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.