WOUNDS / TISSUES / ASPIRATES / MISCELLANEOUS CULTURE MANUAL

TABLE OF CONTENTS

SWABS AND DRAINAGE SPECIMENS

Intraoperative/Interventional Swabs

I. Introduction

All intraoperative and interventional swab cultures may yield bacteria and fungi. Both aerobic and anaerobic bacteria may be present.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

.

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

If fungus is requested, **add**:
Fungi Fluor stain - Refer to Mycology Manual.

b) Culture:

Media	**Incubation**
Blood Agar (BA)[1,2]	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)[1,2]	CO_2 or O_2, 35°C x 48 hours
Chocolate Agar (CHOC)[1,2]	CO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)[1,2]	O_2, 35°C x 7 days
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 fungus 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
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

[1] If organisms were seen in direct Gram stain and cultures yield no corresponding growth after 48 hours of incubation, check direct Gram stain (if discrepant compared to original report, check with the Charge technologist), and re-incubate all aerobic plates and broth for 7 days. If there is no evidence of corresponding growth after 7 days, subculture the THIO to CHOC and BRUC.

[2] If both aerobic swab and anaerobic swab are received, use the aerobic swab to inoculate the aerobic plates, use the anaerobic swab to inoculate the anaerobic plates and the Fastidious Anaerobic Broth (THIO).

* Forward fungus culture media to 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 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 CHOC and BRUC (plus additional media as appropriate) and incubate and process as above.

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. Other organisms will be worked up only if there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes.

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"

Positive Report:

- **Significant isolates** - *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa*, yeasts or other organisms ≤3 different bacterial types - Report all isolates with appropriate susceptibilities.

- **>3 types non-significant isolates** – Report as TEST COMMENT – "Mixed growth of …….list morphotypes."

Telephone results of positive Gram stain or isolates not seen in Gram to the ward / ordering physician.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Microbiological Assessment of Orthopedic Surgery Sites p. 13.14.1 – 13.14.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

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5. H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

6. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

7. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

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

Wound/Abscess Swabs and Drainage

I. Introduction

This section includes specimens from wound swabs, abscess swabs, 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 but are most commonly associated with *S. aureus*, β-hemolytic streptococci, *Streptococcus anginosus* group, *P. aeruginosa* and enteric Gram negative bacilli. 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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:
See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain - Quantitate the presence of pus cells, squamous epithelial cells, and organisms.

b) Culture:

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

B. Interpretation of Cultures:
1. Examine the aerobic plates after 24 and 48 hours incubation and anaerobic plates after 48 hours incubation.
2. Count the number of types of organisms.
   a. If there are **<3 types** of organisms isolated, perform MALDI/rapid tests on each:
      i. Workup any amount of **Probable Pathogens**
      ii. Workup **Possible Pathogens** if pure growth **OR** moderate to heavy **AND** obviously predominant growth over commensal flora.
      iii. Do not workup skin flora.
   b. If there are **≥3 types** of organisms isolated:
      i. Workup any amount of **Probable Pathogens**
      ii. Do not work up other organisms, rule out probable pathogen only.

Organisms for workup are categorized as follows:

Probable Pathogens	Possible Pathogens	Commensal Skin flora
Staphylococcus aureus *Staphylococcus lugdunensis* β-haemolytic streptococcus *Streptococcus anginosus* group (except tracheal swabs) *Pseudomonas aeruginosa* **For chest tube drainage and tracheal swabs**, include: *Haemophilus influenzae* *Streptococcus pneumoniae* **For sternal wounds**, include: Any amount of Probable and Possible Pathogens and Coagulase-negative-*Staphylococcus* **For bite wounds, include:** *Pasteurella multocida*	*Enterococcus* species Aerobic gram-negative-bacilli other than *P. aeruginosa* Yeasts Anaerobes *Corynebacterium jeikeium* **For eye samples, include:** *Corynebacterium macginleyi* **For breast samples, include:** *Corynebacterium kroppenstedtii*	Coagulase-negative-*Staphylococcus* (except sternal wound) viridans *Streptococcus* group * *Micrococcus* species *Corynebacterium* species (not jk) *Bacillus* species not *B. anthracis* *Cutibacterium* species Nonpathogenic *Neisseria* species

*usually associated with commensal oral flora but often isolated as probable skin contaminant
For organisms not listed, consult the charge technologist.

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"

Report "Mixed flora" with quantitation

Positive report: Quantitate all significant isolates; report with appropriate susceptibility results.
If other organisms are also present, report as "Mixed flora" with quantitation.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C
2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.
3. H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.
4. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

5. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

6. Cumitech 5A Practical anaerobic bacteriology December 1991

Bite Wound Swabs

I. Introduction

Bite wounds may become infected with many different organisms but most commonly include *S. aureus, Pasteurella* spp., *S. anginosus* 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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

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

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2/O_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
If anaerobic culture requested, **add**:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C 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., *Streptococcus anginosus* group, beta-haemolytic streptococci and *Pseudomonas aeruginosa* is significant. For other organisms such as Enterobacterales, other Gram negative bacilli, and anaerobes - a significant result is determined by the isolation of a moderate to heavy predominant growth.

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 "mixed growth of skin flora/list morphotypes"

Positive Report: Quantitate all significant isolates with appropriate susceptibilities. If other mixed 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 (enter under the TEST field in the LIS): "No anaerobic swab received; anaerobic culture not done".

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

4. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

5. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

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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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35^oC x 48 hours
MacConkey Agar (MAC)	CO_2/O_2 35^oC 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*, *Streptococcus anginosus* group, *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 "Mixed growth of skin flora/list morphotypes"

Positive report: Quantitate all significant isolates with appropriate susceptibilities. If other mixed flora is also present, report with quantitation.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004 Catheter Tip Cultures. p. 3.6.1 – 3.6.6. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

4. H.D. Isenberg. 2004. Culture of Intravascular Devices p.13.12.1 – 13.12.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

ABSCESSES SPECIMENS

Intraoperative/Interventional Abscess (Pus, Cyst Fluid or Aspirate)

I. Introduction

All intraoperative and interventional abscess cultures may yield bacteria and fungi. Both aerobic and anaerobic bacteria may be present.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

If fungus is requested, **add**:
Fungi Fluor stain - Refer to Mycology Manual.

b) Culture:

Media	Incubation
Blood Agar (BA)[1]	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)[1]	CO_2/O_2 35°C x 48 hours
Chocolate Agar (CHOC)[1]	CO_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)[1]	O_2, 35°C x 7 days
If fungus 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
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

[1] If organisms were seen in direct Gram stain and cultures yield no corresponding growth after 48 hours of incubation, check direct Gram stain (if discrepant compared to original report, check with the Charge technologist), and re-incubate all aerobic plates and broth for 7 days. If there is no evidence of corresponding growth after 7 days, subculture the THIO to CHOC and BRUC.

* Forward fungus culture media to 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 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 CHOC and BRUC (plus additional media as appropriate) and incubate and process as above.

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. Other organisms will be worked up only if there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes as broad groups (coliforms, coag neg staph, enterococci, anaerobes etc).

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"

Positive Report:

- **Significant isolates** - *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa*, yeasts or other organisms ≤3 different bacterial types - Report all isolates with appropriate susceptibilities.

- **>3 types non-significant isolates** – Report as **TEST COMMENT** – "Mixed growth of …….list morphotypes"

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

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Microbiological Assessment of Orthopedic Surgery Sites p. 13.14.1 – 13.14.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

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6. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

7. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

8. Cumitech 5A Practical anaerobic bacteriology December 1991

Pus & Abscess Material (other than Intraoperative/Interventional, Rectal or Bartholin)

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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

Direct Examination:

a) Gram stain-

- Quantitate the presence of pus cells and organisms.
- Note presence of epithelial cells if seen.
- Note presence of branching GPB seen
    1. If Actinomyces or Nocardia is suggested on Gram stain – setup Kinyoun and Modified Kinyoun stain

b) Fungi Fluor 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)	CO_2/O_2 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)[1]	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)[1]	AnO_2, 35°C x 48 hours
If Nocardia is requested, **add**:	
Pyruvate Agar (PYRU)[2]	O_2, 28°C x 4 weeks
If fungus culture is requested, **add**:	
Inhibitory Mold Agar (IMA)[2]	O_2, 28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)[2]	O_2, 28°C x 4 weeks

NOTE:
1. If Actinomyces is requested, set up a second set of anaerobic media to be incubated for 10 days before opening jar.
2. Forward the fungus culture media and PYRU to the Mycology section for incubation and work-up

B. Interpretation of Cultures:

Examine the aerobic culture plates after 24 and 48 hours incubation and the anaerobic plates after 48 hours and the second set of anaerobic media after 10 days of incubation (if Actinomyces requested or suggested on Gram stain).
Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. Other organisms will be worked up only if there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes.

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 10 days incubation"
If Nocardia is requested, report: "No Nocardia isolated".

Positive report:

- **Significant isolates** - *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa*, yeasts or other organisms ≤3 different bacterial types - Report all isolates with appropriate susceptibilities.
- **>3 types non-significant isolates** – Report as **TEST COMMENT** – "Mixed growth of …….list morphotypes".

VI. Referencess

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

4. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

5. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

6. Cumitech 5A Practical anaerobic bacteriology December 1991

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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

b) Culture:

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

B. Interpretation of Cultures:

Examine the culture plates after 24 and 48 hours incubation.
Work up any growth of *S. aureus*, beta-haemolytic Streptococci, *S. anginosus* group or *Pseudomonas aeruginosa*. Screen non-lactose fermenters (NLF) for *Salmonella* species and *Shigella* species.
Ignore organisms that are usually part of the faecal flora (i.e. Lactose fermenting 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 susceptibilities. Report "Mixed faecal flora" if also present.

References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C
2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

Bartholin's Abscess Swab/Aspirate

I. Introduction

Bartholin's glands are small mucous-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.

Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

II. Reagents and Media

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

Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain. - Quantitate the presence of pus cells, squamous epithelial cells, and organisms.

b) Culture:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35^oC x 48 hours
Chocolate Agar (CHOC)	CO_2, 35^oC x 48 hours
Martin –Lewis Agar (ML)	CO_2, 35^oC x 72 hours
MacConkey Agar (MAC)	CO_2/O_2 35^oC x 48 hours

If anaerobic culture is requested, discuss with the Microbiologist or Charge Technologist.

B. Interpretation of cultures:

a) Examine the BA, CHOC, and MAC plates after 24 and 48 hours incubation and the ML plate after 24, 48 and 72 hours incubation. Quantitate the bacterial growth.

b) All potential pathogens should be identified.

Any growth of *S. aureus*, beta-haemolytic Streptococci, *S. anginosus* group, *Pseudomonas aeruginosa* or *Neisseria gonorrhoeae* should be identified.
Other organisms that are usually part of the faecal flora (i.e. Gram negative bacilli) do not require workup.

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 Charge Technologist.

c) For GC work-up, refer to Bacteria and Yeast Work-Up.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

III. Reporting Results

Gram Stain:	Report with quantitation the presence of pus cells and organisms. Note presence of epithelial cells if seen.
Culture:	
Negative Report:	"No significant growth" or "No growth" "No *Neisseria gonorrhoeae* isolated".
Positive Report:	Quantitate all significant isolates with appropriate susceptibilities. Report "Mixed faecal flora" if also present.

"*Neisseria gonorrhoeae* isolated (do not quantitate). Quantitate and report all other significant isolates with appropriate sensitivity results.

For all positive GC cultures: 1. Telephone floor/ordering Physician
2. Send a Communicable Disease Report to the Medical Officer of Health by the microbiologist or supervisor.

IV. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C
2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.
3. H.D Isenberg. 2004. Guidelines for Performance of Genital Cultures – Neisseria gonorhoeae Cultures p. 3.9.3.1 – 3.9.3.14 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.
4. Cumitech 17A, 1993. Laboratory Diagnosis of Female Genital Tract Infections, ASM Press.
5. Cumitech 4A Laboratory Diagnosis of Gonorrhea April 1993
6. QMP-LS Survey B-9412, Feb. 21, 1995. Microbiology Handling of Female Genital Specimens. A pattern of Practice Survey.

TISSUES, BIOPSIES, TRANSPLANTS AND PROSTHETIC DEVICES

Tissues/Biopsies (other than skin or transplant tissues)

I. Introduction

Surgical biopsies, tissues should be considered sterile specimens and therefore the isolation of any organism(s) should be considered significant.
EBUS tissue (endobronchial ultrasound guided biopsies of tissue, primarily mediastinal lymph nodes primarily) may contain oral flora as part of the specimen collection process. Isolates consistent of oral flora are not considered as significant from these specimens.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain. - Quantitate the presence of pus cells, and organisms.

b) Culture:

Inoculate the following media with the remaining sample:

Media	Incubation
Blood Agar (BA)[1]	CO_2, 35^oC x 48 hours[1]
MacConkey Agar (MAC)[1]	CO_2/O_2 35^oC x 48 hours[1]
Chocolate Agar (CHOC) [1]	CO_2, 35^oC x 48 hours[1]
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35^oC x 48 hours

Kanamycin/Vancomycin Agar (KV) Fastidious Anaerobic Broth (THIO)[1]	AnO_2, 35°C x 48 hours O_2, 35°C x 7 days[1]
If Fungus 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
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

[1] If organisms were seen in direct Gram stain and cultures yield no corresponding growth after 48 hours of incubation, check direct Gram stain (if discrepant compared to original report, check with the Charge technologist), and re-incubate all aerobic plates and broth for 7 days. If there is no evidence of corresponding growth after 7 days, subculture the THIO to CHOC and BRUC.

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

B. Direct Examination:

a) Gram stain – Quantitate the presence of pus cells and organisms.
b) Fungi Fluor 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 CHOC and BRUC (and other media as appropriate) and incubate and process as above.

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. Other organisms will be worked up only if there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes.

EBUS tissues: do not work up oral flora isolates.

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"
EBUS tissue: "Mixed growth of oral flora"

Positive Report:

- **Significant isolates** - *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa*, vancomycin-resistant-*Enterococcus*, yeasts or other organisms ≤3 different bacterial types - Report all isolates with appropriate susceptibilities.

- **>3 types non-significant isolates** – Report as TEST COMMENT – (Quantitation) Mixed growth of …….list morphotypes.

Telephone results of positive Gram stain or isolates not seen in gram to the ward/ordering physician.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. Cumitech 23 Infections of the Skin and Subcutaneous Tissues June 1988

4. H.D. Isenberg. 2004. Microbiological Assessment of Orthopedic Surgery Sites p. 13.14.1 – 13.14.6. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

5. H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

6. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

7. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

8. Cumitech 5A Practical anaerobic bacteriology December 1991

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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials /Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

c) Direct Examination: Gram stain: Quantitate the presence of pus cells and organisms.

If fungus is requested, **add**:
Fungi Fluor stain - Refer to Mycology Manual.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2/O_2 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
If fungus is requested, **add**::	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 4 weeks

Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2,	30°C x 4 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 Commensal Skin flora 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 "Mixed growth of skin flora/list morphotypes"

Positive Report: Quantitate all significant isolates with appropriate susceptibilities. If other organisms are also present, report as “Mixed skin flora” with quantitation.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. Cumitech 23 Infections of the Skin and Subcutaneous Tissues June 1988

Transplant Specimens - Bone Graft & Cadaver Fascia/Tissue/ Swab Specimens/Donor Amniotic Fluid/Membrane; Donor Corneal Ring Material

I. Introduction

Specimens collected for transplantation 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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Material / Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Not indicated

b) Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)*	O_2, 35°C x 7 days

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

B. Interpretation of Culture:

Examine the THIO daily for evidence of growth. If evidence of growth in THIO, then perform Gram stain and subculture THIO onto BA, MAC, CHOC and BRUC (plus additional media as appropriate) and incubate in CO_2 and anaerobically for the BRUC.

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. Other organisms will be worked up only if there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

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

Positive Report:

- **Significant isolates** - *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa*, yeasts or other organisms ≤3 different bacterial types - Report all isolates with appropriate susceptibilities.
  - For bone and joint fluids specimens, report organisms to the species level. If not identified in lab, send to PHOL.
- **>3 types non-significant isolates** – Report as **TEST COMMENT** – " Mixed growth of …….list morphotypes".

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Microbiological Assessment of Orthopedic Surgery Sites p. 13.14.1 – 13.14.6. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

Prosthetic Devices (e.g. Pacemaker Wire, Dacron Graft, Prosthetic Valve)

I. Introduction

Prosthetic devices e.g. pacemaker wire, Dacron graft. Prosthetic valve removed from patients may be sent for sterility testing. Medical devices which penetrate the skin significantly increase the risk of device related infection. These devices become colonized by bacteria on the patient's skin or bacteria carried on the hands of medical personnel. Prosthetic devices may also be infected by skin and other bacteria when implanted. These invading bacteria colonize the surface forming a biofilm producing localized infection and may lead to significant infections such as bacteremia and septic thrombosis.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Processing of Specimens

A. Processing of Specimens

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Not indicated.
b) Culture:

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

B. Interpretation of Culture:

Examine the THIO daily for evidence of growth. If evidence of growth in THIO, then perform Gram stain and subculture THIO onto BA, MAC, CHOC and BRUC (plus additional media as appropriate) and incubate in CO_2 and anaerobically for the BRUC.

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. Other organisms will be worked up only if there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes.

C. Susceptibility Testing:

Susceptibility 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:

- **Significant isolates** - *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa*, yeasts or other organisms ≤3 different bacterial types - Report all isolates with appropriate susceptibilities.

- **>3 types non-significant isolates** – Report as **TEST COMMENT** – "Mixed growth of …….list morphotypes".

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Microbiological Assay of Environmental and Medical-Device Surfaces p.13.10.1 – 13.10.12 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

4. H.D. Isenberg. 2004. Culture of Intravascular Devices p.13.12.1 – 13.12.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

Autopsy Specimens

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. For blood culture taken from autopsy, see the Blood Culture Manual.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain - Quantitate the presence of pus cells, squamous epithelial cells, and organisms.

b) Culture:

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

Media	Incubation
For all lung tissue or if fungal culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30^oC x 3 weeks

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

B. Interpretation of Cultures:

i. Examine plates after 24 and 48 hours incubation.
ii. Count the number of types of organisms.
    1. If there are **<3 morphotypes**, perform MALDI/rapid tests on each
    2. If there are **≥3 types** of organisms isolated:
        a. Workup any amount of **Probable Pathogens**
        b. Do not work up other organisms, rule out probable pathogen only.

iii. Freeze significant isolates in BC box.

C. 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 quantity of all significant isolates **without** susceptibilities. Mention "with _____ (quantity) mixed flora suggesting contamination" if observed.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

CATHETER SPECIMENS

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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials/ Media

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

IV. Processing of Specimens

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

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, *Streptococcus anginosus* group, *Pseudomonas aeruginosa*, other Gram negative bacilli, vancomycin-resistant-*Enterococci* and yeasts are significant; quantitate and identify. Other organisms should be quantitated and identified only if ≥15 colonies of that organism are present and there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes with quantitation.

C. Susceptibility Testing:

Susceptibility testing is only performed on ≤3 significant isolates. Susceptibility Testing Manual.

V. Reporting Results

Negative Report: "No growth"

For non-significant organisms:
Report as TEST Comment: "<15 colonies of (list morphotypes of non-significant organisms)". No susceptibility required.
Report as TEST Comment: ">15 colonies of (list morphotypes of mixed non-significant organisms)". No susceptibility required.
For >3 morphotypes: List morphotypes with quantitation

Positive Report: **For significant organisms:**
Report as ISOLATE: "<15 colonies of (organism name)" or "≥15 colonies of (organism name)". Report with appropriate susceptibilities.

For *Staphylococcus aureus*, gram negative bacilli and yeast in any amount, call ward.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004 Catheter Tip Cultures. p. 3.6.1 – 3.6.6In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

4. H.D. Isenberg. 2004. Culture of Intravascular Devices p.13.12.1 – 13.12.6. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

Peritoneal Dialysis Catheter/Canula

I. Introduction

Peritoneal dialysis catheters or canula (PD Canula) removed from patients may be sent for sterility testing.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Not indicated.

b) Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 7 days

B. Interpretation of Culture:

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. Other organisms will be worked up only if there are ≤3 different bacterial types. Otherwise (>3 types), simply list the morphotypes.

Examine THIO daily for up to 7 days. If there is evidence of growth, perform Gram stain and subculture THIO onto BA, MAC, CHOC and BRUC (plus other media as appropriate).

C. Susceptibility Testing:

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

V. Reporting Results

Negative Report: "No growth"

Positive Report:

- **Significant isolates** - *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa*, yeasts or other organisms ≤3 different bacterial types - Report all isolates with quantitation and appropriate susceptibilities.

- **>3 types non-significant isolates** – Report as **TEST COMMENT** – "Mixed growth of …….list morphotypes"

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

3. H.D. Isenberg. 2004. Culture of Intravascular Devices p.13.12.1 – 13.12.6. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

BILE SPECIMENS

Bile and Bile Stents

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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Material / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain – Examine for the presence of pus cells and organisms.

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2/O_2 35°C x 48 hours
If anaerobic culture is requested or bile is collected by PTC, **add**:	
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 7 days

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*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up. For other organisms, a significant result is determined by the isolation of ≤2 organisms. For non-lactose fermenters (NLF), screen for *Salmonella* species.

Examine THIO daily for up to 7 days. If there is evidence of growth in THIO and no growth on plates, perform Gram stain and subculture THIO onto CHOC and BRUC (plus other media as appropriate).

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

a) Gram stain: Report without quantitation the presence of pus cells and organisms.

b) Culture:

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

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

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

4. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

5. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

6. Cumitech 5A Practical anaerobic bacteriology December 1991

MISCELLANEOUS FLUID SPECIMENS

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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials/ Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Not required

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2/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 "Mixed growth of skin flora"

Positive Report: Quantitate all significant isolates with appropriate susceptibilities. If mixed skin flora is also present, report with quantitation.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

Total Parental Nutrition (TPN)

I. Introduction

Total parenteral nutrition fluids are normally sterile.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimen:

See Specimen Processing Procedure QPCMI06003

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 7 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. Examine THIO daily for up to 7 days. If there is evidence of growth, perform Gram stain and subculture THIO onto CHOC and BRUC (plus other media as appropriate).

Any growth should be considered significant.

Freeze all isolates at -70°C and put into Study "BC" box.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Result

Culture:

Negative Report:	"No growth"
Positive Report:	Report all organisms with appropriate susceptibilities. Do not quantitate.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

EAR SPECIMENS

Ear Swab

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 *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae,* Group A streptococcus or fungus / yeast.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

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

b) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35^oC x 48 hours
MacConkey Agar (MAC)	CO_2/O_2 35^oC x 48 hours
Colistin Nalidixic Acid Agar (CNA)	CO_2/O_2 35^oC x 48 hours
If fungus culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30^oC 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 fungus/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: "Mixed growth of skin flora/list morphotypes" or "No growth".

Positive Report: Quantitate all significant isolates with appropriate susceptibilities. If mixed skin flora is also present, report with quantitation.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Otitis Cultures p. 3.11.5.1 – 3.11.5.6. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

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 SPECIMENS

Eye / Conjunctival / Lid Swabs

I. Introduction

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

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

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

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 24, 48 and 72 hours incubation.

Any growth of probable pathogens including *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/heavy predominant growth of a possible pathogen (note *Corynebacterium macginleyii* in eye samples). Possible pathogen morphotype should also be predominant with pus cells seen on the Gram stain.

For work-up and identification of *N. gonorrhaeae*, refer to the Bacteria and Yeast Work up Manual.

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: "Mixed growth of skin flora/list morphotypes" or "No growth". If GC culture was set up, report "No *N. gonorrhaeae* isolated"

Positive Report: Quantitate all significant isolates with appropriate susceptibilities. If other mixed flora is also present, report with quantitation.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Ocular Cultures p. 3.10.1. – 3.10.8. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

Eye / Corneal Scrapings

I. Introduction

Eye / corneal scrapings are collected for the diagnosis of keratitis caused by bacterial, fungal, viral, chlamydial or acanthamoeba infection.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain – Examine for the presence of pus cells and organisms. Fungi Fluor stain (if two smears are provided). Refer to Mycology Manual.

NB: If pre-inoculated plates are received and no smear or additional specimen is received, direct smear stains will not be performed.

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 7 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. Examine THIO daily for up to 7 days. If there is evidence of growth, perform Gram stain and subculture THIO onto BA, MAC, CHOC and BRUC (plus other media as appropriate).

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up.

Other possible pathogens will be worked up only if there are ≤3 different bacterial types. Note: *Corynebacterium macginleyii* is considered possible pathogen for eye samples

Otherwise (>3 types), simply list the morphotypes.

C. Susceptibility Testing:

Refer to Respiratory and Misc non-sterile reporting section in Susceptibility Testing Manual.

V. Reporting Results

For conjunctival scrapings, see Eye / Conjunctival / Lid Swabs.

For corneal scrapings:

a) Gram stain: Report, without quantitation, the presence of pus cells and organisms. Report positive Gram stain as an isolate

NB: If pre-inoculated plates are received and no smear or additional specimen is received. Result in the "TEST" field in the LIS as "No smear received, test not performed."

b) Culture:

Negative report: "No growth."

Positive report:

- **Significant isolates -** Report with appropriate susceptibilities.

- **>3 types non-significant isolates** – Report as **TEST COMMENT** – " Mixed growth of …….list morphotypes".

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Ocular Cultures p. 3.10.1. – 3.10.8. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

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 charge 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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct examination: Examine for pus cells and organisms especially branching gram positive bacilli resembling *Actinomyces* species.

a) Culture:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)[1]	AnO_2, 35°C x 48 hours
Kanamycin/Vancomycin Agar (KV)[1]	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 7 days

[1]If Actinomyces is suggested on direct Gram stain, set up a second set of anaerobic media to be incubated for 10 days before opening jar.

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 CHOC and BRUC (as appropriate) and incubate and process as above.

Any growth of *S. aureus*, β-haemolytic streptococci, *Streptococcus anginosus* group, P*seudomonas aeruginosa* and yeasts are significant; work up.

Other possible pathogens will be worked up only if there are ≤3 different bacterial types. Note: *Corynebacterium macginleyii* is considered possible pathogen for eye samples

Otherwise (>3 types), simply list the morphotypes.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

a) Gram stain: Report presence of organisms.

b) Culture:

Negative Report: "Mixed growth of skin flora/list morphotypes" or "No growth".

Positive Report:

- **Significant isolates** - Report with appropriate susceptibilities.
- **>3 types non-significant isolates** – Report as TEST COMMENT – (Quantitation) Mixed growth of …….list morphotypes.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

3. H.D. Isenberg. 2004. Ocular Cultures p. 3.10.1. – 3.10.8. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

4. H.D. Isenberg. 2004. Culture for anaerobes p. 4.3.1 – 4.3.9 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

5. H.D. Isenberg. 2004. Examination of Primary Culture plates for anaerobic bacteria. p. 4.4.1 – 4.4.6 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

6. H.D. Isenberg. 2004. Incubation techniques for anaerobic bacteriology specimens. p. 4.5.1 – 4.5.4 In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

7. Cumitech 5A Practical anaerobic bacteriology December 1991

FACIAL SPECIMENS

Facial Swabs

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

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials/ Media

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

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.
Important to note branching GPB suggestive of Actinomyces
Fungi Fluor stain (If fungus is requested).

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)	CO_2/O_2 35°C x 48 hours
If Actinomyces is requested or suggested on Gram stain or an anaerobic swab collected or thick pus is received, **add**:	
Fastidious Anaerobic Agar (BRUC)[1]	AnO_2, 35°C x 10 days
Kanamycin/Vancomycin (KV) [1]	AnO_2, 35°C x 10 days
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 10 days

[1]If Actinomyces is requested or suggested on direct Gram stain, set up a second set of anaerobic media to be incubated for 10 days before opening jar.

Media	Incubation
If fungus culture is requested, **add**:	
Inhibitory Mold Agar (IMA)*	O_2, 30°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 30°C x 4 weeks

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

B. Interpretation of Cultures:

Examine the aerobic culture plates after 24 and 48 hours incubation and the anaerobic plates after 48 hours and second set of anaerobic media after 10 days incubation (if Actinomyces is requested or suggested on Gram stain). Examine THIO daily for up to 10 days incubation.
In general, these specimens are handled as Wound/Abscess Swabs and Drainage, 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: "Mixed growth of skin flora/list morphotypes" or "No growth".
If Actinomyces culture requested: "No Actinomyces isolated"

Positive Report: Quantitate significant isolates with appropriate susceptibilities.
If other mixed flora is also present, report with quantitation.

VI. References

1. H.D. Isenberg. 2004. Specimen Collection, Transport and Acceptability p. 2.1.1 – 2.1.28. In Clinical Microbiology Procedures handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C

2. H.D. Isenberg, 2004. Wound Cultures – Wound and Soft Tissue Cultures, p. 3.13.1.1 – 3.13.1.16. In Clinical Microbiology Procedures Handbook, 2nd Edition, Vol 1 ASM Press, Washington, D.C.

Record of Edited Revisions

Manual Section Name: **Wounds / Tissues / Aspirates Culture Manual**

Page Number/Item	Date of Revision	Signature of Approval
Page 3 – Introduction	June 15, 2004	Dr. T. Mazzulli
Reorganized table of contents categories	July 27, 2009	Dr. T. Mazzulli
Removed Appendices II to IV	July 27, 2009	Dr. T. Mazzulli
New sections added – intraoperative/interventional swabs and aspirates	July 27, 2009	Dr. T. Mazzulli
Wounds/abscess/drainage – added Probable and Possible pathogens section	July 27, 2009	Dr. T. Mazzulli
Streptococcus anginosus group added to work up list for catheter tip, prosthetic devices, bile	July 27, 2009	Dr. T. Mazzulli
Extended Thio incubatin to 5 days for intraoperative swabs, tissues	July 27, 2009	Dr. T. Mazzulli
Added instructions for extra anaerobic plates and extended THIO incubation if Actinomyces or organisms seen in gram and no growth in culture	July 27, 2009	Dr. T. Mazzulli
Contact Lens and Contact Lens solution moved to Sterility Manual	July 27, 2009	Dr. T. Mazzulli
Annual Review	July 27, 2009	Dr. T. Mazzulli
Annual Review	July 27, 2010	Dr. T. Mazzulli
Annual Review	August 01, 2011	Dr. T. Mazzulli
Annual Review	September 01, 2012	Dr. T. Mazzulli
Added mixed growth comments to sterile sites	September 01, 2012	Dr. T. Mazzulli
Add *β-haemolytic Streptococcus and Staphylococcus lugdunensis* to probable and possible pathogens	December 22, 2013	Dr. T. Mazzulli
Annual Review	December 22, 2013	Dr. T. Mazzulli
For sternal wounds, include: Any amount of Probable and Possible Pathogens and Coagulase-negative-*Staphylococcus*	June 15, 2014	Dr. T. Mazzulli
EBUS tissue comment added to "Tissue" section	June 15, 2014	Dr. T. Mazzulli
Create proper headers	July 30, 2014	Dr. T. Mazzulli
Annual Review	July 30, 2014	Dr. T. Mazzulli
Specimen collection changed to ESwab	August 30, 2014	Dr. T. Mazzulli
Removed setting up if requested MKS and Kinyoun reporting on Lacrimal and Facial specimens	April 30, 2015	Dr. T. Mazzulli

Page Number/Item	Date of Revision	Signature of Approval
Annual Review	April 30, 2015	Dr. T. Mazzulli
Mixed growth of …….list morphotypes." P.9	April 30, 2015	Dr. T. Mazzulli
Removed all text in all sections under specimen collection and transportation and replaced it with link to Specimen collection manual **QPCMI02001** where info is now housed.	May 26, 2015	Dr. T. Mazzulli
In all sections, under processing of specimen added link to Specimen Processing Procedure QPCMI06003 For sections: Intraoperative/Interventional Abscess (Pus, Cyst Fluid or Aspirate) & Tissues/Biopsies (other than skin or transplant tissues) & Autopsy specimens, & Bile specimens & TPN --Moved processing of specimen steps to specimen processing manual, replaced with link to QPCMI06003 Tissues biopies other than skin – added gram stain procedure	August 18, 2015	Dr. T. Mazzulli
For "Refer to Susceptibility Manual" Added hyperlink to actual manual For Tissues removed "add isolate if positive gram stain and notify ward" with "Telephone results of positive Gram stain or isolates not seen in gram to the ward/ordering physician." Under section "Eye/corneal scraping" Removed instructions (already in specimen collection manual QPCMI02001) and added link to this manual.	August 28, 2015	Dr. T. Mazzulli
Transplant Specimens: For Bone specimens added: For bone and joint fluids specimens, report organisms to the species level. If not identified in lab, send to PHOL	January 7, 2016	Dr. T. Mazzulli
Annual Review Changed Actino incubation time from 7 days to 10 days minimum	April 4, 2016	Dr. T. Mazzulli
Annual Review Replaced Calcofluor with Fungi Fluor Stain	April 20, 2016	Dr. T. Mazzulli
Annual Review	April 15, 2017	Dr. T. Mazzulli
Annual Review	April 10, 2018	Dr. T. Mazzulli
Removed blank pages	September 14, 2018	Dr. T. Mazzulli
Annual Review	April 05, 2019	Dr. T. Mazzulli

Page Number/Item	Date of Revision	Signature of Approval
Annual Review	May 01, 2020	Dr. T. Mazzulli

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

Page Number / Item	Date of Revision	Edited by:
Changed Fastidious Anaerobic Broth (THIO) incubation time from 5 days to 7 days	July 26, 2020	Dorna Zareianjahromi
Changed MAC and CNA incubation to CO2/O2, updated THIO incubate time to 7 days	Jan 21, 2021	Dorna Zareianjahromi
Various edits to clarify extent of commensal workup, reporting of epithelial cells if seen, for facial culture report "no actinomyces isolated" if requested.	Feb 3, 2021	Dorna Zareianjahromi
Minor formatting change	April 11, 2021	Jessica Bourke
Nomenclature update – cutibacterium, enterobacterales	April 19, 2021	Wayne Chiu
Reformatted heading for autopsy specimens, updated culture workup of autopsy to match non-sterile wording. Specified corneal ast reporting refer to resp and misc nonsterile section	June 24, 2021	Wayne Chiu
Table "Organisms for workup" update Moved viridans strep from possible pathogen to commensal skin flora. Added Corynebacterium jeikeium to possible pathogen	August 31, 2021	Wayne Chiu
Removed "Commensal Flora" from routine reporting, replaced with "Mixed growth of skin flora/list morphotypes"	Sep 21, 2021	Wayne Chiu
Added Staph lug to probable, C mcginleyi and C koppenstedtii to possible pathogen chart	Oct 7, 2021	Wayne Chiu
Included Pasteurella as probable in bite wounds and added hyperlink under wound SOP	Oct 19, 2021	Wayne Chiu
Updated abscess nocardia media section Updated eye sample section	April 29, 2022	Wayne Chiu
Updated wording for autopsy reporting	November 30, 2022	Wayne Chiu
Removed fungal media for nocardia requests in abscess	Feb 7, 2023	Wayne Chiu