Ministry of Health

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Acknowledgment

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Acronyms:

BA	Blood agar
HAI	Healthcare associated infection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
MDRO	Multidrug Resistant Organism
MRSA	Methicillin Resistant Staph. Aureus
SOP	Standard operating procedure
OR	Operation room
IP&C	Infection prevention and control
HSE	Health safety and environment
WHO	World Health Organization

1. **Purpose**

   This document describes the procedure of processing microbiological environmental screening and monitoring samples from environmental surfaces and other environmental sources like air .

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 Nosocomial/healthcare associated infection (HAI): infections acquired after 48hrs of admission which are not present or incubating at admission.

   3.2 Active air sampling (air sampler): Sampling of known volumes of air and is carried out using special equipment, by trained staff, and should be performed according to the manufacturer's instructions for the used air sampling equipment.

   3.3 Passive air sample Sedimentation Method: Particles of micro-organisms settle onto surfaces of agar plate via gravity.

4. **Procedure**

   4.1.Clinical background:

   4.1.1 The hospital environment is an important factor in infection risk as it may act as a reservoir for Nosocomial/healthcare associated infection (HAI). Microbiologic sampling of air, water, and environmental surfaces is an expensive and time-consuming process that is complicated by many variables in protocol, analysis, and interpretation. Routine sampling is not usually indicated for healthcare environments. It is therefore indicated for only the following situations:

   - To support investigation of an outbreak when environmental reservoirs are implicated epidemiologically in disease transmission.
   - To monitor a potentially hazardous environmental condition, confirm the presence of a hazardous chemical or biological agent, and validate the successful abatement of the hazard. This type of sampling can be used to detect bioaerosols released from the operation of health-care equipment (e.g., an ultrasonic cleaner) and detect bioterrorism agent in an indoor environmental setting.

- To evaluate the effects of a change in infection-control practice or to ensure that equipment or systems perform according to specifications and expected outcomes. Examples of sampling for quality-assurance purposes may include the use of air sampling during major construction periods to qualitatively detect breaks in environmental infection-control measures, commissioning newly constructed space in special care areas (i.e., ORs and units for immunosuppressed patients) or assessing a change in housekeeping practice.
- Environmental sampling may be warranted in research that can provide new information about the spread of healthcare associated diseases. A classic example is the study of environmental microbial contamination that compared healthcare associated infection rates in an old hospital and a new facility before and shortly after occupancy.

4.2.Principle:

4.2.1 In order to be able to carry out the appropriate microbiological examinations on a sample and provide a meaningful interpretation of test results, it is essential that samples are collected in a suitable manner using the correct equipment.

4.2.2 The selection of agar depends on the sampling objective and the incubation temperature of the agar depends upon the microbe under investigation. Once the media is selected and the incubation temperature is determined, the colonies can grow. As they mature, they are quantified and identified to the genus level for fungal and bacterial isolates and speciated when indicated. The concentrations are expressed in terms of colony forming units per swab or colony forming units per area sampled.

4.3.Pre – analytical stage:

Sample:

- Samples are collected by IP&C or HSE staff according to their protocol. If sent by other departments, need approval from IP&C.
- Prior communication with the laboratory is needed.
- Environmental samples receiving form must be submitted along with the samples. appendix1.

- Samples are generally processed immediately, within 4 h or stored in transport media at 4_C for no more than24 h.

- **Air samples :**
  - Air samples are collected directly on media plates.
  - Media containing selective or non-selective agar, depending on organism(s) of interest.
- **Environmental swabs**
  - Types: cotton, rayon, polyester, calcium alginate. Sponges, and wipe methods can be used if available.
  - Recovery of microorganism can be enhanced by pre-wetting prior to surface.
  - There are many wetting agents can be used ranging from sterile saline, buffered peptone water, various strengths of Ringer solution.

4.3.1. Material:

Reagents	**Consumables/Supplies**	**Equipment**
Selective or non-selective plates Gram stain reagent	Microscopic slides Sterile loops Labels Swabs permanent waterproof marker	Microscope Safety cabinet class II Incubators Air sampler

4.3.2. Safety precaution:

- All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.

4.3.3. 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.
- Record the quality control results in the appropriate QC sheet.

4.4.Analytical stage:

4.4.1 Procedure for air sampling:

- Sampling may be either passive through settle plates or active by using air sampler Requirements

4.4.1.1 **Exposure Settle plates (Sedimentation Method)**

- Agar plates should be at room temperature prior to sampling.
- Expose the plate medium for 30-60 minutes by removing the lid (longer may result in agar drying out).
- Replace the lid, place the plates in a sterile plastic bag, seal and label clearly with date, area of collection and return to the laboratory as soon as possible to ensure that they are processed on the day of collection or at least within 24 hours of collection.
- Once sample is received in the laboratory, immediately incubate under appropriate condition.
- Check read and process plates after 24- and 48-hours incubation.
- Count actual colonies isolated in the exposed plates. Document in working sheet appendix 2 .

4.4.1.2 Procedure for Active air sampling (air sampler)

- Choose the suitable medium for each type of microbial contaminants that is able to process the specimens.
- Place the sampler in the middle of the room. Average size of approximately 5 x 5 m^2.
- After sampling has been completed, wrap the plate (s) with Para film, to reduce the likelihood of contamination and keep them inverted to prevent condensation.
- Plates should be kept cool after collection and during transportation to laboratory to prevent growth of contaminates.

- Samples “Agar plates” are then incubated as soon as possible at 37ºC for 24 – 48hours, for the environmental bacteria. And at 25 ºC for 5 – 14 days for maximum recovery of fungi.
- After incubation, the total number, and the type of colonies in each plate are counted, and analyzed for their species.
- The mean number of viable bacteria or fungi by cubic meter (m^2) of air can be calculated by knowing the air sample flow rate (L/m^3) and the sampling time, and the results will be written as colony forming unit per cubic meter of air (CFU/m^3). Refer to air sampler manufacturer recommendation for calculation.
- Documentation of all the results should be done and sent to the infection control department as soon as possible.

4.4.2 Procedure for Environmental Surface Sampling:

- Once sample is received in the laboratory, inoculate sample swab on the appropriate medium.
- Types of plates will be decided according to the targeted organisms.
- Most probably will be for screening of Multi-Drug Resistant Organisms (MDROs)

4.4.2.1. Sample extraction:

- Extraction solutions AND Vortexing, agitation, or sonication of the swab or sponge are three methods that may increase recovery of microorganism.
- Extraction solutions include: phosphate-buffered saline, Butterfield’s buffer, Butterfie
- An optimum time of 2 min vortexing was shown to be superior over 12 min of sonication.

4.4.2.2. Sample enrichment:

- Enrichment can be useful for slower-growing organisms. It involves placing the sample directly into a broth and incubating, providing time to grow in favorable conditions.

- Broth composition and incubation time and temperature vary depending on the organism of interest. Brain-heart- infusion broth is widely used.

4.4.2.3. Sample collection:

- Aseptic techniques must be strictly applied.
- Prior to collection, swabs should be moistened with sterile water or in the transport fluid in the swab container to enhance particle collection.
- Return to recapped swab immediately to the transport tube media, or use enrichment media.
- Label the swabs including location and date.
- Swabs should be delivered to the laboratory as soon as possible and protected from temperature extremes during transport.
- Once sample is received in the laboratory, inoculate sample swab on the following medium;. BAP, MAC).
- Consider use of medium specific for mold's if shown to be a problem in the environment (e.g. Sabuoraud).
- Incubate under CO2 at 35-37ºC.
- .Check, read and process plates after 24 and 48 hours incubation.

4.5.Post – analytical stage:

4.5.1. **Reporting of result.**

- For incubated plates without growth, report as "No growth after 48 hours".
- For incubated plates with growth, report isolated organism with quantitation.
- Results of sedimentation air sampling are typically expressed as numbers of viable particles or viable bacteria per unit area per the duration of sampling time (i.e., CFU/area/time).

- Results obtained from either sampling device can be expressed as organisms or particles per unit volume of air (CFU/m^3).
- The acceptable level of colony forming units (CFUs) depend on many factors.it should be interpreted by infection control.
- Send completed report to Infection Control Office.

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 test 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
Review How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence	S. Rawlinson a, L. Ciric a, E. Cloutman-Green a,b, *	Journal of Hospital Infection 103 (2019) 363e374	
Guidelines of Microbiological Environmental Sampling in Healthcare Settings	State of Kuwait Ministry of Health	2011	
Guidelines for Environmental Infection Control in Health-Care Facilities	Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC)	2003.	
Environmental sampling of hospital surfaces: Assessing methodological quality	Jocelyn Chai, BSc (Pharm);1 Tysha Donnelly, BSc;2 Titus Wong, MD, MHSc, FRCPC;2,3 Elizabeth Bryce	*Canadian Journal of Infection Control* | Fall 2018 | Volume 33 |	
Examining food, water and environmental samples from healthcare environments Microbiological guidelines	Public Health England	February 2020	

8. Annexes: 1: Environmental samples receiving form

NOTE: This request must be submitted along with the samples.

Sample type: Air sample / Swabs / others (specify)
No. of samples / plates:
Collection date / time :
Collection source :
Collection site in each source :
Collected By:
Collection reason:
Notification to microbiologist / Supervisor : Notified by:
Receiving date at LAB: Received by:

Annexes 2: Environmental samples - Infection control work list

Main site of collection: **Type of Environmental screen :** **Processing date:**

Sample number	**Site of sample**	**Culture result (24hrs and 48 hrs)**				**Microbiologist comment and Further workup**
		mold colony count		**Bacterial colony count**		
		24 hrs	**48 hrs**	**24 hrs**	**48hrs**	

*Yeast is not included in the count. Aspergillus .spp needs to be specified

Authorized by HOD or supervisor ……..