# Aim

To describe the processing of clinical specimens collected for investigation of potential lower respiratory tract infections

# Principle

Investigation of the bacterial causes of lower respiratory tract infection (i.e. pneumonia) is hampered by access to the site of infection. Sputum is the standard specimen but is often of poor quality as a result of collection technique. Sputum specimens are often contaminated with upper respiratory tract and mouth flora. Additionally young children are unable to reliably expectorate (cough up) sputum. In intubated patients, access to the lower respiratory tract is gained via the endotracheal tube (ETT): a variety of sampling strategies exist, of which the simplest is simple aspiration of secretions (the endotracheal aspirate, ETT-A). Unfortunately, ETT-A specimens are also frequently contaminated by commensals meaning interpretation of significance of cultured isolates is difficult.

Lower respiratory tract infections may be caused by a variety of organisms depending on patient factors and whether the infection is acquired in the community (community-acquired pneumonia) or in hospital (hospital-acquired pneumonia):

* Community-acquired infection: *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Klebsiella pneumoniae,* atypical organisms (*Mycoplasma pneumoniae, Chlamydia pneumoniae,* etc.*)*, *Legionella pneumophila,* viruses, others (fungal and parasitic pathogens in certain hosts).
* Hospital acquired infection: *Pseudomonas aeruginosa, Acinetobacter* spp., coliforms, *S. aureus* (incl. MRSA).
* Lung abscess: *Burkholderia pseudomallei* (see SOP MIC-013)*, S. aureus, K. pneumoniae,* oral streptococci and anaerobes.
* *Mycobacterium tuberculosis* and other non-TB mycobacteria (NTM) infections (see SOP MIC-016).

# Method

## Specimen collection

Sputum specimens should be collected into wide mouthed sterile leak proof containers: younger children cannot expectorate sputum and in these cases sputum induction using nebulised saline may be used.

Endotracheal aspirates should be collected using aseptic technique into sterile leak proof containers.

Swabs are not an appropriate specimen and should not be processed.

## Specimen transport and storage

Specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if immediate processing is not possible. If specimens are not processed on the same day as they are collected, interpretation of results should be made with care, since *S. pneumoniae* and *H. influenzae* may be rendered non-viable and GNB overgrowth may occur.

## Specimen processing

Specimens should be processed in the Class II biosafety cabinet.

### Reception

Log the specimen in the appropriate specimen book and assign a specimen number.

### Specimen preparation

For ETT aspirates, flush the suction tubing through with a small amount (0.5 – 1ml) of sterile saline using a sterile syringe to release the specimen into a sterile container.

All subsequent steps are identical for both sputum and ETT aspirates: purulent portions of specimen should be selected for microscopy and culture.

Record the macroscopic appearance of the specimen: salivary; purulent; blood-stained.

### Microscopic examination

Prepare a thin smear of ETT aspirate or sputum on a clean labeled microcopy slide for Gram stain. Allow the smear to air dry and heat-fix on the electric hotplate before staining.

For TB processing, refer to SOP MIC-016.

If parasite infection (e.g. *Paragonimus* spp.) is suspected, prepare a wet prep of sputum with a drop of sterile saline, cover with a coverslip and examine for ova under low power magnification (x10).

### Culture

Inoculate and incubate culture media as indicated in Table 1.

**Table 1.** Culture media, conditions, and target organisms

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Clinical / Gram strain** | **Standard media** | **Incubation** | **Cultures read** | **Target organism(s)** |
| **Temp (°C)** | **Atmosphere** | **Time** |
| All | Blood agar + optochin disc | 35 – 37 | 5 – 10% CO2 | 40 - 48h | Daily | *H. influenzae, M. catarrhalis, S. aureus, S. pneumoniae,* Beta-haemolytic streptococciOther organisms in pure growth may be significant |
| Chocolate agar | 35 – 37 | 5 – 10% CO2 | 40 - 48h | Daily |
| If ICU patient or suspected hospital-acquired infection | MacConkey agar | 35 – 37 | Air | 40 - 48h | Daily | EnterobacteriaceaePseudomonads |
| If yeast or fungal elements seen or suspected fungal infection | Sabouraud agar | 35 – 37 | Air | 40 - 48h | Daily | Fungi |
| If ?melioid | ASHSB | 35 – 37 | Air | 48h (Sub SB to ASH at 48h) | Daily | *B. pseudomallei* |

# Interpretation

Record the semi-quantitative growth of all colonies (i.e. +/- to ++++).

## Minimum level of identification in the laboratory

In general significant isolates should be identified as fully as possible: potentially significant organisms are summarised in SOP MID-004.

Significant isolates:

* *S. pneumoniae*
* *S. aureus*
* *H. influenzae*
* *M. catarrhalis*
* Group A, C, and G beta-haemolytic streptococci
* *B. pseudomallei*
* Yeasts should be reported at the “yeasts” level
* Moulds should be identified to species level if possible (lactophenol blue sellotape prep)
* Generally (especially in patients without chronic lung disease), coliforms, pseudomonads, and other Gram negative non-fermenters (e.g. *Acinetobacter* spp., *B. cepacia,* and *Stenotrophomonas maltophilia*) are only possibly significant when present in moderate to large numbers, when in pure growth or when they are the predominant organism in a mixed culture.

Heavily mixed cultures should be interpreted with caution (interpret in conjunction with Gram stain result): the presence of many epithelial cells implies upper respiratory tract contamination.

## Antimicrobial susceptibility testing

All significant isolates should have antimicrobial susceptibilities determined according to SOP MIC-001.

## Reporting

If the sputum was salivary, comment “Poor quality specimen/salivary specimen received. Please repeat if clinically indicated”.

Gram stain results: Epithelial cells, WBC and organisms detected.

ZN stain results: presence (and quantity) or absence of AFB (see SOP MIC-016).

Wet prep results (if done): presence or absence of named organisms (e.g. paragonimus ova not seen).

Culture results: presence of significant isolates (e.g. *S. pneumoniae*); no significant growth; absence of growth.

# Quality assurance

Media and identification tests should be quality controlled according to the relevant SOP.

# Limitations

Prior antimicrobial use may result in negative cultures. Absence of specimen homogenisation may affect culture results.

# References

1. Health Protection Agency, UK SOP B57: Investigation of Bronchoalveolar Lavage, Sputum, and Associated Specimens (Issue 2.4; August 2012).
2. Hawkey, P and Lewis, D. Medical Bacteriology. 2nd Edition (2004). Oxford University Press.
3. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edition Update (2006). Cambridge University Press.

# Synopsis / Bench aid



# Risk assessment

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| **COSHH risk assessment - University of Oxford COSHH Assessment Form** |
| **Description of procedure**Culture of sputum and endotracheal aspirates | **Substances used**Variable, depending on organism cultured (may include Gram stain reagents; 3% hydrogen peroxide (catalase test); N,N,N',N'-tetramethyl-1,4-phenylenediamine (oxidase test); sodium deoxycholate (bile solubility test); bioMerieux API reagents) |
| **Quantities of chemicals used**Small | **Frequency of SOP use**Daily |
| **Hazards identified**1. Autoclaved liquid2. Potentially infectious material in sample 3. Potentially pathogenic bacteria4. Chemical exposure form bacterial identification tests | **Could a less hazardous substance be used instead?** No |
| **What measures have you taken to control risk?** 1. Training in good laboratory practices (GLP)2. Appropriate PPE (lab coat, gloves, eye protection)3. Use of biosafety cabinet for reading of plates / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) |
| **Checks on control measures**Observation and supervision by senior staff |
| **Is health surveillance required?**No | **Training requirements:**GLP |
| **Emergency procedures**:1. Report all incidents to Safety Adviser2. Use eyewash for splashes3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:1. Sharps discarded into appropriate rigid containers for incineration2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration3. Chemical waste disposed of according to manufacturer’s instructions |