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.

<table>
<thead>
<tr>
<th>Type</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic bacteria</td>
<td>Veillonella, Peptostreptococcus, Fusobacterium, Porphyromonas, Bacteroides, Prevotella, Actinomycetes, Eubacterium, Bifidobacterium, Cutibacterium</td>
</tr>
</tbody>
</table>

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Management System\UHN Mount Sinai Hospital Microbiology\Standard Operating Procedures\Bacteriology Procedures\
Fungi

*Candida* spp.

Parasites

*Entamoeba gingivalis, Trichomonas tenax*

**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 or if mold isolated) - 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>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.

Inoculated with sediment from the spun specimen:

If Fungus is requested OR specimen is from lung transplant patients, add:

- Inhibitory Mold Agar (IMA) | O₂, 28°C x 4 weeks
- Esclusin 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

If Nocardia is requested, add:

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

* 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).
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. If there is no fungal culture ordered on the sample, setup direct Fungi-fluor stain and document result on workcard.

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* (*Note: exception for Probable pathogens labelled with an asterisk)*
- *Nocardia*
- Filamentous fungus
- *Cryptococcus neoforms/gattii*
- *Screen diphtheroid-like organism if predominant compared to commensal flora*

**Possible respiratory pathogens:**
- Yeast not *Cryptococcus neoforms/gattii*
- Group C and G streptococcus
- Other gram negative bacilli (not listed above) of single morphological type
- *Corynebacterium pseudodiphtheriticum*
- *Neisseria meningitidis*
- *Mycoplasma hominis*
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 predominate 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: $\geq 10y$

For *Candida* species: “*ISOLATE name*” “$\geq 10 \times 10^6$ 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: $\geq 10C$

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 µL 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)

Keep the BA, HI and MAC plates

days

$O_2$, $35^\circ C \times 5$ day

$CO_2$, $35^\circ C \times 5$
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>&gt;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 x E6 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.
<table>
<thead>
<tr>
<th>Department of Microbiology Quality Manual</th>
<th>Policy # MI_RESP</th>
<th>Page 18 of 55</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Section:</strong> Bacteriology Procedures</td>
<td><strong>Subject Title:</strong> Respiratory Culture Manual</td>
<td></td>
</tr>
<tr>
<td><strong>Version:</strong> 1.10  CURRENT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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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). Identification must be confirmed by PHOL. Refer to *Bacteria and Yeast Workup for identification*.

3. Freeze isolates as per Isolate Notification and Freezing Table QPCMI15003.

C. Susceptibility Testing:

   Refer to Susceptibility Testing Manual.

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” with appropriate susceptibilities.
VI. References


https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/62769
https://www.mayomedicallaboratories.com/it-mmfiles/Microbiology_Test_Request_Form.pdf
http://www.eucast.org/clinical_breakpoints/
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, ≥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
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
      
      Pus cells: Examine for presence or absence
      
      Yeast: Examine for presence of pseudohyphae and/or budding yeasts.
      
      Vincent’s angina: Examine for presence of spirochetes and/or fusiform bacilli.

   b) Culture: Not indicated.
V. Reporting

<table>
<thead>
<tr>
<th>Negative Report:</th>
<th>OR</th>
<th>Positive Report – Yeast only:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus cells seen</td>
<td></td>
<td>Pus cells seen</td>
</tr>
<tr>
<td>No yeast seen on direct examination.</td>
<td></td>
<td>No pus cells seen</td>
</tr>
<tr>
<td>No organisms suggestive of Vincent’s</td>
<td></td>
<td>Yeast seen on direct examination. Fungal culture not</td>
</tr>
<tr>
<td>angina seen</td>
<td></td>
<td>done</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive Report – Vincent’s organisms only:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus cells seen</td>
</tr>
<tr>
<td>No yeast seen on direct examination. Fungal culture not done</td>
</tr>
</tbody>
</table>

Organisms suggestive of Vincent’s angina seen

Positive Report – Yeast with vincent’s organisms:

<table>
<thead>
<tr>
<th>Pus cells seen</th>
<th>OR</th>
<th>Yeast seen on direct examination. Fungal culture not done</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pus cells seen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No yeast seen on direct examination. Fungal culture not done</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 QPCM102001

III. Reagents / Material / Media

See Analytical Process – Bacteriology Reagents_Materials_Media List QPCM11001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCM106003

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**:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></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 7 Days</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
- Brain Heart Infusion Agar with 5% Sheep O₂, 28°C x 4 weeks
- Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*

If *B. cepacia* is requested or the 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 is requested, **add**:

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

* 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**:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fastidious Anaerobic Agar (BRUC)</td>
<td>AnO₂, 35°C x 10 days</td>
</tr>
<tr>
<td>Kanamycin / Vancomycin Agar (KV)</td>
<td>AnO₂, 35°C x 10 days</td>
</tr>
</tbody>
</table>
B. Interpretation of cultures:

Examine the BA and HI plates after 24 and 48 hours incubation for any growth of Group A streptococcus, *S. aureus* and *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)*
Esulin Base Medium (EBM)*

If anaerobic culture requested, **add:**
Fastidious Anaerobic Agar (BRUC)
Kanamycin Vancomycin Agar (KV)
Fastidious Anaerobic Broth (THIO)

*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>

*lpf = low power field

- ii) Fungi-fluor stain (if fungus is requested)
- iii) 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**:

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UNIVERSITY HEALTH NETWORK/MOUNT SINAI HOSPITAL, DEPARTMENT OF MICROBIOLOGY

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Any documents appearing in paper form that do not state “CONTROLLED COPY” in red print are not controlled and should be checked against the document (titled as above) on the server prior to use.
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***

---

* Forward inoculated fungal media to Mycology section for incubation and work-up.
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*  
*Mycoplasma hominis*

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.

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.

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

For Yeast NOT Cryptococcus neoformans or Cryptococcus gattii: report as “ISOLATE name POSSIBLY significant. Yeasts other than Cryptococcus neoformans/gattii are NOT commonly associated with pneumonia. Histopathologic and clinical correlation is required.”

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:

<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 Agar (CHOC)</td>
<td>CO₂, 35°C x 72 hours</td>
</tr>
</tbody>
</table>

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:

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

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

4. 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”.
“No Neisseria gonorrhoeae, beta-lactamase negative or positive” (enter beta lactamase result under “Breakpoint Panel” in LIS Isolate Screen).
“No 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


H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 in Clinical Microbiology
<table>
<thead>
<tr>
<th>Department of Microbiology Quality Manual</th>
<th>Policy # MI_RESP</th>
<th>Page 49 of 55</th>
</tr>
</thead>
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<tr>
<td>Section: Bacteriology Procedures</td>
<td>Subject Title: Respiratory Culture Manual</td>
<td></td>
</tr>
</tbody>
</table>


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.

}STMN 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.

}STMD 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**

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<th>Page Number / Item</th>
<th>Date of Revision</th>
<th>Signature of Approval</th>
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</thead>
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<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>
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<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>
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<tr>
<td>Page 41 Gastric aspirates/swabs from neonates or stillborn – new</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 48 Yeast identification</td>
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<td>November 25, 2004</td>
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<tr>
<td>Specimen collection procedure – see Pre-analytical Procedure – Specimen Collection QPCM102001</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Specimen processing procedure – See Specimen Processing Procedure QPCM106003</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<td>Yeast ID – removed. See Bacteria and Yeast Work-up manual</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<td>Germ tube, removed. See Technical manual</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<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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<tr>
<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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<tr>
<td>Annual Review</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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### Management System

**UHN Mount Sinai Hospital Microbiology**

**Standard Operating Procedures**

**Bacteriology Procedures**

**Subject Title:** Respiratory Culture Manual

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<tr>
<th>Page Number / Item</th>
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<tr>
<td>Annual Review</td>
<td>July 23, 2006</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Reporting statement for BAL with pathogen(s) and predominant commensal flora</td>
<td>September 15, 2006</td>
<td>Dr. T. Mazzulli</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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<tr>
<td>Annual Review</td>
<td>August 15, 2008</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>August 15, 2009</td>
<td>Dr. T. Mazzulli</td>
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<td>August 15, 2010</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>November 07, 2011</td>
<td>Dr. T. Mazzulli</td>
</tr>
<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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<tr>
<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>
</tr>
<tr>
<td>BAL – updated reporting to specify C gattii</td>
<td>November 21, 2013</td>
<td>Dr. T. Mazzulli</td>
</tr>
<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>
</tr>
<tr>
<td>CMV Surveillance: fixed numbering Updated Heading and numbering</td>
<td>June 12, 2014</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Inserted new UHN Logo</td>
<td>August 5, 2014</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>New media code BCSA for B. cepacia add on</td>
<td>September 25, 2014</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Added section: <em>Stenotrophomonas maltophilia</em> Detection in Legionella Indeterminate/Positive Respiratory Specimens Added BAL reference for Rhodococcus</td>
<td>June 09, 2015</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Sputum Possible pathogens: Added <em>Neisseria meningitidis</em> Sputum Probable/possible listed in order as BAL</td>
<td>October 20, 2015</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<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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</table>
### Respiratory Culture Manual

**Department of Microbiology**

**Quality Manual**

**Section:** Bacteriology Procedures

**Subject Title:** Respiratory Culture Manual

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<th>Page Number / Item</th>
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<tr>
<td>Annual Review</td>
<td>June 09, 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>Annual Review</td>
<td>April 04, 2017</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Updated Actino incubation time from 7 days to 10 days</td>
<td></td>
<td></td>
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<tr>
<td>Annual Review</td>
<td>May 05, 2018</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>BAL reporting section; updated reporting comment for yeast to exclude Candida.</td>
<td></td>
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<tr>
<td>Added reference to now set up susceptibility and report as per susceptibility manual.</td>
<td>November 30, 2018</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review</td>
<td>June 30, 2019</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Changed Fastidious Anaerobic Broth (THIO) incubation time from 5 days to 7 days</td>
<td>July 26, 2020</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Annual Review</td>
<td>September 14, 2020</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Addition of <em>Mycoplasma hominis</em> as possible respiratory pathogen under sputum and BAL</td>
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</table>

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

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<tr>
<th>Page Number / Item</th>
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<th>Edited by:</th>
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<tbody>
<tr>
<td>Updated reporting of mouth swab</td>
<td>January 21, 2021</td>
<td>Dorna Zareianjahromi</td>
</tr>
<tr>
<td>Added yeast not crypto comment to sputum section</td>
<td>February 26, 2021</td>
<td>Wayne Chiu</td>
</tr>
<tr>
<td>Minor formatting change</td>
<td>April 11, 2021</td>
<td>Jessica Bourke</td>
</tr>
<tr>
<td>Nomenclature update – cutibacterium</td>
<td>April 19, 2021</td>
<td>Wayne Chiu</td>
</tr>
<tr>
<td>Added BAL mold workup. If mold isolated and there is no fungal culture ordered, setup fungifluor</td>
<td>April 23, 2021</td>
<td>Wayne Chiu</td>
</tr>
</tbody>
</table>