RESPIRATORY TRACT CULTURE MANUAL

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INTRODUCTION

A. Upper Respiratory Tract (above the larynx) Specimens include:

- Throat swabs
- Epiglottal swabs
- Nasal/nasopharyngeal aspirates / swabs
- Mouth swabs
- Oral abscess swabs / aspirates
- Sinus or antral aspirates

B. Lower Respiratory Tract Specimens include:

- Sputum
- Bronchial aspirates (washings)
- Bronchial brushings
- Bronchoalveolar lavage (BAL)
- Lung biopsies
- Lung Aspirates
- Open Lung biopsies

Lower respiratory tract specimens may be contaminated with organisms found in the upper respiratory tract.

## COMMENSAL FLORA - RESPIRATORY TRACT

<table>
<thead>
<tr>
<th>Type</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic bacteria</td>
<td>Veillonella, Peptostreptococcus, Fusobacterium, Porphyromonas, Bacteroides, Prevotella, Actinomyces, Eubacterium, Bifidobacterium, Propionibacterium</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Candida</em> spp.</td>
</tr>
<tr>
<td>Parasites</td>
<td><em>Entamoeba gingivalis</em>, <em>Trichomonas tenax</em></td>
</tr>
</tbody>
</table>
References:


BRONCHOALVEOLAR LAVAGE (BAL)

I. Introduction

Bronchoalveolar lavage (BAL) specimens are collected when sputum specimens fail to identify an etiologic agent of pneumonia or the patient is unable to produce sputum. Lavages are especially suitable for detecting *Pneumocystis jirovecii* and fungal elements. For Bronchoscopy Aspirates/Washings specimens see BRONCHOSCOPY ASPIRATES / WASHINGS

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:

i) Gram stain - Cytospin on unspun specimen

ii) Fungi-fluor stain (if fungus is requested) - with sediment of the spun specimen.

iii) Acid-fast stain (if requested STAT and approved by microbiologist) - Direct smear from sediment of the spun specimen.
b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculate with unspun specimen using 1 uL loop:</td>
<td></td>
</tr>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Haemophilus Isolation Medium (HI)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>If <em>B. cepacia</em> is requested or specimen is from a patient with Cystic Fibrosis, add:</td>
<td></td>
</tr>
<tr>
<td><em>B. cepacia</em> Selective Agar (OCBL.BCSA)</td>
<td>O₂, 35°C x 5 days</td>
</tr>
<tr>
<td>Keep the BA, HI and MAC plates</td>
<td>CO₂, 35°C x 5 days</td>
</tr>
<tr>
<td>Inoculated with sediment from the spun specimen:</td>
<td></td>
</tr>
<tr>
<td>If Fungus is requested OR specimen is from lung transplant patients, add:</td>
<td></td>
</tr>
<tr>
<td>Inhibitory Mold Agar (IMA) *</td>
<td>O₂, 28°C x 4 weeks</td>
</tr>
<tr>
<td>Escculin Base Medium (EBM)*</td>
<td>O₂, 28°C x 4 weeks</td>
</tr>
<tr>
<td>Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*</td>
<td>O₂, 28°C x 4 weeks</td>
</tr>
<tr>
<td>If Nocardia is requested, add:</td>
<td>O₂, 35°C x 4 weeks</td>
</tr>
<tr>
<td>Pyruvate Agar (PYRU)*</td>
<td></td>
</tr>
</tbody>
</table>

* Forward inoculated fungal media to Mycology Section for incubation and work-up.

B. Interpretation of cultures:

1. Examine BA, HI and MAC after 24 and 48 hours incubation. If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, examine BA, HI, MAC and OCBL.BCSA daily for 5 days. Record the number of commensal flora (as <10, 10-100 or >100; the count for commensal flora should be based on the count of the predominant commensal flora species) and record the number of colonies of **Probable** or **Possible** respiratory pathogens (as <10, 10-100 or >100).
### Inoculation Loop size

<table>
<thead>
<tr>
<th>Inoculation Loop size</th>
<th>No. of colonies</th>
<th>Colony count/L</th>
<th>Reporting Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 uL</td>
<td>1-9 colonies</td>
<td>1-8 x 10^6 cfu/L</td>
<td>&lt;10 x E6 cfu/L</td>
</tr>
<tr>
<td></td>
<td>10-100 colonies</td>
<td>10-100 x 10^6 cfu/L</td>
<td>≥10 x E6 cfu/L</td>
</tr>
<tr>
<td></td>
<td>&gt;100 colonies</td>
<td>&gt;100 x 10^6 cfu/L</td>
<td>&gt;10 x E6 cfu/L</td>
</tr>
</tbody>
</table>

2. Work up any amount of **Probable** respiratory pathogens. Workup **Possible** respiratory pathogens only if predominant. Refer to Bacteria and Yeast Workup for identification. (*Note: exception for Probable pathogens labelled with an asterisk*).

3. For filamentus fungus, seal the agar plate and send the culture to Mycology for identification.

4. If there is a question regarding the significance of an isolate, consult the senior/charge technologist or microbiologist.

**Probable respiratory pathogens:**
- Streptococcus pneumoniae
- Moraxella catarrhalis
- Hemophilus influenzae
- Staphylococcus aureus
- Pseudomonas aeruginosa
- Group A streptococcus
- Burkholderia cepacia
- Rhodococcus equi*
- Nocardia
- Filamentous fungus
- Cryptococcus neoformans/gattii
- *Screen diphtheroid-like organism if predominant compared to commensal flora

**Possible respiratory pathogens:**
- Yeast not Cryptococcus neoformans/gattii
- Group C and G streptococcus
- Other gram negative bacilli (not listed above) of single morphological type
- Corynebacterium pseudodiphtheriticum
- Neisseria meningitidis

C. Susceptibility Testing:
Refer to Susceptibility Testing Manual.

### For cystic fibrosis patients:
For B. cepacia and slow growing mucoid P. aeruginosa, susceptibilities can be referred back 4 weeks.
V. Reporting

a) Direct Examination:
   Gram Stain: Report WITHOUT quantitation:
   - presence or absence of pus cells;
   - presence or absence of squamous epithelial cells;
   - presence of predominante respiratory pathogens;
   - presence of “Commensal flora”;
   - “No bacteria seen” if no organism is seen.

   Fungi-fluor Stain: Refer to Fungi-fluor Stain

   Acid-fast stain: Refer to Fluorochrome Stain

b) Culture:

   Negative Report:

   For Commensal flora, the count for commensal flora should be based on the count of the predominant commensal flora species:

   “<10 x E6 cfu/L Commensal Flora, NOT significant”
   LIS TEST Comment Code: }<10c

   “≥10 x E6 cfu/L Commensal Flora, POSSIBLY significant. Commensal flora isolated in this amount might represent aspiration pneumonia. Clinical correlation required.”
   LIS TEST Comment Code: }>10c

   “No growth”

   “No B. cepacia isolated.” If B. cepacia culture is requested or specimen from a patient with Cystic Fibrosis.

   “No Nocardia isolated.” If Nocardia culture is requested.
Positive Report

If commensal flora is also present, report:
“Commensal flora” with quantitation (“<10 x E6 cfu/L” or “≥10 x E6 cfu/L” LIS TEST Comment Code: }<10b OR }≥10) WITHOUT negative report commensal flora comment.

For <10 colonies of **Probable** or **Possible** respiratory pathogens isolated:
“ISOLATE name” “<10 x E6 cfu/L. NOT significant. Organisms cultured in quantities <10 x E6 cfu/L are suggestive of commensal flora. Treatment for pneumonia given before a BAL is obtained may reduce counts. Clinical correlation required.”
LIS ISOLATE Comment Code: \<10B
Report with appropriate susceptibilities.

For ≥10 colonies of **Probable** or **Possible** respiratory pathogens isolated:
“ISOLATE name” “≥10 x E6 cfu/L SIGNIFICANT RESULT. Organisms cultured in quantities ≥10 x E6 cfu/L are consistent with pneumonia.”
LIS ISOLATE Comment Code: \>10B
Report with appropriate susceptibilities.

For *Rhodococcus equi, Nocardia species, Cryptococcus neoformans/gattii* or *B. cepacia* report as “SIGNIFICANT GROWTH consistent with pneumonia.” (without quantitation).
LIS ISOLATE Comment Code: \SIGB

For Yeast **NOT** *Cryptococcus neoformans* or *Cryptococcus gattii*:
report as “ISOLATE name” “≥10 x E6 cfu/L POSSIBLY significant. Yeasts other than *Cryptococcus neoformans/gattii* are NOT commonly associated with pneumonia. Histopathologic and clinical correlation is required.”
LIS ISOLATE Comment Code: \>10y

For *Candida* species: “ISOLATE name” “≥10 x E6 cfu/L. Candida species isolated from respiratory specimens, even in high quantities, most commonly reflects benign colonization or contamination from commensal flora.”
LIS ISOLATE Comment Code: \>10C

For “Filamentous fungus” “SIGNIFICANT GROWTH consistent with pneumonia.” “identification to follow” (DO NOT quantitate).
LIS ISOLATE Comment Code: \SIGB
References


http://cid.oxfordjournals.org/content/38/2/161.full

http://cid.oxfordjournals.org/content/34/10/1379.long

BRONCHIAL BRUSH SPECIMENS

I. Introduction

Protected brush specimens are obtained free of oral contamination. However, some studies have shown that quantitative cultures are necessary to distinguish pathogens from non-pathogens. These studies have demonstrated that colony counts of \( \geq 1 \times 10^6/L \) (\( \geq 100/mL \)) i.e. growing more than 10 colonies on a plate streaked with a 10 \( \mu \)L loop may be 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 Specimens

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Not indicated.

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculate with 10ul loop:</td>
<td></td>
</tr>
<tr>
<td>Blood Agar (BA)</td>
<td>( CO_2 ), 35°C x 48 hour</td>
</tr>
<tr>
<td>Haemophilus Isolation Medium (HI)</td>
<td>( CO_2 ), 35°C x 48 hour</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>( CO_2 ), 35°C x 48 hour</td>
</tr>
</tbody>
</table>

If \( B. \) cepacia is requested or specimen is from a patient with Cystic Fibrosis, add:

\( B. \) cepacia Selective Agar (OCBL.BCSA) \( O_2 \), 35°C x 5 day

Keep the BA, HI and MAC plates \( CO_2 \), 35°C x 5 days

UNIVERSITY HEALTH NETWORK/MOUNT SINAI HOSPITAL, DEPARTMENT OF MICROBIOLOGY

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Management System\UHN_Mount Sinai Hospital Microbiology\Standard Operating Procedures\Bacteriology Procedures\
B. Interpretation of cultures:

1. Examine BA, HI and MAC after 24 and 48 hours incubation. If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, examine BA, HI, MAC and OCBL.BCSA daily for 5 days. Record the total number of commensal flora (as <10, 10-100 or >100; the count for commensal flora should be based on the count of the predominant commensal flora species) and record the number of colonies for growth of each of Probable or Possible respiratory pathogens (as <10, 10-100 or >100).

<table>
<thead>
<tr>
<th>Inoculation Loop size</th>
<th>No. of colonies</th>
<th>Colony count/L</th>
<th>Reporting Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 uL</td>
<td>1-10 colonies</td>
<td>1-10 x 10^6 cfu/L</td>
<td>&lt;1 x E6 cfu/L</td>
</tr>
<tr>
<td></td>
<td>10-100 colonies</td>
<td>10-100 x 10^6 cfu/L</td>
<td>≥1 x E6 cfu/L</td>
</tr>
<tr>
<td></td>
<td>&gt;100 colonies</td>
<td>&gt;100 x 10^6 cfu/L</td>
<td>&gt;1 x E6 cfu/L</td>
</tr>
</tbody>
</table>

2. Work up any amount of Probable respiratory pathogens. Workup Possible respiratory pathogens only if predominant. Refer to Bacteria and Yeast Workup for identification. (*Note: exception for Probable pathogens labelled with an asterisk).

3. For filamentous fungus, seal the agar plate and send the culture to Mycology for identification.

4. If there is a question regarding the significance of an isolate, consult the senior, charge technologist or microbiologist.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting

If the brush is received in <1 mL of fluid, report in the LIS “Test Comment” field as “Brush received in wrong volume of fluid”.

If a dry brush is received, report in the LIS “Test Comment” as “Dry brush received”.
Negative Report:

For Commensal flora, the count for commensal flora should be based on the count of the predominant commensal flora species:

“<1 x E6 cfu/L Commensal Flora, NOT significant” LIS TEST Comment Code: }<1cf

“>1 x E6 cfu/L Commensal Flora, POSSIBLY significant. Commensal flora isolated in this amount might represent aspiration pneumonia. Clinical correlation required.” LIS TEST Comment Code: }>1cf

“No growth”

“No *B. cepacia* isolated” if *B. cepacia* culture is requested or specimen is from a patient with *Cystic Fibrosis*

Positive Report:

**Note:** Do not quantitate isolates on brushes received dry or in wrong volume of fluid.

For <10 colonies of *Probable* or *Possible* respiratory pathogens isolated:

“*ISOLATE name*” “<1 x E6 cfu/L. NOT significant. Organisms cultured in quantities <1 x E6 cfu/L are suggestive of contamination from commensal flora. Treatment for pneumonia given before a Bronchial Brush Specimen is obtained may reduce counts. Clinical correlation is required.” Report with appropriate susceptibilities.  
LIS ISOLATE Comment Code: }<1BR

For >10 colonies of *Probable* or *Possible* respiratory pathogens isolated:

“*ISOLATE name*” “>1 x E6 cfu/L SIGNIFICANT RESULT. Organisms cultured in quantities >1 x E6 cfu/L are consistent with pneumonia.” Report with appropriate susceptibilities.  
LIS ISOLATE Comment Code: }>1BR
For *Rhodococcus equi*, *Nocardia species*, *Cryptococcus neoformans/gattii* or *B. cepacia*: report as “SIGNIFICANT GROWTH consistent with pneumonia.” (without quantitation). LIS ISOLATE Comment Code: \SIGB

For Yeast *not* *Cryptococcus*: report as “ISOLATE name” “\(>1 \times 10^6\) cfu/L POSSIBLY significant. Yeasts other than *Cryptococcus* species are NOT commonly associated with pneumonia. Histopathologic and clinical correlation is required.”

LIS ISOLATE Comment Code: \>1y

For “Filamentous fungus” “SIGNIFICANT GROWTH consistent with pneumonia.” “identification to follow” (DO NOT quantitate).

**VI. References**


CMV SURVEILLANCE BRONCHOSCOPY SPECIMENS

I. Introduction

Bronchoalveolar lavage (BAL) specimens from bone marrow transplant patients are collected for CMV surveillance on Day 35 post-transplant. These specimens should be processed in the Virology section. BAL specimens other than for CMV surveillance should be processed as outlined on page 3.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

Specimens collected for **routine CMV surveillance** are sent to Virology for processing ONLY. DO NOT set up for other tests.

III. Reagents / Materials / Media

See Analytical Process – Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

See Specimen Processing Procedure QPCMI06003

V. Reporting

Negative Report: No CMV DNA detected.

Positive Report: CMV DETECTED.

VI. References


EPIGLOTTAL SWABS

I. Introduction

Acute epiglottitis is usually caused by *H. influenzae* type b and less commonly by *S. aureus*, Group A streptococcus and viruses.

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: Not indicated

b) Culture:

<table>
<thead>
<tr>
<th>Medium</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Haemophilus Isolation Medium (HI)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>

B. Interpretation of cultures:

Examine the plates after 24 and 48 hours incubation for any growth of *H. influenzae*, Group A streptococcus and *S. aureus*.

Send all *Haemophilus influenzae* isolates to the Public Health Laboratory (PHOL) for typing.
C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

V. Reporting

Negative report: “Commensal flora” or “No growth”.

Positive report: Quantitate all significant isolates with appropriate susceptibilities. Report “Commensal flora” with quantitation if also present.

Telephone all positive Group A streptococcus results to ward / ordering physician as per Isolate Notification and Freezing Table QPCMI15003.

VI. References


GASTRIC ASPIRATES/BIOPSIES (for *Helicobacter pylori*)

I. Introduction

*Helicobacter pylori* is implicated in the etiology of some cases of gastritis and peptic ulcers.

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

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>Microaerophilic, 35°C x 7 days</td>
</tr>
<tr>
<td>Campylobacter Agar (CAMPY)</td>
<td>Microaerophilic, 35°C x 7 days</td>
</tr>
<tr>
<td>Urea (Rapid)</td>
<td>O₂, 35°C x 4 hours</td>
</tr>
</tbody>
</table>
B. Interpretation of cultures:

1. Examine the direct urea slant after 1 and 4 hours incubation. A positive reaction is presumptive evidence of the presence of *H. pylori*.

2. Examine the plates after 3, 5 and 7 days incubation. Colonies of *H. pylori* are grey, translucent and small (0.5 to 1.0 mm in diameter). Refer to Bacteria and Yeast Workup for identification.

C. Susceptibility Testing:

Not required.

V. Reporting

a) Direct Examination:

Gram Stain: Presence or absence of small, curved Gram negative bacilli

b) Culture:

Preliminary Report:

If rapid Urease is positive and small gram negative bacilli seen in Gram stain, report in “ISOLATE window” of the LIS – “*Helicobacter pylori*” “probable identification based on positive urease and Gram stain result, culture confirmation to follow”.

Final Report:

Negative Report: “No *Helicobacter pylori* isolated”

Positive Report: “*Helicobacter pylori* isolated”

VI. References

GASTRIC ASPIRATES/SWABS from Neonates or Stillborn

I. Introduction

In utero the fetus is in a sterile environmental. Therefore, no bacteria should be present in the gastric aspirate of the newborn. The presence of bacteria in a gastric aspirate or swab of a neonate or stillborn may be 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

a) Direct Examination:

i) Gram Stain

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>
B. Interpretation of cultures:

Examine the culture plates after 24 and 48 hours incubation.

Work up:
- any growth of *S. aureus*, beta-haemolytic streptococci group A, B, C and G, *H. influenza*, *Pseudomonas aeruginosa*
- pure growth of a gram-negative bacilli
- pure, $\geq$2+ growth of any other organism

List by gram stain and morphology:
- Pure, <2+ growth of any other organism
- Mixed cultures

C. Susceptibility Testing:

Stillborn – not required

V. Reporting

a) Direct Examination

Gram Stain: Report with quantitation the presence or absence of pus cells and organisms.

b) Culture:

Negative Report: “No growth”
“(Quantitation) mixed growth of list organisms…”

Positive Report: Quantitate all significant isolates with appropriate susceptibilities.

VI. References


MOUTH SWABS

I. Introduction

Mouth swabs are usually obtained in order to identify oral yeast infections (thrush) and less often Vincent’s angina (a rare oropharyngeal infection associated with Borrelia vincentii (a spirochete) and Fusobacterium species (a fusiform bacilli)).

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:

Yeast: Examine for presence of pseudohyphae and/or budding yeasts.

Vincent’s angina: Examine for presence of spirochetes and/or fusiform bacilli and pus cells.

b) Culture: Not indicated.
V. Reporting

Negative Report: “No yeast seen on direct examination. Fungal culture not done”

“No organisms suggestive of Vincent’s angina seen”.

Positive Report: “Yeast seen on direct examination. Fungal culture not done”.

“Yeast (with pseudohyphae) seen on direct examination. Fungal culture not done”

“Organisms suggestive of Vincent’s angina seen”

VI. References


NASAL SWABS FOR Culture and Susceptibilities

I. Introduction

These specimens are submitted to identify nasal carriers of Staphylococcus aureus. Neisseria meningitidis will be screened for only if requested. For specimens that are submitted to identify nasal carriers of Methicillin Resistant S. aureus (MRSA) see the Infection Control Manual.

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: Not indicated.

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin-Nalidixic Agar (CNA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>

If Neisseria meningitidis is requested, add:

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

1. Examine the plate after 24 and 48 hours incubation and the ML and CHOC plate after 48 and 72 hours incubation.
2. Identify *S. aureus*. Identify *N. meningitidis* if requested.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

V. **Reporting**

Negative report: “No *Staphylococcus aureus* isolated”
“*No Neisseria meningitidis* isolated”, if *N. meningitidis* is requested.

Positive report: “*Staphylococcus aureus*” or “Methicillin Resistant *Staphylococcus aureus* “isolated” with appropriate susceptibilities.
“*Neisseria meningitidis* isolated”.

Telephone all positive MRSA and *Neisseria meningitidis* results to ward/ordering physician and Infection Control Practitioner as per Isolate Notification and Freezing Table QPCMI15003.

VI. **References**


NASOPHARYNGEAL SWABS/AUGER SUCTIONS FOR *Bordetella pertussis*

I. **Introduction**

Requests for *Bordetella pertussis* will not be processed in-house. A posterior nasopharyngeal swab should be collected and placed in *B. pertussis* Transport Medium. Routine throat swabs are not acceptable and will not be processed. Auger suctions should be collected using a specialized syringe and tubing. The tubing should be sent to the lab in a sterile container. The specimen should be forwarded to the Provincial Health Laboratory (PHOL) for processing.

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

V. **Reporting**

Negative report: “*Bordetella pertussis* not detected by PCR. Refer to Public Health Report #______________”.

Positive report: “*Bordetella pertussis* detected by PCR. Refer to Public Health Report #______________”.

VI. **References**

Provincial Health Laboratory Procedure
OPEN LUNG/TRANSTHORACIC NEEDLE/TRANSBRONCHIAL LUNG BIOPSIES/ LUNG ASPIRATES

I. Introduction

There are three major lung biopsy specimen types that may be received in the laboratory.

1. **Open lung biopsy** specimen usually consists of a wedge of lung tissue obtained during surgery and submitted in a clean, sterile container.

2. **Transthoracic needle biopsy** specimens are taken by pushing a small bore needle through the chest wall into the lung and aspirating the contents of the needle into a small amount of fluid.

3. **Transbronchial lung biopsy** specimens are taken using a fiberoptic bronchoscope and removing a portion of lung tissue. A much smaller piece of tissue is obtained than with open lung biopsy.

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:
      i) Gram stain
      ii) Fungi-fluor stain (if fungus is requested)
b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Fastidious Anaerobe Agar (BRUC)</td>
<td>AnO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Fastidious Anaerobic Broth (THIO)</td>
<td>O₂, 35°C x 5 Days</td>
</tr>
</tbody>
</table>

If Fungal culture is requested, **add:**

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitory Mold Agar (IMA) *</td>
<td>O₂, 28°C x 4 weeks</td>
</tr>
<tr>
<td>Esculin Base Medium (EBM) *</td>
<td>O₂, 28°C x 4 weeks</td>
</tr>
<tr>
<td>Brain Heart Infusion Agar with 5% Sheep</td>
<td>O₂, 28°C x 4 weeks</td>
</tr>
<tr>
<td>Blood, Gentamicin, Chloramphenicol,</td>
<td></td>
</tr>
<tr>
<td>Cyclohexamide (BHIM)*</td>
<td></td>
</tr>
</tbody>
</table>

If *B. cepacia* is requested or the specimen is from a patient with Cystic Fibrosis, **add:**

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cepacia Selective Agar (OCBL.BCSA)</td>
<td>O₂, 35°C x 5 days</td>
</tr>
<tr>
<td>Keep the BA, HI and MAC plates</td>
<td>CO₂, 35°C x 5 days</td>
</tr>
</tbody>
</table>

If Nocardia is requested, **add:**

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate Agar (PYRU) *</td>
<td>O₂, 35°C x 4 weeks</td>
</tr>
</tbody>
</table>

* Forward inoculated fungal cultures to Mycology for incubation and work-up.

B. Interpretation of culture:

1. Examine aerobic plates after 24 and 48 hours incubation, anaerobic plates after 48 hours and THIO daily for 5 days for any growth. If no growth on aerobic and anaerobic plates, but organisms resembling anaerobic organisms are seen on Gram stain, reincubate the BRUC for an additional 48 hours. If *B. cepacia* is requested or the specimen is from a patient with Cystic Fibrosis, examine the BA, CHOC, MAC and OCBL.BCSA plate daily for 5 days.

2. Work up any growth and identify all isolates including yeast. Refer to [Bacteria and Yeast Workup](#) for identification.

D. Susceptibility Testing:
Refer to Susceptibility Testing Manual.
V. Reporting

a) Direct Examination:

   Gram Stain: Without Quantitation:
   Report presence or absence of pus cells.
   Report presence or absence of organisms.

   Fungi-fluor Stain: Refer to Fungi-fluor Stain.

b) Culture:

   “No B. cepacia isolated” if B. cepacia culture is requested or
   Specimen is from a patient with Cystic Fibrosis.
   “No Nocardia isolated” if Nocardia culture is requested.

   Positive Report: Report all isolates with appropriate susceptibilities. Do not quantitate.

   Telephone all positive results of direct examination and culture to ward / ordering physician.

VI. References


ORAL ABSCESS SWABS

I. Introduction

Oral abscesses are usually caused by a mixture of both aerobic and anaerobic organisms from the oral cavity. However, swabs from an oral abscess will only be processed for *S. aureus*, Group A streptococcus and *H. influenzae* unless otherwise requested.

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

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Haemophilus Isolation Medium (HI)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>

If Actinomyces is requested or suggested on Gram stain, **add**:

- Fastidious Anaerobic Agar (BRUC): AnO₂, 35°C x 10 days
- Kanamycin / Vancomycin Agar (KV): AnO₂, 35°C x 10 days
B. Interpretation of cultures:

Examine the BA and HI plates after 24 and 48 hours incubation for any growth of Group A streptococcus, \textit{S. aureus} and \textit{H. influenzae}. Examine the BRUC and KV plates (if set up for Actinomyces) after 48 hours and 10 days.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

V. Reporting

a) Direct Examination:

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

b) Culture:

Negative Report: “Commensal flora” or “No growth”.
“No Actinomyces isolated.”

Positive Report: Report with quantitation all significant isolates with appropriate susceptibilities.
Report “Commensal flora” with quantitation if also present.

Telephone all positive Group A streptococcus results to ward / ordering physician as per Isolate Notification and Freezing Table QPCMI15003

VI. References


SINUS/ANTRAL SPECIMENS

I. Introduction

Acute sinusitis commonly involves upper respiratory tract organisms such as S. pneumoniae, H. influenzae, M. catarrhalis, S. aureus, B. cepacia, P. aeruginosa, Group A streptococcus and fungus. A moderate to heavy pure growth of other Gram negative bacilli should also be considered significant. Anaerobic culture is done on request only. Nasal and nasopharyngeal specimens are unacceptable for diagnosis of sinusitis since there is a poor correlation with sinusitis and are cultured for MRSA only.

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:

i) Gram stain

ii) Fungi-fluor stain (if fungus is requested)

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Haemophilus Isolation Medium (HI)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>
If Fungal culture is requested **add:**
Inhibitory Mold agar (IMA)* O₂, 28°C x 4 weeks
Esculin Base Medium (EBM)* O₂, 28°C x 4 weeks

If anaerobic culture requested, **add:**
Fastidious Anaerobic Agar (BRUC) AnO₂, 35°C x 48 hours
Kanamycin Vancomycin Agar (KV) AnO₂, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO) O₂, 35°C x 5 days
*Forward inoculated fungal media to Mycology section for incubation and work-up.

B. Interpretation of cultures:
Examine the BA, HI and MAC plates after 24 and 48 hours incubation and the BRUC, KV, after 48 hours incubation and THIO daily for 5 days.

C. Susceptibility testing:
Refer to [Susceptibility Testing Manual](#).

V. **Reporting**

a) Direct Examination:
   i) Gram stain: Report with quantitation the presence of pus cells and organisms.
   ii) Fungi-fluor stain: Refer to [Fungi-fluor stain](#)

b) Culture:
   
   Negative report: “Commensal flora” or “No growth”.
   “No anaerobes isolated” if anaerobic culture is requested.
   
   Positive report: Quantitate and report significant isolates with appropriate susceptibilities.
   Report “Commensal flora” with quantitation if also present.
VI. References


SPUTUM (INCLUDING ENDOTRACHEAL TUBE AND TRACHEOSTOMY SPECIMENS; BRONCHOSCOPY ASPIRATES / WASHINGS)

I. Introduction

Pneumonia may be categorized as: i) Community acquired pneumonia (CAP), ii) Nosocomial or Hospital acquired pneumonia (NAP / HAP), iii) Aspiration pneumonia and iv) pneumonia in immunocompromised patients (e.g. HIV, transplant patients). Generally the etiology of the pneumonia varies depending on the category. The most common organisms to cause CAP include *Streptococcus pneumoniae, Mycoplasma pneumoniae*. Respiratory viruses, *Chlamydia pneumoniae, Haemophilus influenzae* and *Legionella pneumophila*. HAP is more commonly due to aerobic gram negative bacilli, anaerobes, *Staphylococcus aureus, Streptococcus pneumoniae* and others. Aspiration pneumonia may be due to chemical pneumonitis with or without a mixture of oral aerobes and anaerobes. Along with the common organisms noted above, unusual agents such as pneumocystis, dimorphic fungi, *Cryptococcus* may be found in immunocompromised patients. Acute bronchitis may be viral or occasionally bacterial.

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:

i) Gram Stain:

Sputum is always contaminated to some degree with oropharyngeal organisms. Consequently, a screening procedure for routine culture is required to exclude grossly contaminated specimens or saliva.

**DO NOT screen** PMH patients, endotracheal tube (ETT) aspirates, suctioned
samples, Bronchoscopy Aspirates/Washings or any specimens requesting only Mycobacterium tuberculosis (TB) or fungus culture.

**Screening Procedure**

Select the most purulent portion of the specimen for Gram staining and culture. Scan the smear under low power (10X magnification) as soon as possible and examine for epithelial cells.

<table>
<thead>
<tr>
<th>Squamous epithelial cells</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 25 cells/lpf*</td>
<td>Poor quality specimen. Discard culture plates without examining.</td>
</tr>
<tr>
<td>&lt; 25 cells/lpf</td>
<td>Examine and document gram stain results. Continue incubation of culture plates.</td>
</tr>
</tbody>
</table>

*lf = low power field

i) Fungi-flour stain (if fungus is requested)

ii) Acid-fast stain (if requests STAT and approved by microbiologist - Direct smear from an unconcentrated specimen).

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Haemophilus Isolation Medium (HI)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>

If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, **add:**

- *B. cepacia* Selective Agar (OCBL.BCSA)  
  O₂, 35°C x 5 days
- Keep the BA, HI and MAC plates  
  CO₂, 35°C x 5 days

If Nocardia culture is requested, **add:**

- Pyruvate Agar (PYRU)*  
  O₂, 35°C x 4 weeks

If Fungal culture is requested, **add:**

- Inhibitory Mold Agar (IMA)*  
  O₂, 28°C x 4 weeks
- Esculin Base Medium (EBM)*  
  O₂, 28°C x 4 weeks
- Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*  
  O₂, 28°C x 4 weeks

NOTE: This document is Uncontrolled When Printed.
Any documents appearing in paper form that are not stamped in red "MASTER COPY" are not controlled and should be checked against the document (titled as above) on the server prior to use.
Management System\UHN_Mount Sinai Hospital Microbiology\Standard Operating Procedures\Bacteriology Procedures\
* Forward inoculated fungal media to Mycology section for incubation and work-up.

B. Interpretation of Cultures:

Examine the plates after 24 and 48 hours incubation.

1. Identify all **Probable** respiratory pathogens if there is a moderate to heavy growth (≥2+). EXCEPTION: Identify any amount of *Cryptococcus neoformans/gattii* and filamentous fungus.

2. Identify all **Possible** respiratory pathogens if there is a moderate to heavy growth (≥2+) growth AND if obviously predominant.

3. Identify all **Probable** and **Possible** respiratory pathogens if there is a light growth (1+) AND obviously predominant AND if any amount of pus cells are seen in gram stain.

4. Refer to **Bacteria and Yeast Workup** for identification.

5. For filamentous fungus, seal the agar plate and send the culture to Mycology for identification.

6. If there is a question regarding the significance of an isolate, consult the senior/charge technologist or microbiologist.

**Probable respiratory pathogens:**
*Streptococcus pneumoniae*
*Moraxella catarrhalis*
*Hemophilus influenzae*
*Staphylococcus aureus*
*Pseudomonas aeruginosa*
*Group A streptococcus*
*Burkholderia cepacia***
*Rhodococcus equi* *
*Nocardia**
*Filamentous fungus**
*Cryptococcus neoformans/gattii**

*Screen diphtheroid-like organism if predominant compared to commensal flora
**Workup and report any amount
***Workup and report any amount for Cystic Fibrosis Patients

**Possible respiratory pathogens:**
Yeast not *Cryptococcus neoformans/gattii*
Group C and G streptococcus
Other gram negative bacilli (not listed above) of a single morphological type
*Corynebacterium pseudodiphtheriticum*
*Neisseria meningitidis*

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

For cystic fibrosis patients:

For *B. cepacia* and slow growing mucoid *P. aeruginosa*, susceptibilities can be referred back 4 weeks.

V. Reporting

a) Direct Examination

i) Gram Stain:
   Rejected Sputum Report:
   Greater than 25 squamous epithelial cells per low power field
   LIS Test Comment Code: }>25E

   Acceptable Sputum Report:
   Report with quantitation:
   - Presence or absence of pus cells;
   - Presence or absence of squamous epithelial cells;
   - Presence of predominant respiratory pathogens (amount greater than that of commensal flora);
   - Presence of “Commensal flora”;
   - “No bacteria seen” if no organism is seen

ii) Fungi-fluor stain: Refer to Fungi-fluor stain

iii) Acid-fast stain: Refer to Fluorochrome stain.
<table>
<thead>
<tr>
<th>Section: Bacteriology Procedures</th>
<th>Subject Title: Respiratory Culture Manual</th>
</tr>
</thead>
</table>

b) Culture:

Rejected Sputum Report: “Specimen unsuitable for processing due to oropharyngeal contamination”
LIS Test Comment Code: {REJ}
Negative Report: “Commensal flora” (DO NOT quantitate) or “No growth”.
“No B. cepacia isolated” if B. cepacia culture is requested or specimen is from patient with Cystic Fibrosis.

Positive Report: Quantitate and report significant isolates with appropriate susceptibilities. Report “Commensal flora” with quantitation if also present.

“Filamentous fungus” “isolated” “identification to follow” (DO NOT quantitate).

VI. References


THROAT SWABS

I. Introduction

Throat (pharyngeal) swabs are submitted for the diagnosis of Group A streptococcal pharyngitis.

Occasionally, specific requests may be received to rule out the following:
  - Gonococcal pharyngitis
  - Diphtheria pharyngitis
  - Vincent’s angina
  - Candida pharyngitis (thrush)
  - Meningococcal carriers
  - Viral pharyngitis
  - Mycoplasma pharyngitis

If no specific organism or infection is suggested, it should be assumed that the specimen is for the diagnosis of streptococcal pharyngitis and should be processed as such.

Specimens for other infections (e.g. viral, mycoplasma) should be submitted in appropriate transport media. Refer specimens for virology to the virology section. Requests for Diphtheria, or Mycoplasma should be forwarded to the Public Health Lab (PHOL) for processing.

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 for Group A streptococcus, Neisseria gonorrhoeae or Neisseria meningitidis
If yeast (thrush) is suspected / requested: Gram stain. Examine for presence of pseudohyphae and/or budding yeast.

If Vincent’s angina is suspected / requested: Gram stain. Examine for presence of spirochetes and/or fusiform bacilli and pus cells.

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>AnO₂, 35°C x 18-24 hours</td>
</tr>
</tbody>
</table>

If *Neisseria gonorrhoeae / meningitidis* is requested, add:

- Martin-Lewis Agar (ML)  
  CO₂, 35°C x 72 hours
- Chocolate Agar (CHOC)  
  CO₂, 35°C x 72 hours

If *Corynebacterium diphtheriae* is requested, forward swab to Public Health Laboratory (PHOL) for processing.

**Note:** The ML plate is inoculated by rolling the swab in a “Z” pattern over the medium followed by cross streaking with a sterile loop over the entire plate.

**B. Interpretation of Cultures:**

1. Examine the BA plate after 18-24 hours incubation and identify all morphologically distinct beta haemolytic colonies

2. For all specimens processed after 1600 hours, re-incubate BA anaerobically for a further 24 hours.

3. Examine the ML and CHOC plate after 48 and 72 hours incubation.

**C. Susceptibility Testing:**

Refer to Susceptibility Testing Manual.

**V. Reporting**

a) Gram stain

“No yeast seen on direct examination. Yeast culture not done”
“No organisms suggestive of Vincent’s angina seen”.

“Yeast seen on direct examination. Yeast culture not done”

“Many pus cells and organisms suggestive of Vincent’s angina seen”

b) Culture:

Negative report: “No Group A streptococcus isolated”.
“No Neisseria gonorrhoeae isolated” if requested.
“No Neisseria meningitidis isolated” if requested.
“No Corynebacterium diphtheriae isolated” if requested.

Positive report: “Group A streptococcus”.
“Neisseria gonorrhoeae, beta-lactamase negative or positive” (enter beta lactamase result under “Breakpoint Panel” in LIS Isolate Screen).
“Neisseria meningitidis”
“Corynebacterium diphtheriae (toxigenic/non-toxigenic)”.

Telephone all positive N. gonorrhoeae, N. meningitidis and Group A streptococci isolates according to Isolate Notification and Freezing Table QPCMI15003 (For MSH Family Medicine Patients, call the Family Medicine Resident on-call through locating when reporting positives on weekends).

VI. References


Stenotrophomonas maltophilia Detection in Legionella Indeterminate/Positive Respiratory Specimens

I. Introduction

Legionella species causing Legionnaires’ disease in a respiratory specimen can cause serious respiratory illness resulting in pneumonia.

Public Health Ontario Laboratories uses a Legionella PCR assay to detect all Legionella species and L. pneumophila on upper respiratory specimens. This assay may reflect false-positives for Legionella species other than L. pneumophila due to cross reactivity with Stenotrophomonas maltophilia.

Respiratory specimens reported as indeterminate or positive for Legionella species other than L. pneumophila will be tested if sufficient quantities remain for the detection of Stenotrophomonas maltophilia unless a culture was already performed showing “No growth”.

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:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>O2, 35°C x 48 hours</td>
</tr>
</tbody>
</table>

B. Interpretation of Cultures:

1. Examine the MAC plate after 24 and 48 hours incubation. Identify any amount of *Stenotrophomonas maltophilia*.

C. Susceptibility Testing: Not indicated

Negative Report:

UPDATED REPORT:
The bacterial culture was reviewed and *Stenotrophomonas maltophilia* was not detected.

jSTMN for Stenotrophomonas not detected

Positive Report:

UPDATED REPORT:
The bacterial culture was reviewed and *Stenotrophomonas maltophilia* was detected in small numbers. The quantity of growth is not consistent with pneumonia but it may be associated with a false-positive Legionella species PCR result. Results should be interpreted taking this into account.

jSTMD for Stenotrophomonas detected.

V. **References**

Mount Sinai Hospital, Microbiology. 2013. Cross-Reactivity with Legionella PCR. Medical Staff Bulletin. Toronto, ON
### Record of Edited Revisions

**Manual Section Name:** Respiratory Bench Manual

<table>
<thead>
<tr>
<th>Page Number / Item</th>
<th>Date of Revision</th>
<th>Signature of Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Review</td>
<td>June 6, 2001</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review</td>
<td>June 6, 2002</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review</td>
<td>June 6, 2003</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Page 39 Gastric aspirates/biopsies (for <em>Helicobacter pylori</em>) move to this section from Enterics</td>
<td>May 09, 2004</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Page 41 Gastric aspirates/swabs from neonates or stillborn – new</td>
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<td>June 24, 2004</td>
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<td>Page 5, 9, 31 Interpretation of cultures</td>
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<td>Dr. T. Mazzulli</td>
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<td>Page 28-32 Sputum workup and reporting</td>
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</tr>
<tr>
<td>Page 48 Yeast identification</td>
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<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>November 25, 2004</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Specimen collection procedure – see Pre-analytical Procedure – Specimen Collection QPCMI02001</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Specimen processing procedure – See Specimen Processing Procedure QPCMI06003</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Yeast ID – removed. See Bacteria and Yeast Work-up manual</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Germ tube, removed. See Technical manual</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>TB Stains, removed. See Technical manual</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Bronchial Brush – instructions for processing and reporting dry brush added</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Nasal Swab for C&amp;S (not MRSA) added</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<td>Gastric Aspirate for H. pylori reporting – phrase for preliminary reporting added</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>July 23, 2006</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Reporting statement for BAL with pathogen(s) and</td>
<td>September 15, 2006</td>
<td>Dr. T. Mazzulli</td>
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</tbody>
</table>

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<table>
<thead>
<tr>
<th>Page Number / Item</th>
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<tr>
<td>predominant commensal flora</td>
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<tr>
<td>Remove Nasal swab for MRSA section; add hyperlink to Infection Control Manual for these specimens</td>
<td>February 14, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>August 13, 2007</td>
<td>Dr. T. Mazzulli</td>
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<td>August 15, 2008</td>
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<tr>
<td>Annual Review</td>
<td>November 07, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Modified BAL to quantitative workup and reporting</td>
<td>November 07, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Modified Bronchial Brush reporting phrase</td>
<td>November 07, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>BAL from routine lung transplant combined with BAL</td>
<td>November 07, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>New BAL and BAL Brush reporting phrase for Yeasts and Commensal flora</td>
<td>December 13, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Revised BAL Positive Report that has commensal flora isolated</td>
<td>March 23, 2012</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>March 23, 2012</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>BAL – Added reporting category for Candida</td>
<td>December 28, 2012</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 31, 2013</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>BAL – updated reporting to specify C gattii</td>
<td>November 21, 2013</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Lung tissue (THIO) for 5 days</td>
<td>January 29, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>March 31, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>CMV Surveillance: fixed numbering Updated Heading and numbering</td>
<td>June 12, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Inserted new UHN Logo</td>
<td>August 5, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>New media code BCSA for B. cepacia add on</td>
<td>September 25, 2014</td>
<td>Dr. T. Mazzulli</td>
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<td>Added section: Stenotrophomonas maltophilia Detection in Legionella Indeterminate/Positive Respiratory Specimens</td>
<td>June 9, 2015</td>
<td>Dr. T. Mazzulli</td>
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<td>Added BAL reference for Rhodococcus</td>
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<td>Sputum Possible pathogens: Added Neisseria meningitidis</td>
<td>October 20, 2015</td>
<td>Dr. T. Mazzulli</td>
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<td>Sputum Probable/possible listed in order as BAL</td>
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<td>P. 7, 12, 28, 38 added to resulting for b.cepacia “or specimen is from a patient with Cystic fibrosis”</td>
<td>November 30, 2015</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>June 9, 2016</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Updated MSH logo in header</td>
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<tr>
<td>Updated Actino incubation time from 7 days to 10days</td>
<td>April 4, 2017</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 5, 2018</td>
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<td>BAL reporting section; updated reporting comment for yeast to exclude Candida.</td>
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