National Department of Health

Sputum Culture

G_90_SOP_17_A

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1. Purpose & Scope

This SOP describes the procedure for culture using bronchial secretions and sputum specimens to isolate common bacterial pathogens causing lower respiratory tract infection.

Pneumonia is caused by a number of infectious agents, including viruses, bacteria and fungi. Many agents of pneumonia are difficult to isolate in routine laboratories *Legionella pneumophilia, Mycoplasma pneumoniae, Chlamydia pneumoniae,* Respiratory Syncytial Virus and Pneumocystis jiroveci are outside the scope of this Standard Operating Procedure (SOP).

2. Principle & Clinical application

For Community acquired pneumonia (CAP), *Streptococcus pneumoniae* is a major cause of pneumonia. Other important bacterial causes include *Klebsiella pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Burkholderia cepacia complex and Burkholderia pseudomallei* (in some locations)**.**

Nosocomial pneumonia may be caused by a wide range of bacteria including *Pseudomonas aeruginosa, Acinetobacter baumannii complex, Staphylococus aureus,* Enterobacterales species and less often anaerobes.

In immunocompromised people, a wide range of organisms may cause lower respiratory tract infection including viral and fungal pathogens.

Normal flora often seen in respiratory cultures includes:

- Viridans group Streptococci
- *Neisseria spp*.
- *H.parainfluenzae*
- *Corynebacterium spp*. In low numbers
- Low numbers of enteric or environmental flora (these are commonly moderate to heavy in patients who are colonised)
- Yeast
- *Bacillus spp*.
- Coagulase negative *Staphylococcus spp.*
- *Pseudomonas spp.* In low numbers
- *Streptococcus milleri* group
- Fungi other than yeasts – The role of these fungi may be difficult to determine from a single specimen and may represent carriage or transient flora only. Repeat specimens are recommended to determine the fungus' role.

3. Responsibilities

Role	**Responsibility**
Lab bench scientist/ technician	Specimen reception, registration and processing. Identify, interpret and document AMR phenotypes that require storage/ referral
On duty senior scientist	Checking of culture and AST results, cross correlating with request and microscopy Addition of necessary interpretative comments on the LIMS prior to verification (validation) of the report Identify and document AMR phenotypes that require storage/ referral Supervision and sign off of bench scientist/technician competency and media/reagent QC compliance/results

4. Specimens

Specimen	**Indication**
Sputum	Productive cough AND suspicion of pneumonia
Endotracheal aspirate	Suspected ventilator-associated pneumonia
Bronchial washing (blind)	Suspected ventilator-associated pneumonia (e.g. new infiltrate, increased sputum with purulence, fever, WCC > 11 x 10^9/L)

Sputum: Of all specimen types received for microbiological culture, sputum has the greatest potential to produce misleading results. Specimens are commonly salivary and contain normal throat and mouth flora and food particles, and do not represent the lower respiratory tract. Organisms which can cause pneumonia can also be part of the normal throat and mouth flora.

Endotracheal aspirate (ETA): Tracheal aspirates are specimens of sputum collected from patients (usually from ICU) who are intubated or who have a tracheostomy. These specimens are less prone to heavy contamination by oral flora. Enteric organisms are an important cause of nosocomial pneumonia and identification of a predominant organism and/or Pseudomonas should be performed for ICU patients.

Bronchial washings: Secretions aspirated back through a bronchoscope or blindly via a catheter inserted into a ventilated patient's endotracheal tube after injecting saline into a major airway.

Cystic fibrosis (CF) patient specimens (rarely received in PNG): Cystic Fibrosis is a life-limiting genetic disorder which primarily affects the lungs and digestive system because of a malfunction in the exocrine system that's responsible for producing saliva, sweat, tears and mucus. People with CF develop an abnormal amount of excessively thick and sticky mucus within the lungs, airways and the digestive system. This causes impairment of the digestive functions of the pancreas and traps bacteria in the lungs resulting in recurrent infections, leading to irreversible damage. Lungs of CF patients are often colonized or infected in infancy and early childhood with organisms, such as *Staphylococcus aureus* and *Haemophilus influenzae*, that may damage the epithelial surfaces, leading to increased attachment of, and eventual replacement by, *P. aeruginosa*. Chronic infection with *P. aeruginosa* is the main proven perpetrator of lung function decline and ultimate mortality in CF patients.

Specimen is collected into a sterile container with a screw cap.

Refrigerated or placed on ice before transport to laboratory if delays of > 1 hr after collection is anticipated.

Refrigerate the specimen if delay of > 30 minutes before processing is anticipated.

5. Safety

All sputum specimen are processed in a Class II Biosafety cabinet. Wear gloves during handling of primary specimens.

Heat fix slides by preference on hot plate to sterilize same.

For other safety aspects, please review this document G_10_Info_3_A Laboratory Biosafety.

6. Equipment, Materials and Reagents

- Blood Agar Plate (BA)
- Chocolate Agar (CA)
- Mueller Hinton (MH) and Mueller Hinton with 5% human blood (MHF)
- Oxygen (O_2) Incubator and Carbon dioxide (CO_2) incubator or candle jar
- Glass slides
- Gram stain reagents
- Optochin disc
- XV discs
- Sterile loops
- Antibiotic discs
- Saline
- Swab sticks
- Timer

7. Procedure

7.1 Check patient name, MRN and DOB/age matches with specimen and laboratory request form

7.2 Register the specimen on the LIMS and generate labels for the sputum sample container and request form

7.3 Choose the area of sputum which is purulent or blood stained to make a smear and prepare a sputum smear on a glass slide for a direct Gram stain procedure (G_90_T_1)

7.4 Air dry the smear completely on a slide rack or flat surface and heat fix by placing the slide with the smear facing upward over a heating block at 70ºC for approximately one minute .

7.5 Perform Gram stain and scan the slide on low power (10x objective)

7.6 Record the appearance as below and indicate whether the specimen, macroscopically is bloodstained.

Gram stain- 10x objective	**Appearance**
3+ Pus cells >25/LPF	Purulent
2+ Pus cells 10-25/LPF	Mucopurulent
1+ Pus cells >10/LPF	Mucoid
Reject the specimen, if Epithelial cells > 10 per LPF AND Pus cells <25/LPF	Salivary Add comment 1 below. NB. If Doctor's request has '*Burkholderia pseudomallei*' or 'melioidosis culture' do NOT reject the specimen

7.7 Using a loop or sterile swab pick up purulent and/or blood-stained sections of the sputum and inoculate on BA and CA.

7.8 Carefully streak out to use up the full agar surface.

7.9 Place optochin disc on BA plate at the end of first set of streak lines.

7.10 Incubate plates in CO_2 at 35-37ºC for 48hrs.

7.11 Examine plates at 18-24hrs (day 1) and 40-48 hrs (day 2) for bacterial growth[1]

Note that pneumonia caused by common pathogenic organisms is characterised by very high numbers of the causative organism in the specimen, ≥10^6/ml. Therefore culture plates streaked out in the usual way will yield at least a **moderate to heavy growth of the pathogen**. (This does not always apply if the specimen has been diluted or the organism is atypical, e.g. *Nocardia spp.,* or there has been recent antibiotic therapy).

7.12 Describe the colonial morphology of any predominant organism (s) including quantity . Note that in sputum cultures, normal upper respiratory tract flora is expected to be present.

7.13 Examine the BA for a zone of inhibition of > 14 mm around the optochin disc, indicating *Streptococcus pneumoniae*. Note that optochin resistant strains are described and if the colony morphology/Gram stain is highly suggestive, a second method of identification (e.g. MALDI-TOF[2]) is required to confirm identification.

[1] Bronchial washing (from ICU) culture plates should be read daily for 5 days. A report is sent out at 2 days and the report is supplemented if significant growth occurs after 2 days. *Nocardia* plates (if requested) are incubated and read daily for 10 days.

[2] See for example https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(19)30626-3/pdf

7.14 Identify and perform susceptibility according to criteria in the table below under Section 7.

8. Results Recording

8.1 Record all results onto paper worksheet with registered lab number and patient identification.

8.2 Final results recorded into LIMS in accord with the relevant LIMS SOP when developed.

Direct Gram stain	Organisms	Culture[3]	Report	Comment
Any amount of Polys/Orgs	*Cryptococcus neoformans* Filamentous fungi- e.g. *Aspergillus ; must have several colonies*	1+ to 3+	ID	Report Comment 2 below for Aspergillus
Polys/Orgs any	*Strep. pneumoniae* *Haemophilus influenzae*	2+ to 3+	ID+AST	Do not report light (1+) growth with URTF
Polys/Orgs scanty (1+) to profuse (3+) per high power field	*Moraxella catarrhalis* *Neisseria meningitidis*	2+ to 3+	ID+AST	Report if present in significant amounts, even if not predominant
Polys/Orgs 1+ to 3+ ICU patients only	*Pseudomonas aeruginosa* *Up to two different coliforms* *Acinetobacter sp.*	1+ to 3+	ID+AST	For ICU pseudomonas, add comment 7. For ICU Acinetobacter, add comment 8. Non-ICU report with comment 4 below
Polys/Orgs any	*Stenotrophomonas maltophilia*	1+ to 3+	ID	Add comment 5 below
Polys/Orgs 1+ to 3+	*Staph. aureus* Beta haem. Strep B, C or G Gram negative rod (especially *Klebsiella pneumoniae*)	2+ to 3+	ID+AST	Report if **predominant** organism in the culture *Staph. aureus,* add comment 6

[3] 1+ indicates growth in the primary inoculum area of the plate. 2+ indicates colonies into the second and third quarters of the streaked plate; 3+ indicates growth across all quarters of the plate.

Direct Gram stain	Organisms	Culture[3]	Report	Comment
	Acinetobacter sp. Fastidious GNB *e.g.* *Pasteurella* species			
Polys/Orgs any Non ICU patient	>1 type of coliform	1+ to 3+	Mixed coliforms	If ICU patient, and one predominant organism, perform ID+AST; discuss with supervisor if unsure*
Polys/Orgs any	*Candida* species	1+ to 3+	*Candida* species	Add comment 3

* For those labs with a mentored WHATSAPP group, that is also an option for advice.

9. Interpretation

For culture interpretation compare Gram stain, polymorph number and bacterial morphotypes with culture:

A high number of polymorphs in the direct Gram stain may indicate infection or inflammation which can be viral or bacterial.

- If organisms grown on culture are inconsistent with the Gram stain, the correctness of the original direct Gram stain result should be checked by reviewing the slide.
- An organism is more likely a pathogen if it is pure or predominant growth and polymorphs are detected in the Gram stain
- Absence of culture growth despite a purulent sample may indicate viral infection, a patient already on antibiotics or the presence of a fastidious organism or anaerobe that has not been able to be cultured

Comments available for addition to reports within the LIMS

Criteria for addition	Comment text
> 10 squamous epithelial cells per hpf	1. This sputum demonstrated abundant (>10) squamous cells indicating likely contamination with oropharyngeal flora. Culture is not indicated. Please submit another sample of better quality if culture still required.
Aspergillus isolation	2. Repeated detection of Aspergillus species in sputum has significance in the setting of sustained neutropenia, some

Criteria for addition	Comment text
	other immune defects and pre-existing lung disease (e.g. Allergic Bronchopulmonary Aspergillosis condition).
Candida species	3. This isolate represents oropharyngeal and/or respiratory tract colonisation. Treatment not indicated.
Non-ICU patients as noted in section 7 'Result Reporting' above	4. This organism most probably represents respiratory tract colonisation and antibiotic treatment is not usually indicated.
Stenotrophomonas species	5. This organism most probably represents respiratory tract colonisation and antibiotic treatment is not usually indicated.
Staph. aureus predominant	6. Isolation of *Staph. aureus* from respiratory samples frequently represents upper RT colonisation and treatment rarely indicated. Pneumonia due to S. aureus is associated with clinically evident lung abscess, parapneumonic effusion or empyaema; blood cultures are frequently positive.
ICU patient with *Pseudomonas aeruginosa* isolated AND ciprofloxacin susceptible	7. If treatment indicated, use high ciprofloxacin dose 750mg bd oral for adult for maximum 5-7 days or cefazidime 2g 8-hourly; reduce doses in the setting of renal impairment. Pseudomonas aeruginosa is intrinsically resistant to amoxicillin-clavulanate, ceftriaxone, sulfamethoxazole/trimethoprim, nitrofurantoin and chloramphenicol.
ICU patient with *Acinetobacter species* isolated	8. Acinetobacter species are intrinsically resistant to amoxicillin-clavulanate, ceftriaxone and chloramphenicol.

10. Quality Control

10.1 Media QC is done and recorded for each batch of relevant media

10.2 AST QC is to be done weekly

10.3 Bench reagent QC as per SOP

11. Related documents

Antibiotic disc susceptibility testing	G_90_SOP_6
Setup of disc antibiotic susceptibility tests	G_90_J_1

Bench training

In addition to reading this SOP, staff/ students are recommended to view these resources:

- Short (18 minutes) video masterclass on sputum bench reading- https://idmic.net/2019/07/08/video-masterclass-respiratory-plate-reading/
- 2022 Recorded Presentation (50 minutes) by Tessa Oakley on Respiratory sample analysis – see https://idmic.net/2022/07/04/respiratory-specimens-approach-to-analysis-and-interpretation/

12. References

- DMDP Sputum Culture SOP number 025 version 4 Effective 01/01/13
- PMGH Work Instruction 014 Chris Ashhurst-Smith Effective 18/02/13
- Sputum and Respiratory Lecture Tessa Oakley March 2022