar with Oxacillin (MSAOX) – MedPrep
Martin-Lewis Agar (ML) – Quelab
Oxidase Strip - API
Vitek Cards - bioMerieux
Antibiotic Disks - Oxoid
Mueller Hinton Agar - MedPrep
PYR Disks - Remel
LAP Disks - Remel
Rapid Yeast Plus Strips - Oxoid
Rapid Ana II Strips - Oxoid
MRSA Screen - Denka Seiken

APPENDIX II

READING OF GRAM STAIN

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

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

   NB: PMN / WBC will be reported as Pus cells

   < 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 = +++

APPENDIX III

COMMENSAL FLORA – SKIN, EAR, EYE

Type	**Organisms**
Aerobic bacteria	Corynebacterium, Coagulase negative Staphylococcus, Micrococcus, nonpathogenic Neisseria, Acinetobacter, Aerococcus
Anaerobic bacteria	Propionibacterium, Clostridium, Peptostreptococcus
Fungi	*Candida* spp., *Malassezia*

Murray P.A, et al. 1999. Manual of Clinical Microbiology, 7th Edition, ASM Press.

APPENDIX IV

YEAST IDENFIFICATION

All yeast isolates except from voided urines, sputums (if growth is ≤ 1+), superficial sites, wounds,and drainage fluid will be screened using a Germ tube (see Appendix VII). Depending on the result of the Germ tube, proceed as follows:

1) Sterile sites and biopsy speciemens:

   a) Germ tube: **Positive** - Report as "*Candida albicans*".
   b) Germ tube: **Negative** - Perform a yeast strip and chlamydospore / corn-meal agar for full identification. If unable to identify using the yeast strip, and chlamydospore / corn-meal agar, consult charge technologist or medical microbiologist.

2) Sputum and bronchoscopy isolates:
   i) Growth ≤ 1+:
   No Germ tube performed. Report as part of Commensal flora **without** specifically commenting on the presence of yeast.

   ii) Growth ≥ 2+:
   a) Germ tube: **Positive** - Report as "*Candida albicans*"
   b) Germ tube : **Negative** - Rule out Cryptococcus using urease test. If organism is not cryptococcus, then report as "Yeast, not *Candida albicans* or Cryptococcus".

3) Voided urines, superficial sites, wounds and drainage fluids:

   No Germ tube performed. Report as "Yeast isolated".

4) All other isolates:

a) Germ tube: **Positive** - Report as "*Candida albicans*".
b) Germ tube: **Negative** - Report as "Yeast, not *Candida albicans*".