RESPIRATORY TRACT CULTURE MANUAL

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Note: Lower respiratory tract specimens may be contaminated with organisms found in the upper respiratory tract (Commensal Flora – Respiratory Tract). Sputum samples are screened to assess the amount of oropharyngeal contamination before processing.

BRONCHOALVEOLAR LAVAGE (BAL), BRONCHOSCOPY ASPIRATES / WASHINGS - ROUTINE

I. Introduction

Bronchoalveolar lavage (BAL) specimens, including aspirates and washings 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 carinii* and fungal elements.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimens

See Specimen Processing Procedure QPCMI06003

Direct Examination: Prepare 3 smears for:

i) Gram stain
ii) Fungifluor stain
iii) Extra smear held in Mycology Section for special stains.

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
Inihibitory Mold Agar (IMA) *	O_2, 28°C x 4 weeks
Esculin Base Medium (EBM)*	O_2, 28°C x 4 weeks
Blood Egg Albumin Agar (BEAA)*	O_2, 28°C x 4 weeks

Media	**Incubation**	
If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, **add**:		
OF Base, Colistin, Bacitracin & Lactose Agar (OCBL)	O_2,	35°C x 5 days
Keep the BA, HI and MAC plates	CO_2,	35°C x 5 days
If Nocardia is requested, **add**:		
Sodium Pyruvate Agar (PYRA)	O_2,	35°C x 4 weeks

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

B. Interpretation of cultures:

Examine the plates after 24 and 48 hours incubation.

1. Identify any growth of **Probable** respiratory pathogens.
2. Identify any growth of **Possible** respiratory pathogens if predominant (i.e. amount of pathogen growth greater than that of commensal flora).
3. For yeast grown in culture on bacterial culture plates see Yeast Identification.
4. For filamentus fungus, seal the agar plate and send the culture to Mycology for identification.
5. If there is a question regarding the significance of an isolate, consult the charge technologist or microbiologist.

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

Possible respiratory pathogens:
Yeast not *Cryptococcus neoformans*
Group C and G streptococcus
Other gram negative bacilli (not listed above) of single morphological type

For cystic fibrosis patients:
Report any amount of *B. cepacia.* For *B. cepacia* and slow growing mucoid *P. aeruginosa*, identification and sensitivities can be referred to previous specimens processed within the

preceding 4 weeks.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting

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.
Fungifluor Stain:	Refer to Mycology Manual

Acid-fast stain (if **STAT** request): Refer to Reporting of Acid-fast smears, Appendix IV.

Culture:

Negative Report:	"Commensal Flora" (DO NOT quantitate) or "No growth" "No *B. cepacia* isolated" if *B. cepacia* culture is requested. "No *Nocardia* isolated" if *Nocardia* culture is requested.
Positive Report:	DO NOT quantitate. Report all significant isolates with appropriate sensitivities. Report "Commensal flora" if also present. "Filamentous fungus" "isolated" "identification to follow" (DO NOT quantitate).

Telephone all Group A streptococcus 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

ROUTINE LUNG TRANSPLANT BRONCHOSCOPY SPECIMEN

I. Introduction

Following a lung transplant, patients are followed routinely in the clinic and undergo regular bronchoscopies looking mainly for bacterial viral (particularly CMV) or fungal pathogens (particularly Aspergillus). These specimens will be processed similarly to other routine bronchoscopies except that Mycobacteria (TB) culture will not be performed unless specifically requested. As well, fungal cultures will be kept for only a maximum of 2 weeks before discarding if negative.

II. Specimen Collection and Transplant

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents/Materials/Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

Direct Examination: Prepare 3 smears for:

i) Gram stain
ii) Fungifluor stain
iii) Extra smear held in Mycology Section for special stains.

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
Inihibitory Mold Agar (IMA) *	O_2,	28°C x 2 weeks
Esculin Base Medium (EBM)*	O_2,	28°C x 2 weeks
Blood Egg Albumin Agar (BEAA)*	O_2,	28°C x 2 weeks
If *B. cepacia* is requested or specimen is from a patient with Cystic Fibrosis, **add**:		
OF Base, Colistin, Bacitracin & Lactose Agar (OCBL)	O_2,	35°C x 5 days
Keep the BA, HI and MAC plates	CO_2,	35°C x 5 days
If Nocardia is requested, **add**:		
Sodium Pyruvate Agar (PYRA)	O_2,	35°C x 4 weeks

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

B. Interpretation of cultures:

Examine the plates after 24 and 48 hours incubation.

1. Identify any growth of **Probable** respiratory pathogens.
2. Identify any growth of **Possible** respiratory pathogens if predominant (i.e. amount of pathogen growth greater than that of commensal flora).
3. For yeast grown in culture on bacterial culture plates see Yeast Identification.
4. For filamentus fungus, seal the agar plate and send the culture to Mycology for identification.
5. If there is a question regarding the significance of an isolate, consult the charge technologist or microbiologist.

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

Possible respiratory pathogens:
Yeast not *Cryptococcus neoformans*
Group C and G streptococcus
Other gram negative bacilli (not listed above) of single morphological type

For cystic fibrosis patients:

Report **any** amount of *B. cepacia.* For *B. cepacia* and slow growing mucoid *P. aeruginosa*, identification and sensitivities can be referred to previous specimens processed within the preceding 4 weeks.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting

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.
Fungifluor Stain:	Refer to Mycology Manual

Acid-fast stain (if **STAT** request): Refer to Reporting of Acid-fast smears, Appendix IV.

Culture:

Negative Report:	"Commensal Flora" (DO NOT quantitate) or "No growth" "No *B. cepacia* isolated" if *B. cepacia* culture is requested. "No *Nocardia* isolated" if *Nocardia* culture is requested.
Positive Report:	DO NOT quantitate. Report all significant isolates with appropriate sensitivities. Report "Commensal flora" if also present. "Filamentous fungus" "isolated" "identification to follow" (DO NOT quantitate).

Telephone all Group A streptococcus 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.

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 isolated.

Positive Report: CMV isolated.

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.

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 nonpathogens. These studies have demonstrated that colony counts of >1 x 10^6/L (>100/ml) i.e. growing more than 10 colonies on a plate streaked with a 0.01 ml 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**	
Blood Agar (BA)	CO_2,	35°C x 48 hours
Haemophilias Isolation Medium (HI)	CO_2,	35°C x 48 hours
MacConkey Agar (MAC)	CO_2,	35°C x 48 hours

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

Of Base, Colistin, Bacitracin & Lactose Agar (OCBL)	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:

Examine BA, HI and MAC after 24 and 48 hours incubation. Examine OCBL daily for 5 days. Identify with quantitation growth of any potential pathogens, including yeast.

V. Reporting

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

If a dry brush is received, report in the "Test Comment" field of the LIS as "Dry brush received".

Issue a preliminary report after 18-24 hours incubation.

Negative Report: "No growth"
"No *B. cepacia* isolated" if *B. cepacia* culture is requested.

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

Report all isolates quantitatively: $<1 \times 10^6$/L if <10 colonies
$>1 \times 10^6$/L if ≥10 colonies

All potential pathogens in any number with appropriate susceptibilities.

">1×10^6/L or <1×10^6/L Commensal flora, including (list all organisms)."

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 (PHL) 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 sensitivities. Report "Commensal flora" if also present.

Telephone all positive Group A streptococcus results 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 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

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

   This specimen usually consists of a wedge of lung tissue obtained during surgery and submitted in a clean, sterile container.

2. Transthoracic needle biopsy

   These 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

   These 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: Prepare 3 smears for:
   i) Gram stain
   ii) Fungifluor stain
   iii) Extra smear held in Mycology Section for special stains.

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 48 hours
Inhibitory Mold Agar (IMA) *	O_2, 28°C x 4 weeks
Esculin Base Medium (EBM) *	O_2, 28°C x 4 weeks
Blood Egg Albumin Agar (BEAA) *	O_2, 28°C x 4 weeks
If *B. cepacia* is requested or the specimen is from a patient with Cystic Fibrosis, **add**:	
OF Base, Colistin, Bacitracin & Lactose Agar (OCBL)	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**:	
Sodium Pyruvate Agar (PYRA)	O_2, 35°C x 4 weeks

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

B. Interpretation of culture:

Examine aerobic plates after 24 and 48 hours incubation and anaerobic plates after 48 hours incubation for any growth and identify all isolates including yeast. If no growth on aerobic and anaerobic plates, but organisms resembling anaerobic organisms is seen on gram stain, reincubate the BRUC and THIO for an additional 48 hours. Examine the OCBL plate daily for 5 days. If yeast grown, perform Germ Tube test (Refer to Appendix VII) and identify at the Respiratory Tract Culture Bench (i.e. **DO NOT** forward bacterial culture plates to Mycology section for identification). Refer to Appendix VI for yeast identification.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

VI. Reporting

Direct Examination:

Gram Stain:	Report presence or absence of pus cells. Report presence or absence of organisms. DO NOT quantitate.
Fungifluor Stain:	Refer to Mycology Manual.

Culture:

Negative Report:	"No growth." "No *B. cepacia* isolated" if *B. cepacia* culture is requested. "No *Nocardia* isolated" if *Norcardia* culture is requested.
Positive Report:	Report all isolates with appropriate sensitivities. 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.

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).

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

.

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination: Gram stain:

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

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

b) Culture: Not indicated.

V. Reporting

Negative Report:	"No fungal element seen on direct examination. Fungal culture not done"
	"No organisms suggestive of Vincent's angina seen".
Positive Report:	"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 Sensitivities

I. Introduction

These specimens are submitted to identify nasal carriers of *Staphylococcus aureus. Neisseria meningitidis* will be screened for only if requested.

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 *N. meningitidis* is requested, **add**:	
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours

B. Interpretation of cultures:

1. Examine the CNA plate after 24 and 48 hours incubation and the ML 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*" with appropriate sensitivities. "Methicillin Resistant *Staphylococcus aureus*" with appropriate sensitivities. "*Neisseria meningitidis*"

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.

NASAL SWABS FOR METHICILLIN RESISTANT *S. aureus* (MRSA)

I. Introduction

These specimens are submitted to identify nasal carriers of **Methicillin Resistant** *S. aureus* (MRSA).

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**
Mannitol Salt Agar with Oxacillin (MSAOX)	O_2, 35°Cx 48 hours

B. Interpretation of cultures:

1. Examine the MSAOX after 24 and 48 hours incubation.
2. Sub-culture yellow colonies of each phenotypic type from the MSAOX plate to BA prior to identification.

NB: Erroneous slide agglutination results may occur if using colonies directly from the MSAOX plate.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

V. Reporting

Negative report: "No Methicillin Resistant *Staphylococcus aureus* isolated"

Positive report: "Methicillin Resistant *Staphylococcus aureus*" with appropriate sensitivities.

Notify the Infection Control Practitioner of all **new** MRSA isolates and telephone all positive MRSA 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. 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 a clean, sterile container. 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 (PHL) 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

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 – Quantitate the presence of pus cells and organisms.

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 requested or suggested on Gram stain, **add**:

Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 7 days
Kanamycin / Vancomycin Agar (KV)	AnO_2, 35°C x 7 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 7 days.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

V. Reporting

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

Culture:

Negative Report:	"Commensal flora" or "No growth". "No Actinomyces isolated."
Positive Report:	Quantitate and report all significant isolates with appropriate susceptibilities. Report "Commensal flora" if also present.

Telephone all positive Group A streptococcus results 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.

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 - Examine for and quantitate the presence of pus cells and organisms.
   ii) Fungifluor - Forward to Mycology Section for staining and interpretation.

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

Media	**Incubation**
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**:	
Kanamycin Vancomycin Agar (KV)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 48 hours

*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 KV, BRUC and THIO after 48 hours incubation.

C. Susceptibility testing:

Refer to Susceptibility Testing Manual.

VII. Reporting

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

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 sensitivities. Report "Commensal flora" if also present.

Telephone all positive Group A streptococcus isolates 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. 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

I. Introduction

Pneumonia may be divided into four broad categories including: 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 ± a mixture of oral aerobes and anaerobes. Along with the common organisms noted above, unusual agents such as pneumocystis, dimorphic fungi, cryptococcus may be found in immunocompromised patients. Acute bronchitis may be viral or occasionally bacterial.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimens:

See Specimen Processing Procedure QPCMI06003

a) Direct Examination:

i) Gram Stain

Sputum is always contaminated to some degree with oropharyngeal organisms.

Consequently, a screening procedure for routine culture is required to exclude grossly contaminated specimens or saliva.

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

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*	Discard culture plates without examining.
< 25 cells/lpf	Examine and report, with quantitation, routine Gram stain results. Continue incubation of culture plates.

*lpf = low power field

NB: If yeast is predominant organism seen, then report with quantitation.

If yeast is seen mixed with other organisms and is not the predominant organism, then report as Commensal flora without specifically commenting on the presence of yeast.

ii) Approved requests for **STAT** acid fast stain: Direct smear from an unconcentrated specimen.

iii) Fungus requests: Prepare smear for Fungifluor and forward to Mycology Section for staining and interpretation.

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**:	
OF base, colistin, bacitracin & lactose Agar (OCBL)	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**:	
Sodium Pyruvate Agar (PYRA)	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
Blood Egg Albumin Agar (BEAA)*	O_2, 28°C x 4 weeks

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

B. Interpretation of Cultures:

Routine 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* and filamentous fungus (refer to 4. and 5.)
2. Identify all **Possible** respiratory pathogens if there is a moderate to heavy growth (≥2+) growth **AND** if predominant (i.e. amount of pathogen growth greater than that of commensal flora).
3. Identify all **Probable** and **Possible** respiratory pathogens if there is a light growth (1+) **AND** predominant (i.e. amount of pathogen growth greater than that of commensal flora) **AND** if any amount of pus cells are seen in gram stain.
4. For yeast grown in culture on bacterial culture plates see Yeast 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 charge technologist or microbiologist.

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

Possible respiratory pathogens:
Yeast not *Cryptococcus neoformans*
Group C and G streptococcus
Other gram negative bacilli (not listed above) of single morphological type

*** <u>For cystic fibrosis patients</u>:**

Report <u>any</u> amount of *B. cepacia.* For *B. cepacia* and slow growing mucoid *P. aeruginosa*, identification and sensitivities can be referred to previous specimens processed within the preceding 4 weeks.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting

Gram Stain:
Rejected Sputum Report:
Greater than 25 squamous epithelial cells per low power field

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

Acid-fast stain (if **STAT** request): Refer to Reporting of Acid-fast smears, Appendix IV.

Culture:

Rejected Sputum Report:	"Specimen unsuitable for processing due to oropharyngeal contamination"
Negative Report:	"Commensal flora" (DO NOT quantitate) or "No growth". "No *B. cepacia* isolated" if *B. cepacia* culture is requested.
Positive Report:	Quantitate and report significant isolates with appropriate sensitivities. Report with quantitation "Commensal flora" if also present. "Filamentous fungus" "isolated" "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. 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 (PHL) 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* requested, **add**:	
Martin-Lewis Agar (ML)	CO_2, 35°C x 72 hours
If *Corynebacterium diphtheriae* requested, forward swab to Public Health Laboratory (PHL) 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:

a) Examine the BA plate after 18-24 hours incubation and identify all morphologically distinct beta hemolytic colonies by performing:

i) Gram stain: Gram positive cocci in pairs and chains
ii) Strep grouping: Identify only group A

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

c) Examine the ML plate after 48 and 72 hours incubation.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting

a) Gram stains: "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"
"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 to ward / ordering physician. (For MSH Family Medicine Patients, call the Family Medicine Resident on-call through locating when reporting positives on weekends).

For Centenary Health Centre (CHC) in-patients, inform CHC infection control of all positive GC isolates.

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.

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:

i) Use one half of the tissue to prepare a smear for Gram stain and to directly inoculate a urea slant.

ii) Incubate the urea slant at 35°C for 1 – 4 hours.

iii) Macerate the remaining tissue for culture:

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:

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

b) Examine the plates after 3, 5 and 7 days incubation. Colonies of *H. pylori* are grey, translucent and small (0.5 to 1.0 mm in diameter). See Identification of *H. pylori*.

C. Susceptibility Testing:

Not required.

V. Reporting

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

Culture:

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

Final Report:
Negative Report: "No *Helicobacter pylori* isolated"

Positive Report: "*Helicobacter pylori* isolated"

VI. References

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.

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: Gram Stain - Quantitate the presence and absence of pus cells, squamous epithelial cells, and organisms.

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.
Any growth of *S. aureus*, beta-haemolytic streptococci group A, B, C and G, *H. influenzae* and *Pseudomonas aeruginosa* is significant. A pure growth of other gram-negative bacilli is also significant. Full identification is required for all significant organisms.
Mixed cultures and/or growth of ≤1+ non-significant organisms do not require workup (identify by gram stain and morphology).

C. Susceptibility Testing:

Neonates - Refer to Susceptibility Testing Manual for significant organisms.
Stillborn - not required

V. Reporting

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

Culture:

Negative Report: "No growth"
"(*Quantitation*) mixed growth of *list organisms*…"

Positive Report: Quantitate all significant isolates with appropriate sensitivities.

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.

Appendix I

Commensal Flora – Respiratory Tract

Type	**Organism**
Aerobic bacteria	*Streptococcus pyogenes* (and other hemolytic 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, Propionibacterium
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.