RESPIRATORY TRACT CULTURE MANUAL

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Sinus or antral aspirates

B. Lower Respiratory Tract Specimens include:

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Bronchial brushings
Bronchoalveolar lavage (BAL)
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Lower respiratory tract specimens may be contaminated with organisms found in the upper respiratory tract.

COMMENSAL FLORA - RESPIRATORY TRACT	
Type	**Organism**
Aerobic bacteria	*Streptococcus pyogenes* (and other haemolytic streptococci), *S. pneumoniae*, *S. aureus*, Coagulase negative Staphylococci, *Neisseria* spp., *Haemophilus* spp., *Moraxella* spp., *Corynebacterium* spp., Stomatococcus, enteric organisms, Micrococcus, Lactobacillus, Mycoplasma
Anaerobic bacteria	Veillonella, Peptostreptococcus, Fusobacterium, Porphyromonas, Bacteroides, Prevotella, Actinomyces, Eubacterium, Bifidobacterium, Cutibacterium

Fungi	*Candida* spp.
Parasites	*Entamoeba gingivalis, Trichomonas tenax*

References:

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

Media	**Incubation**
Inoculate with **unspun** specimen using **1 uL loop**:	
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours
If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, **add**:	
B. cepacia Selective Agar (OCBL.BCSA)	O_2, 35°C x 5 days
Keep the BA, HI and MAC plates	CO_2, 35°C x 5 days
Inoculated with **sediment** from the spun specimen:	
If Fungus is requested OR specimen is from lung transplant patients, **add**:	
Inihibitory Mold Agar (IMA) *	O_2, 28°C x 4 weeks
Esculin Base Medium (EBM)*	O_2, 28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 28°C x 4 weeks
If Nocardia is requested, **add**: Pyruvate Agar (PYRU)*	O_2, 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).

Inoculation Loop size	No. of colonies	Colony count/L	Reporting Count
1 uL	1-9 colonies	1-8 x 10^6 cfu/L	<10 x E6 cfu/L
	10-100 colonies	10-100 x 10^6 cfu/L	≥10 x E6 cfu/L
	>100 colonies	>100 x 10^6 cfu/L	≥10 x E6 cfu/L

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

Possible respiratory pathogens:

Yeast not *Cryptococcus neoformans/gattii*	*Neisseria meningitidis*
Group C and G streptococcus	*Mycoplasma hominis*
Other gram negative bacilli (not listed above) of single morphological type	*Rhodococcus equi*
Corynebacterium pseudodiphtheriticum	

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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2010. Lower Respiratory Tract Cultures, 3.11.2 in Clinical Microbiology Procedures Handbook, 3rd ed. Vol.1 ASM Press, Washington, D.C.

Mayhall CG. Ventilator-Associated Pneumonia or Not? Contemporary Diagnosis. Emerging I Infectious Diseases. 2001:7(2):200-204.

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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:

Media	**Incubation**
Inoculate with **10ul loop:**	
Blood Agar (BA)	CO_2, 35°C x 48 hour
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours

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

B. cepacia Selective Agar (OCBL.BCSA) O_2, 35°C x 5 day
Keep the BA, HI and MAC plates CO_2, 35°C x 5 days

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).

Inoculation Loop size	No. of colonies	Colony count/L	Reporting Count
10 uL	1-10 colonies	1-10 x 10^6 cfu/L	<1 x E6 cfu/L
	10-100 colonies	10-100 x 10^6 cfu/L	≥1 x E6 cfu/L
	>100 colonies	>100 x 10^6 cfu/L	≥1 x E6 cfu/L

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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2010. Lower Respiratory Tract Cultures, 3.11.2 in Clinical Microbiology Procedures Handbook, 3rd ed. Vol.1 ASM Press, Washington, D.C.

Mayhall CG. Ventilator-Associated Pneumonia or Not? Contemporary Diagnosis. Emerging I Infectious Diseases. 2001:7(2):200-204.

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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 – 3.11.3.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

Medium	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours

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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

Media	Incubation
Blood Agar (BA)	Microaerophilic, 35°C x 7 days
Campylobacter Agar (CAMPY)	Microaerophilic, 35°C x 7 days
Urea (Rapid)	O_2, 35°C x 4 hours

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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. *Helicobacter pylori* Cultures, 3.8.4.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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http://www.health.gov.on.ca/en/public/programs/ohip/outofcountry/prior_approval.aspx
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:

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

B. Interpretation of cultures:

Examine the culture plates after 24 and 48 hours incubation.

Work up:

- any growth of *S. aureus*, beta-haemolytic streptococci 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:

Neonates – Refer to Susceptibility Testing Manual for significant organisms.
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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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

Negative Report:			
	Pus cells seen	OR	No pus cells seen
	No yeast seen on direct examination. Fungal culture not done		
	No organisms suggestive of Vincent's angina seen		
Positive Report – Yeast only:			
	Pus cells seen	OR	No pus cells seen
	Yeast seen on direct examination. Fungal culture not done	OR	Yeast (with pseudohyphae) seen on direct examination. Fungal culture not done
	No organisms suggestive of Vincent's angina seen		
Positive Report – Vincent's organisms only:			
	Pus cells seen	OR	No pus cells seen
	No yeast seen on direct examination. Fungal culture not done		
	Organisms suggestive of Vincent's angina seen		
Positive Report – Yeast with vincent's organisms:			
	Pus cells seen	OR	No pus cells seen
	Yeast seen on direct examination. Fungal culture not done	OR	Yeast (with pseudohyphae) seen on direct examination. Fungal culture not done
	Organisms suggestive of Vincent's angina seen		

VI. References

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

Media	Incubation
Colistin-Nalidixic Agar (CNA)	CO_2, 35°C x 48 hours
If *Neisseria meningitidis* is requested, **add**:	
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours
Chocolate (CHOC)	CO_2, 35°C x 72 hours

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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Nasal Sinus Cultures, 3.11.9.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours
Fastidious Anaerobe Agar (BRUC)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 7 Days
If Fungal culture is requested, **add:**	
Inhibitory Mold Agar (IMA) *	O_2, 28°C x 4 weeks
Esculin Base Medium (EBM) *	O_2, 28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2, 28°C x 4 weeks
If *B. cepacia* is requested or the specimen is from a patient with Cystic Fibrosis, **add**:	
B. cepacia Selective Agar (OCBL.BCSA)	O_2, 35°C x 5 days
Keep the BA, HI and MAC plates	CO_2, 35°C x 5 days
If Nocadia is requested, **add**:	
Pyruvate Agar (PYRU) *	O_2, 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 *Norcardia* 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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 – 3.11.3.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

Abdulmassih, R., Makadia, J., Como, J., Paulson, M., Min, Z., & Bhanot, N. (2016, December). Propionibacterium acnes: Time-to-Positivity in Standard Bacterial Culture From Different Anatomical Sites. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27829959

Shannon, S., Mandrekar, J., Gustafson, D., Rucinski, S., Dailey, A., Segner, R., Patel, R. (2013, February). Anaerobic thioglycolate broth culture for recovery of Propionibacterium acnes from shoulder tissue and fluid specimens. Retrieved July 27, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3553932/

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

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:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours

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

Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 10 days
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C x 10 days

B. Interpretation of cultures:

Examine the BA and HI plates after 24 and 48 hours incubation for any growth of Group A streptococcus, *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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 – 3.11.3.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours

If Fungal culture is requested **add:**		
Inhibitory Mold agar (IMA)*	O_2,	28°C x 4 weeks
Esculin Base Medium (EBM)*	O_2,	28°C x 4 weeks
If anaerobic culture requested, **add**:		
Fastidious Anaerobic Agar (BRUC)	AnO_2,	35°C x 48 hours
Kanamycin Vancomycin Agar (KV)	AnO_2,	35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2,	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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Nasal Sinus Cultures, 3.11.9.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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.

Squamous epithelial cells	Action
> 25 cells/lpf*	Poor quality specimen. Discard culture plates without examining.
< 25 cells/lpf	Examine and document gram stain results. Continue incubation of culture plates.

*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:

Media	Incubation
Blood Agar (BA)	CO_2, 35°C x 48 hours
Haemophilus Isolation Medium (HI)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours

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

B. cepacia Selective Agar (OCBL.BCSA)	O_2, 35°C x 5 days
Keep the BA, HI and MAC plates	CO_2, 35°C x 5 days

If Nocardia culture is requested, **add**:		
Pyruvate Agar (PYRU)*	O_2,	35°C x 4 weeks
If Fungal culture is requested, **add**:		
Inhibitory Mold Agar (IMA)*	O_2,	28°C x 4 weeks
Esculin Base Medium (EBM)*	O_2,	28°C x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)*	O_2,	28°C x 4 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 scant or light growth AND pure or obviously predominant.
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	

* Workup and report any amount

** Workup and report any amount for Cystic Fibrosis Patients

Possible respiratory pathogens:

Yeast not *Cryptococcus neoformans/gattii*	*Neisseria meningitidis*
Group C and G streptococcus	*Mycoplasma hominis*
Other gram negative bacilli (not listed above) of single morphological type	*Rhodococcus equi*
Corynebacterium pseudodiphtheriticum	

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:

Rejected Sputum Report: "Specimen unsuitable for processing due to oropharyngeal contamination"
LIS Test Comment Code: **}REJ**

Negative Report: "Commensal flora" (DO NOT quantitate) or "No growth".
"No *B. cepacia* isolated" if *B. cepacia* culture is requested or specimen is from patient with Cystic Fibrosis

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

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

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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology,

8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 – 3.11.3.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

Media	**Incubation**
Blood Agar (BA)	AnO_2, 35°C x 18-24 hours
If *Neisseria gonorrhoeae* / *meningitidis* is requested, **add**:	
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 72 hours
If *Corynebacterium diphtheriae* is requested, forward swab to Public Health Laboratory (PHOL) for processing.	

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

B. Interpretation of Cultures:

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).
"*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

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Respiratory Tract Cultures, 3.11.1.1 in Clinical Microbiology

Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Group A Streptococcus Culture, 3.11.8.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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:

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

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 Stenotorphomonas 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

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	June 6, 2001	Dr. T. Mazzulli
Annual Review	June 6, 2002	Dr. T. Mazzulli
Annual Review	June 6, 2003	Dr. T. Mazzulli
Page 39 Gastric aspirates/biopsies (for *Helicobacter pylori*) move to this section from Enterics	May 09, 2004	Dr. T. Mazzulli
Page 41 Gastric aspirates/swabs from neonates or stillborn – new	May 09, 2004	Dr. T. Mazzulli
Page 4 Volume of specimen to send to PHL	June 24, 2004	Dr. T. Mazzulli
Page 5, 9, 31 Interpretation of cultures	September 23, 2004	Dr. T. Mazzulli
Page 6, 10, 18, 32 Gram Stain reporting	September 23, 2004	Dr. T. Mazzulli
Page 49 Yeast identification	September 23, 2004	Dr. T. Mazzulli
Page 53 Respiratory Tract Pathogen (new)	September 23, 2004	Dr. T. Mazzulli
Page 36 Incubate urea slant at 35^{o}C added	September 23, 2004	Dr. T. Mazzulli
Page 3-10 BAL workup and reporting	November 25, 2004	Dr. T. Mazzulli
Page 28-32 Sputum workup and reporting	November 25, 2004	Dr. T. Mazzulli
Page 48 Yeast identification	November 25, 2004	Dr. T. Mazzulli
Annual Review	November 25, 2004	Dr. T. Mazzulli
Specimen collection procedure – see Pre-analytical Procedure – Specimen Collection QPCMI02001	April 6, 2005	Dr. T. Mazzulli
Specimen processing procedure – See Specimen Processing Procedure QPCMI06003	April 6, 2005	Dr. T. Mazzulli
Yeast ID – removed. See Bacteria and Yeast Work-up manual	April 6, 2005	Dr. T. Mazzulli
Germ tube, removed. See Technical manual	April 6, 2005	Dr. T. Mazzulli
TB Stains, removed. See Technical manual	April 6, 2005	Dr. T. Mazzulli
Bronchial Brush – instructions for processing and reporting dry brush added	April 6, 2005	Dr. T. Mazzulli
Nasal Swab for C&S (not MRSA) added	April 6, 2005	Dr. T. Mazzulli
Gastric Aspirate for H. pylori reporting – phrase for preliminary reporting added	April 6, 2005	Dr. T. Mazzulli
Annual Review	April 6, 2005	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	July 23, 2006	Dr. T. Mazzulli
Reporting statement for BAL with pathogen(s) and predominant commensal flora	September 15, 2006	Dr. T. Mazzulli
Remove Nasal swab for MRSA section; add hyperlink to Infection Control Manual for these specimens	February 14, 2007	Dr. T. Mazzulli
Annual Review	August 13, 2007	Dr. T. Mazzulli
Annual Review	August 15, 2008	Dr. T. Mazzulli
Annual Review	August 15, 2009	Dr. T. Mazzulli
Annual Review	August 15, 2010	Dr. T. Mazzulli
Annual Review	November 07, 2011	Dr. T. Mazzulli
Modified BAL to quantitative workup and reporting	November 07, 2011	Dr. T. Mazzulli
Modified Bronchial Brush reporting phrase	November 07, 2011	Dr. T. Mazzulli
BAL from routine lung transplant combined with BAL	November 07, 2011	Dr. T. Mazzulli
New BAL and BAL Brush reporting phrase for Yeasts and Commensal flora	December 13, 2011	Dr. T. Mazzulli
Revised BAL Positive Report that has commensal flora isolated	March 23, 2012	Dr. T. Mazzulli
Annual Review	March 23, 2012	Dr. T. Mazzulli
BAL – Added reporting category for Candida	December 28, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli
BAL – updated reporting to specify *C gattii*	November 21, 2013	Dr. T. Mazzulli
Lung tissue (THIO) for 5 days	January 29,2014	Dr. T. Mazzulli
Annual Review	March 31, 2014	Dr. T. Mazzulli
CMV Surveillance: fixed numbering Updated Heading and numbering	June 12, 2014	Dr. T. Mazzulli
Inserted new UHN Logo	August 5, 2014	Dr. T. Mazzulli
New media code BCSA for *B. cepacia* add on	September 25, 2014	Dr. T. Mazzulli
Added section: *Stenotrophomonas maltophilia* Detection in Legionella Indeterminate/Positive Respiratory Specimens Added BAL reference for Rhodococcus	June 09, 2015	Dr. T. Mazzulli
Sputum Possible pathogens: Added *Neisseria meningitidis* Sputum Probable/possible listed in order as BAL	October 20, 2015	Dr. T. Mazzulli
P. 7, 12, 28, 38 added to resulting for b.cepacia "or specimen is from a patient with Cystic fibrosis"	November 30, 2015	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Annual Review Updated MSH logo in header	June 09, 2016	Dr. T. Mazzulli
Annual Review Updated Actino incubation time from 7 days to 10days	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.

Page Number / Item	Date of Revision	Edited by:
Updated reporting of mouth swab	January 21, 2021	Dorna Zareianjahromi
Added yeast not crypto comment to sputum section	February 26, 2021	Wayne Chiu
Minor formatting change	April 11, 2021	Jessica Bourke
Nomenclature update – cutibacterium	April 19, 2021	Wayne Chiu
Added BAL mold workup. If mold isolated and there is no fungal culture ordered, setup fungifluor	April 23, 2021	Wayne Chiu
Updated wording for possible resp pathogen. Added note regarding commensal flora in BAL sputum workup. minor formatting.	July 13, 2021	Wayne Chiu
Added note regarding screening of expectorated and induced sputum	May 5, 2022	Wayne Chiu
Removed confirmation of H. pylori ID by PHOL	July 12, 2022	Wayne Chiu
Updated wording for reporting of yeast (not neoformans/gattii) in sputum	November 1, 2022	Wayne Chiu

Page Number / Item	Date of Revision	Edited by:
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 expose the plate 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	November 10, 2023	Qin Liu
In the interpretation section of "SPUTUM (INCLUDING ENDOTRACHEAL TUBE AND TRACHEOSTOMY SPECIMENS; BRONCHOSCOPY ASPIRATES / WASHINGS", modified the interpretation to "Identify all Probable and Possible respiratory pathogens if there is a scant or light growth AND pure or obviously predominant."	May 21, 2024	Oliver Li
Minor formatting changes.	June 13, 2024	Oliver Li