Ministry of Health

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Document Author	Dr Mahmood AL Subhi
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Document Reviewer	Microbiology documents development team
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Validated by		**Approved by**	
Name	Dr. Muna Habib	Name	Dr.Badryah Al Rashidi
Designation	Director Department Development & Conterol (DGQAC)	Designation	Director General of Primary Health Care
Signature		Signature	
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Member name	**Institution**	**Designation**
Dr.Mahmoud Al Subhi	Rustaq Hospital	Team Leader Consultant medical microbiologist
Ms. Zainab Al Hadhrami	Directorate General of Specialized Medical Care	Team Coordinator Senior technologist specialist A
Ms. Saleh Al Shukairi	Ibra Hospital	Senior technologist specialist A
Dr. Hanaa Al Auraimi	Royal Police of Oman Hospital	Consultant medical microbiologist
Dr. Nawal AL Kindi	Khoula Hospital	Consultant medical microbiologist
Dr. Al Warith Al Kharusi	Nizwa Hospital	Consultant medical microbiologist
Dr.Abdulrahman Al Mahrouqi	Ibri Hospital	Specialist microbiologist pathologist
Dr. Nada Al Tamimi	Al Massara Hospital	Consultant medical microbiologist
Dr. Wafaa Al Tamtami	Armed Forces Hospital	Specialist senior microbiologist

Contributors:

- Ms. Hanaa Al Shaqsii, Rustaque Hospital, specialist

Acronyms:

BA	Blood agar
CA	Chocolate agar
CF	Cystic fibrosis
MAC	MaConkey
ET	Endotracheal
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
ICU	Intensive care unit
SOP	Standard operating procedure
WBC	White Blood cells
LPF	Low power field
HPF	High power field
VTM	Viral transport media
MOH	Ministry of health

1. **Purpose**

   This document describes the procedure for culture and isolation of organisms known to cause respiratory infection from sputum and Eendotracheal (ET) samples. This document does not include nasopharyngeal, throat, viral swabs and molecular testing, and samples from cystic fibrosis patients.

2. **Scope**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions**

   3.1 Oropharyngeal flora: Bacteria and other microorganisms that normally inhabit the oral cavity

   3.2 Tracheal Aspirate: samples collected via endotracheal tube.

   3.3 Prevalence: In epidemiology, prevalence is the proportion of a particular population found to be affected by a medical condition at a specific time

   3.4 Alveoli: Tiny air sacs at the end of the bronchioles

   3.5 Aspiration of colonizing flora: Inhalation of orppharyngeal flora.

   3.6 leak-proof sterile container: a container or enclosure that is constructed in such a manner that it will not allow its contents to spill out without being opened and physically discharging the contents

4. **Procedure**

   4.1. Clinical background:

   - Sputum and ET samples always contaminated to some degree with oropharyngeal flora. Different flora may be seen in patients with underlying conditions.
   - Although the normal aerobic oropharyngeal flora consists primarily of gram-positive organisms, the prevalence of gram-negative bacilli increases dramatically in acutely ill patients receiving antimicrobial therapy and hospitalized for several days, in chronic alcoholics, in diabetics, and in the institutionalized elderly population.
   - Aspiration of colonizing flora into the alveoli is the most common mechanism initiating a pneumonic infection. Therefore, Organisms that causes pneumonia are often organisms that colonize the upper respiratory tract.

- Inhalation of aerosols is a second, less frequent, mechanism for microorganism access to the lower respiratory tract.
- Droplet and airborne organisms' important mode of transmission of pneumonia.
- In general, pneumonia classified as Community Acquired or Hospital Acquired. Hospital Acquired can be Ventilator Associated.

4.2. Principle:

Recovery and recognition of organisms responsible for pneumonia depends on:

- The quality and adequacy of the lower respiratory tract specimen.
- Collection time: preferably early morning sputum culture
- Avoidance of contamination by upper respiratory tract flora during collection of the sample.
- Current usage of antimicrobial treatment.
- It is difficult to differentiate between contamination and significant growth, microscopy screening for the quality of sample is useful tool to decide in such situation.
- The ratio of polymorph to epithelial cell indicates the quality of sputum and ET sample. Therefore, it paramount to do microscopy screening.

4.3. Pre – analytical stage:

4.3.1. Sample:

Sputum and ET specimen:

- Sample collection: early morning fresh specimens to be collected before starting the antibiotic when possible.
- Should be collected in a leak-proof sterile container and transported in a sealed plastic bag immediately.
- If viruses and atypical bacteria suspected, refer to nasopharyngeal and throat swabs transported in VTM. For more details, refer to nasopharyngeal and throat swabs SOP.
- Transportation: specimens should be transported and processed as soon as possible. If processing is delayed more than two hours, refrigerate the specimen to avoid overgrowth of colonised flora and loos of significant

pathogens. Delays of over 48 hours are undesirable and should be rejected with comment.

- Specimens of unacceptable quality are reported the same day they are received. These criteria do not apply to sputum requested for any other culture types such as Mycobacteria, Legionella, or Fungi. They also do not apply to specimens from CF patients.

4.3.2. Material:

Reagents	**Consumables/Supplies**	**Equipment**
MacConkey plate Blood Agar plate Chocolate plate Gram stain reagent Sputasol	Microscopic slides Sterile loops	Microscope Slide dryer Safety cabinet class II Incubators

4.3.3. Safety precaution:

- All specimens need to be treated as potentially infectious.
- All sputum and associated specimens are to be processed in the biohazard safety cabinet.
- Standard procedures for handling of biohazard material must be followed at all times.

4.3.4. Quality control:

- Check the expiry dates of all media, reagents and stains before use.
- All media, reagents, kits, and stains **MUST** be quality controlled before use.
- Identification tests should be run with appropriate controls.
- Check the sterility / turbidity of the used sputasol by culturing once needed.
- Record the quality control results in the appropriate QC sheet.

4.4. Analytical stage:

4.4.1 Sample reception:

- After checking the sputum / ET sample acceptability criteria, add sputum / ET microscopy as reflex test.
- Microscopy should be performed for sputum samples regardless the specimen appearance.

4.4.2 Appearance exam:

4.4.2.1 Describe the sputum appearance using the following terms: salivary, mucosalivary, mucoid, mucopurulent and/or blood stained.

- Salivary: colourless and foam
- Mucoid: clear or gray-white viscid
- (Mucopurulent): thicker, yellowish to green
- Blood stained: streak of blood

4.4.2.2 Add an equivalent amount of sputazol to the volume of sputum/ ET sample.

4.4.2.3 Mix well every 5 min for 10-15 minutes. A representative portion of the specimen is chosen to prepare a film for Gram stain

4.4.3 Microscopy (Gram stain) Screening protocol:

4.4.3.1 Examine the specimen for polymorphs and epithelial cells under low power field (LPF) with the 10x objective and proceed as follows in table (1) :

Table (1): Microscopy (Gram stain) Screening protocol:

Sputum / ET rejection criteria (LPF)				
Epithelial cells/ LPF	Polymorphs/ LPF	Reject	C/S	Number of organisms to be processed
0	0- >25	N*	Y**	3 isolates
10- 24	>25	N	Y	2 isolates
10- 24	10- 24	Y	N	
≥ 25	≥ 25	Y	N	

*N: No

* *Y: Yes

- The sample should be rejected without culturing if the above microscopy criteria are not met.
- Release the microscopy finding of the rejected sample without bacterial count.
- If the specimen is found to be acceptable for culture, proceed with Gram stain reporting by quantifying the number of WBC and bacterial count. under HPF as follows in table ((2):

Table (2): Quantification of WBC/ Bacterial count under HPF microscopy

Sputum / ET reporting (HPF)		
WBC or Bacteria count	1- 5, some fields without WBC/ Bacteria	Scanty
	5- 10/ HPF	+
	10- 25/HPF	++
	>25 /HPF	+++

4.4.4 Culture of the sputum / ET samples:

4.4.4.1 Culture the specimen on the surface of plates as followed using sterile loop and streak:

Media	**Incubation**
Blood agar Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	O_2, 35°C x 48 hours
If fungus culture is requested or seen in gram stain, **add** 2 Sabroud agar	1. O_2, 30°C x 7 to 14 days 2. O_2, 35°C x 7 to 14 days

4.4.4.2 Add optochin disc in blood agar plates.

4.4.4.3 Set-up for Nocardia, fungi, and Legionella only upon request or suggested on direct exam.

4.4.5 Samples Identification and Isolation:

4.4.5.1 Pure isolate of pathogenic organism is considered significant.

4.4.5.2 In case of more than 1 isolate:

- Work-up only a moderate to heavy growth of potentially pathogenic organisms according to the microscopy evaluation in table (1) and culture growth: *S. pneumoniae*, *M. catarrhalis*, *Streptococcus* (A, B, C, G, **not** S. *anginosus*), *H. influenzae*, *S. aureus*, *GNB, Yeast, Rhodococcus spp*.(This is not meant to be a complete list).
- Work-up a maximum of 3 organisms.
- Consult in charge technologist or a microbiologist if > 3 pathogens.

4.4.6 Susceptibility Testing:

- As per the national antibiotic susceptibility testing guidelines.

4.5. Post – analytical stage:

4.5.1 Reporting:

- Microscopy report: For the rejected samples based on microscopy result, report rejected sputum / ET using the following comment: “Specimen unsuitable for culture, Microscopic appearance of saliva or contaminated with oropharyngeal flora. Please send a repeat sample, if clinically indicated, with proper collection and a new test order”.
- To report the result of culture:
  - Negative report:
    - If only normal respiratory flora is present, then report “Normal respiratory flora". Consult microbiologist in case of any doubtful culture.
    - Report as "No growth” if no growth.
  - Positive report: Significant isolates with appropriate susceptibilities.
  - Rejected Specimen: as per sputum / ET screening protocol.

5. Responsibilities

5.1. Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2. Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended
- To report failures or incident.

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Department of microbiology Standard Operating Procedures. Bacteriology Procedures. Respiratory manual procedure.	University health network/mount sinai hospital,	2021	
Public Health England. Investigation of bronchoalveolar lavage, sputum and associated specimens.	UK Standards for Microbiology Investigations	2019	B 57 Issue 3.5.