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>
</table>
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>Inoculate with <strong>unspun</strong> specimen using <strong>1 ul loop</strong>:</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>
</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  |
| 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 |

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 over commensal flora. Refer to **Bacteria and Yeast Workup** for identification.

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:

<table>
<thead>
<tr>
<th>Streptococcus pneumoniae</th>
<th>Burkholderia cepacia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraxella catarrhalis</td>
<td>Cryptococcus neoformans/gattii</td>
</tr>
<tr>
<td>Hemophilus influenzae</td>
<td>Nocardia</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Filamentous fungus</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Group A streptococcus</td>
<td></td>
</tr>
</tbody>
</table>

### Possible respiratory pathogens:

<table>
<thead>
<tr>
<th>Yeast not <em>Cryptococcus neoformans/gattii</em></th>
<th><em>Neisseria meningitidis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C and G streptococcus</td>
<td><em>Mycoplasma hominis</em></td>
</tr>
<tr>
<td>Other gram negative bacilli (not listed above) of single morphological type</td>
<td><em>Rhodococcus equi</em></td>
</tr>
<tr>
<td><em>Corynebacterium pseudodiphtheriticum</em></td>
<td></td>
</tr>
</tbody>
</table>

### Commensal Flora:

Other oral flora (ex. Coag neg staph, viridans strep, etc) or non-predominant growth of possible respiratory pathogens.

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: \>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 \( \geq 1 \times 10^6 \text{/L} (\geq 100/\text{mL}) \) 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>Blood Agar (BA)</td>
<td>( \text{CO}_2 ), 35°C \times 48 hour</td>
</tr>
<tr>
<td>Haemophilus Isolation Medium (HI)</td>
<td>( \text{CO}_2 ), 35°C \times 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>( \text{CO}_2 ), 35°C \times 48 hours</td>
</tr>
</tbody>
</table>

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

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

- **O₂**, 35°C x 5 day
- **CO₂**, 35°C x 5 days
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>&lt;1 x 10^6 cfu/L</td>
<td>&lt;1 x 10^6 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 10^6 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 10^6 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.”

UNIVERSITY HEALTH NETWORK/MOUNT SINAI HOSPITAL, DEPARTMENT OF MICROBIOLOGY

NOTE: This document is Uncontrolled When Printed.
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.
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 4 and 7 days incubation. Being careful not to expose the plates to ambient air for longer than 1-2 min at most when examining the plates as high oxygen concentration will kill microaerophiles. 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

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”.

Interim Report: (Both negative and positive reports are finalized by seniors)

Negative Report: “No Helicobacter pylori isolated”

Negative Report: for biopsy samples not collected in Portagerm

“No Helicobacter pylori isolated”

“Specimen received in Starplex container as opposed the Portagerm pylori collection container. This may reduce the viability of H. pylori resulting in a false negative culture. Please take this into account when interpreting this result.”
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
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

   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>
</tr>
</thead>
<tbody>
<tr>
<td>Pus cells seen OR No pus cells seen</td>
</tr>
<tr>
<td>No yeast seen on direct examination. Fungal culture not done</td>
</tr>
<tr>
<td>No organisms suggestive of Vincent’s angina seen</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Positive Report – Yeast with vincent’s organisms:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus cells seen OR No pus cells seen</td>
</tr>
<tr>
<td>Yeast seen on direct examination. Fungal culture not done OR Yeast (with pseudohyphae) seen on direct examination. Fungal culture not done</td>
</tr>
<tr>
<td>Organisms suggestive of Vincent’s angina seen</td>
</tr>
</tbody>
</table>

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</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<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>

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

- Martin-Lewis Agar (ML)
- Chocolate (CHOC)
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 Nocadia 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.

C. 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:
      Negative Report:
      “No growth.”

      “No B. cepacia isolated” if B. cepacia culture is requested or if 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)*
  - 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

Processing of Specimens:
See Specimen Processing Procedure QPCMI06003

a) Direct Examination:

i) Gram Stain:

Sputum (including expectorated and induced 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
1) PMH patients,
2) endotracheal tube (ETT) aspirates,
3) suctioned samples,
4) Bronchoscopy Aspirates/Washings
5) 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

---

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\
If Nocardia culture is requested, **add**:  
Pyruvate Agar (PYRU)*  
\[O_2, \quad 35^\circ C \times 4 \text{ weeks} \]

If Fungal culture is requested, **add**:  
Inhibitory Mold Agar (IMA)*  
Esculin Base Medium (EBM)*  
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*  
\[O_2, \quad 28^\circ C \times 4 \text{ weeks} \]

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

**A. 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, Nocardia and filamentous fungus

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

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 | Burkholderia cepacia** |
| Moraxella catarrhalis   | Cryptococcus neoformans/gattii* |
| Hemophilus influenzae   | Nocardia* |
| Staphylococcus aureus   | Filamentous fungus* |
| Pseudomonas aeruginosa  | |
| Group A streptococcus   | |

NOTE: This document is Uncontrolled When Printed.
* Workup and report any amount
** Workup and report any amount for Cystic Fibrosis Patients

### Possible respiratory pathogens:

<table>
<thead>
<tr>
<th>Yeast not Cryptococcus neoformans/gattii</th>
<th>Neisseria meningitidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C and G streptococcus</td>
<td>Mycoplasma hominis</td>
</tr>
<tr>
<td>Other gram negative bacilli (not listed above) of single morphological type</td>
<td>Rhodococcus equi</td>
</tr>
<tr>
<td>Corynebacterium pseudodiphtheriticum</td>
<td></td>
</tr>
</tbody>
</table>

### Commensal Flora:
Other oral flora (ex. Coag neg staph, viridans strep, etc) or non-predominant growth of possible respiratory pathogens.

### B. 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:

Reject 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 Significant growth of Yeast NOT Cryptococcus neoformans/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”.
“Neisseria gonorrhoeae, beta-lactamase negative or positive” (enter beta lactamase result under “Breakpoint Panel” in LIS Isolate Screen).
“No Neisseria meningitidis”
“No 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

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>
</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 48 Yeast identification</td>
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</tr>
<tr>
<td>Annual Review</td>
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<td>Dr. T. Mazzulli</td>
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<td>Specimen collection procedure – see Pre-analytical Procedure – Specimen Collection QPCM02001</td>
<td>April 6, 2005</td>
<td>Dr. T. Mazzulli</td>
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<td>Specimen processing procedure – See Specimen Processing Procedure QPCM06003</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>
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<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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<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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<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>
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<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 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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<td>Annual Review</td>
<td>August 13, 2007</td>
<td>Dr. T. Mazzulli</td>
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<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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<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>
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<tr>
<td>BAL – updated reporting to specify <em>C. gattii</em></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>
</tr>
<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 <em>B. cepacia</em> add on</td>
<td>September 25, 2014</td>
<td>Dr. T. Mazzulli</td>
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<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: <em>Neisseria meningitidis</em> Sputum Probable/possible listed in order as BAL</td>
<td>October 20, 2015</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>P. 7, 12, 28, 38 added to resulting for <em>b.cepacia</em> “or specimen is from a patient with Cystic fibrosis”</td>
<td>November 30, 2015</td>
<td>Dr. T. Mazzulli</td>
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### Page Number / Item

| Annual Review | Updated MSH logo in header | June 09, 2016 | Dr. T. Mazzulli |
| Annual Review | Updated Actino incubation time from 7 days to 10 days | April 04, 2017 | Dr. T. Mazzulli |
| Annual Review | BAL reporting section; updated reporting comment for yeast to exclude Candida. | May 05, 2018 | Dr. T. Mazzulli |
| Added reference to now set up susceptibility and report as per susceptibility manual. | November 30, 2018 | Dr. T. Mazzulli |
| Annual Review | | June 30, 2019 | Dr. T. Mazzulli |
| Changed Fastidious Anaerobic Broth (THIO) incubation time from 5 days to 7 days | July 26, 2020 | Dr. T. Mazzulli |
| Annual Review | Addition of *Mycoplasma hominis* as possible respiratory pathogen under sputum and BAL | September 14, 2020 | Dr. T. Mazzulli |

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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<tbody>
<tr>
<td>Updated reporting of mouth swab</td>
<td>January 21, 2021</td>
<td>Dorna Zareianjahromi</td>
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<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>
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<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>
<tr>
<td>Updated wording for possible resp pathogen. Added note regarding commensal flora in BAL sputum workup. minor formatting.</td>
<td>July 13, 2021</td>
<td>Wayne Chiu</td>
</tr>
<tr>
<td>Added note regarding screening of expectorated and induced sputum</td>
<td>May 5, 2022</td>
<td>Wayne Chiu</td>
</tr>
<tr>
<td>Removed confirmation of H. pylori ID by PHOL</td>
<td>July 12, 2022</td>
<td>Wayne Chiu</td>
</tr>
<tr>
<td>Updated wording for reporting of yeast (not neoformans/gattii) in sputum</td>
<td>November 1, 2022</td>
<td>Wayne Chiu</td>
</tr>
</tbody>
</table>
Updated GASTRIC ASPIRATES/BIOPSIES (for *Helicobacter pylori*) (pg19-20)

- Interpretation of cultures:
  Examine the plates after 4 and 7 days with 1-2 min at most to reduce exposure to ambient air
- Reporting:
  Both negative and positive reports are **finalized by seniors**
- Added can message for negative report for biopsy samples not collected in portagerm

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<td>Updated GASTRIC ASPIRATES/BIOPSIES (for <em>Helicobacter pylori</em>) (pg19-20)</td>
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