GUIDELINES FOR HOSPITAL ENVIRONMENTAL SURVEILLANCE AIIMS KALYANI

[Standard Operating Procedure]

Prepared by HICC-AIIMS Kalyani

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I. INTRODUCTION

Environmental surveillance is the process of monitoring the air, water, and surface quality in a hospital setting, is an important component of hospital infection control to identify and prevent potential sources of infection. It involves collecting and analyzing samples from various sources, such as operation theatres, labor rooms, equipment's, floors, walls, and table tops, to detect the presence of microorganisms that may pose a risk to patients and staff. It also helps to evaluate the effectiveness of infection control measures, such as disinfection, ventilation, waste management and can provide valuable information for outbreak investigation and management, as well as for quality improvement and accreditation.

II. OBJECTIVE OF ENVIRONMENTAL MICROBIOLOGIC SAMPLING

Usually rates of healthcare–associated infection (HAI) does not correlate with levels of general microbial contamination of air or environmental surfaces. Thus, random, undirected sampling (referred to as "routine") should be discouraged. Targeted microbiologic sampling (recommended by CDC) connotes a monitoring process that includes:

1. A written, defined, multidisciplinary protocol for sample collection and culturing
2. Analysis and interpretation of results using scientifically determined or anticipatory baseline values for comparison; and
3. Expected actions based on the results obtained.

III. INDICATIONS FOR ENVIRONMENTAL SURVEILLANCE/SAMPLING

Microbiologic sampling of air, water, and inanimate surfaces (i.e., environmental sampling) is an expensive and time-consuming process that is complicated by many variables in protocol, analysis, and interpretation. It is therefore indicated for only four situations.

1. To support an investigation of an outbreak of disease or infections when environmental

reservoirs or fomites are implicated epidemiologically in disease transmission.

2. For research purposes—Well-designed and controlled experimental methods and approaches can provide new information about the spread of health-care associated diseases.

3. To monitor a potentially hazardous environmental condition: To confirm the presence of a hazardous biological agent, and to validate the successful abatement of the agent after any work that may affect air flow supply rates or distribution patterns E.g. Duct work.

4. Quality assurance 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.

Bioaerosol: Biological contaminants occur in the air as aerosols and may include bacteria, fungi, viruses, and pollens. Aerosols are characterized as solid or liquid particles suspended in air. Particles in a biological aerosol usually vary in size from <1 μm to ≥50 μm

IV. PROTOCOL FOR ENVIRONMENTAL SAMPLING IN AIIMS KALYANI

MICROBIOLOGIC AIR SAMPLING

It is used to determine the numbers and types of microorganisms, or particulates, in indoor air. The results represent indoor air quality at singular points in time, and these may be affected by a variety of factors, including:

a. Indoor traffic
b. Visitors entering the facility
c. Temperature
d. Time of day or year
e. Relative humidity
f. The relative concentration of particles or organisms
g. Performance of the air-handling system components.

4.1.1. Preliminary concerns for conducting air sampling:

1. Possible characteristics and conditions of the aerosol, including size range of particles, relative amount of inert material, concentration of microorganisms, and environmental factors should be considered.
2. Type of sampling instruments, sampling time, and duration of the
3. sampling program should be determined.
4. Number of samples to be taken should be determined.
5. Determine the method of assay that will ensure optimal recovery of microorganisms.

4.1.2. Areas where Air Surveillance is to be Performed

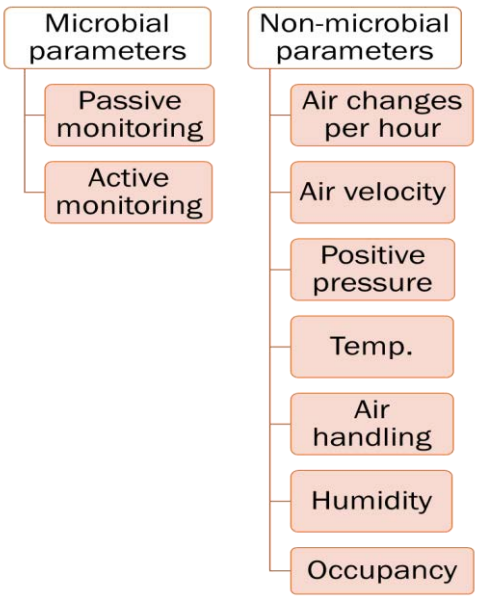
1. Operation theatres with laminar flow installed (HEPA Filtered Operating Room).
2. Intensive Care Unit (ICU)
3. Inpatient wards requiring a protective environment (positive pressure ventilation).

4.1.3. When to Perform:

The targeted air surveillance is done only in case of the following situations as per recommendations of CDC.

1. After new construction and commissioning of HEPA Filtered OTs
2. After any reconstruction/ repair work inside the Operating rooms.
3. After regular maintenance work of HEPA filters to check filter efficiency
4. Investigation of an outbreaks,
5. For research purpose (well designed and controlled experimental methods)
6. When potentially hazardous environmental condition is suspected.
7. For quality assurance to evaluate the effects of a change in infection control practice.

Evaluation of air quality:



4.1.4. How to perform:

Air sampling methods:

1. **Active monitoring:** Impaction on solid surfaces -– Sieve sampler
2. **Passive monitoring:** Sedimentation –-Settle plate method
3. Impingement in liquids
4. Filtration
5. Centrifugation
6. Electrostatic precipitation
7. Thermal precipitation

1. Passive monitoring – Settle plate method

1,1,1 method of sampling

- Performed to determine the Index of Microbial Air Contamination (IMA).
- This index corresponds to the number of CFU counted on
  - A Petri dish with a diameter of 9 cm (90 mm)

- Placed according to the 1/ 1/1 scheme
- For 1 hour, 1 m above the floor, about 1 m away from walls or any major obstacles
- Incubation @ 37ºC for 24 hours

2. Active monitoring – Sieve sampler

- Colonies counted do not reflect the actual organism load in air.
- Nutrient agar or blood agar
  - Insertion of slit
  - Place the agar plate and raise the platform
  - Start the motor
  - Sampling time: 10 mins / 100 lt./min
  - Remove plate
  - Incubate at 37°C for 24 hrs.
- Actual no of organisms counted per m^3 of air sample

$P_r = N (1/N+1/N-1+1/N-2…1/N-r+1)$

P_r – Probable number

r – Number of colonies on 90 mm plate

N – Total no of holes in sampling head

Positive hole conversion table:

CONVERSION TABLE for LA637

To correlate the CFUs (Colony Forming Units) present on the agar plate to the most probable number (MPN) of micro-organisms per cubic meter of air sampled, the following formula is used:

$$P_r = N\,[1/N + 1/(N-1) + 1/(N-2) + \ldots + 1/(N-r+1)]$$

Where:
P_r = most probable number of micro-organisms in the volume of air sampled
N = number of holes on Microflow sampling head
R = number of CFUs on the agar plates after incubation

r	P_r	r	P_r
1	3	46	51
2	4	47	52
3	5	48	54
4	6	49	55
5	7	50	56
6	8	51	57
7	9	52	58
8	10	53	59
9	11	54	60
10	12	55	62

Acceptable levels: (Index of Microbial Air Contamination (IMA) by Swiss Hospital Association)

- Measured as CFU/m^2/hr.
- At rest: ≤ 5 CFU/9 cm diameter/hour
- When operational: ≤ 25 CFU/9 cm diameter/hour

4.1.5. Reporting and Action Plan for Air Surveillance:

The reports of air surveillance to be communicated immediately to the concerned area with following action plan suggested in case of unacceptable reports.

Action Plan:

- In case of positive microbiological sampling report, the area/site should be cleaned and scrubbed thoroughly with soap/detergent and water followed by cleaning with disinfectant (phenolic agents/ Bacillocid 1%). This should be

followed by repeat fogging and repeat microbiological testing.

- OT/room/area should be used only after microbiological surveillance cultures are reported as negative.
- **In case of repeated unacceptable reports—the HEPA filters should be checked** for filter efficiency by the concerned engineer and changed if required.

TABLE-1: Non-microbial parameters in OT

Properties	Recommendations	Frequency of testing
Air changes /hour	20, of which 4 are fresh air	Every 3 months
Air velocity	25-35 feet per min Downwards, unidirectional flow	Every 3 months
Positive pressure	2.5 pascal	Daily
Temperature	21 ± 3°C - General OT 18 ± 2°C - Ortho OT	Daily
Humidity	20-60% (Ideal: 55%)	Daily
Occupancy	5-8 persons	At any time
Air handling	**NO** window/split ACs; Separate AHU for all OTs; HEPA efficacy.	
General consideration	Paints (Antibacterial, antifungal), Automatic doors (touch free), Flooring (Seamless, should not be of porous stone)	

4.2. WATER SAMPLING:

Water sampling in health-care settings is used to detect waterborne pathogens of clinical significance or to determine the quality of water in a facility's distribution system.

TABLE-2: Waterborne pathogen and their reservoirs in healthcare settings

Organisms	Water reservoirs
Gram-negative bacteria	
Coliforms	Fecal contamination of Hospital drinking water
Pseudomonas	Potable (tap) water, distilled water, antiseptic solutions contaminated with tap water, sinks, hydrotherapy pools, lithotripsy therapy tanks, dialysis water, eyewash stations, flower vases, and endoscopes with residual moisture in the channels.
Burkholderia cepacia *Stenotrophomonas spp.* *Sphingomonas spp.*	Distilled water, solutions, disinfectants, dialysis machines, nebulizers, mouth wash, ventilator temperature probes
Ralstonia pickettii	Fentanyl solutions, chlorhexidine , distilled water, respiratory therapy solution
Serratia marcescens	Potable water, antiseptics (e.g., benzalkonium chloride and chlorhexidine), disinfectants (e.g., quaternary ammonium compounds, glutaraldehyde)
Acinetobacter spp.	Medical equipment that collects moisture (e.g., mechanical ventilators, cool mist humidifiers, vaporizers), room humidifiers, environmental surfaces
Enterobacter spp.	Humidifier water, intravenous fluids, unsterilized cotton swabs, ventilators, suctioning machine, blood gas analysers
Legionella pneumophila	Found in various man-made aquatic environments such as cooling towers, showers, faucets, respiratory therapy equipment, and room-air humidifiers.

Nontuberculous mycobacteria	
M. abscessus	Inadequately sterilized medical instruments
M. chelonae	Dialysis, reprocessed dialyzers, inadequately-sterilized medical instruments, jet injectors, contaminated solutions,
M. fortuitum	Aerosols from showers or other water sources, inadequately sterilized medical instrument
M. gordonae	Deionized water, laboratory solution, potable water ingestion prior to sputum specimen collection

Others	
Cryptosporidium species	Highly resistant to disinfectants and not killed by chlorination of water
Giardia lamblia	Recreational water in pediatric units
Norovirus	Caused outbreaks of gastroenteritis, with possible transmission via hand contact and contaminated items within toilets and the bedside environment
Fungal pathogens	Common among patients with hematological malignancies or stem cell transplantation The fungi recovered from several water reservoirs of the hospitals causing outbreaks include: *Aspergillus* - hospital water samples Mucor- water-damaged plaster Fusarium- showers and sinks in a hospital water distribution system

4.2.1. Modes of transmission for waterborne infections include:

1. Ingestion of water
2. Contact transmission [e.g., from an improperly reprocessed medical device]
3. Inhalation of aerosols dispersed from water sources
4. Aspiration of contaminated water.

4.2.2. Microbiological testing of water samples:

Routine testing of the water in a health-care facility is usually not indicated, but sampling in support of outbreak investigations can help determine appropriate infection-control measures.

4.2.3. Indications:

1. Outbreak investigation - When environmental reservoirs are implicated epidemiologically in disease transmission.
2. Research purpose - To study the spread of HAIs
3. For quality assurance:
   - Sampling for short duration – To evaluate change in infection-control practice
   - Extended sampling - Construction and renovation activities

4.2.4. Sampling points in hospitals:

Must represent different sources from which water is obtained.

- **Portable water**: Pipelines, hot and cold water systems, endoscopy rinse water, dialysis water, dental chair unit waterline.
- **High risk of contamination**: Water from Unprotected sources, loops, reservoirs, low-pressure zones etc.

4.2.5. Water analysis:

Category 1 - Contamination with enteric pathogens - From faecal contamination of drinking water.

Category 2 - Contamination with *Legionellae* sp., MDR-GNB, NTM- Present in hospital environment, may get contaminated with various hospital water reservoirs.

TABLE-3: Methods of detection

Test	Use
Multiple-tube method	• Extensively used for drinking water analysis • For highly turbid samples
Membrane filtration method	• For testing dialysis water • For testing clean water • For testing large volume of water • When bacterial count in water is expected to be low • Not recommended for highly polluted water which may block membrane pores
Plate count method - pour plate method & spread plate method	• If bacterial count is expected to be high (during water-born outbreak investigations) • To assay small quantity-
Presence absence methods	• Liquid cultivation technique used for detection of target organism

4.2.6. Collection and transport of water sample

Water specimens are not "static specimens" at ambient temperature. Potential changes in both numbers and types of microbial populations can occur during transport. The collected water should be processed within 2 hours. If that is not possible, then it should be refrigerated at 2-8˚C within 2 hours and processed within 24 hours.

- **Container-** Screw capped borosilicate glass or autoclavable plastic bottles (Capacity -500 ml)
- **Volume -** At least 150-200 ml of water
- Extreme care should be taken to avoid contamination of bacteria present in the surrounding environment or hands of collecting person.
- **Sodium thiosulfate** should be added to neutralize the bactericidal effect of residual chlorine present in water if any.
    - ❖ 0.1 mL of a 3% sodium thiosulfate solution in a 120mL bottle will give a

final concentration of 18 mg/L in the sample and neutralize up to 5 mg/L residual chlorine.

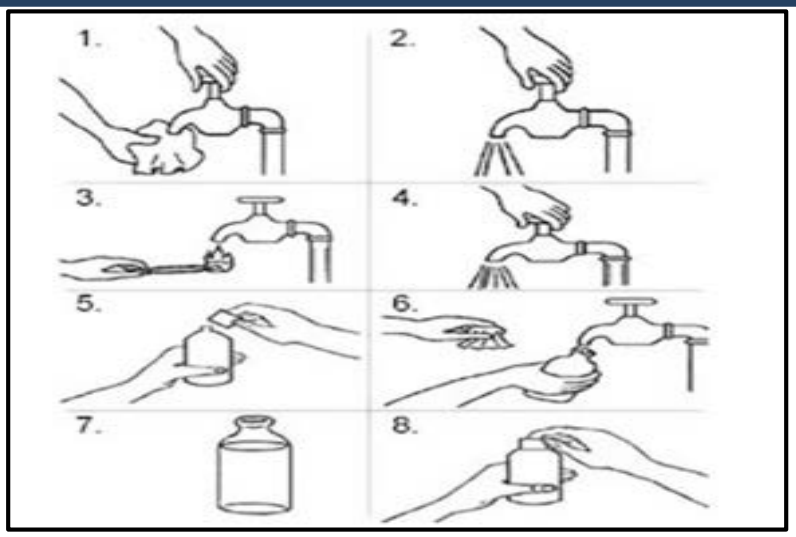
4.2.7. Sampling method from tap:

Pre flush technique: sample is taken from tap when it is not in use at least for ≥ 2 hours (preferably early morning or at the time of lowest usage). Water should be collected immediately after opening the tap.

Post flush technique: The sample is collected after running for 2–3 minutes i.e. discarding initial free-flow water from tap. (most frequently used). When *Legionella or Pseudomonas* outbreak is suspected, both Pre flush and post flush water sampling is recommended.

TABLE-4: The comparison of Pre flush and post flush water sampling will help to point out the source of contamination

Bacterial count	Interpretation
Pre-flush> post-flush	Contamination is near the tap and associated pipework and fittings near to that outlet.
Post-flush> pre-flush	Contamination is remote from the point of delivery and may suggest stagnation in the water system
Both Similar bacterial	Attention should focus on the whole water supply, storage, and distribution system.



Procedure for sampling water from a tap (WHO)

1. Clean the tap/outlet using a clean cloth to remove any dirt.
2. Turn on the tap and let the water run at maximum flow for 1 to 2 minutes; then turn it off.
3. Sterilize the tap outlet for a minute with the flame from a cigarette lighter or an ignited alcohol-soaked cotton-wool swab.
4. Turn on the tap again and allow the water to flow for 1 to 2 minutes at a medium flow rate.
5. Open a sterilized bottle by carefully unscrewing the cap.
6. Immediately hold the bottle under the water jet and fill.
7. While filling the bottle, hold the cap face downwards to prevent entry of dust, which may contaminate the sample.
8. Screw on the cap. A small air space should be left so that the contents can be shaken more easily before analysis.

4.2.8. Presumptive or Total coliform count:

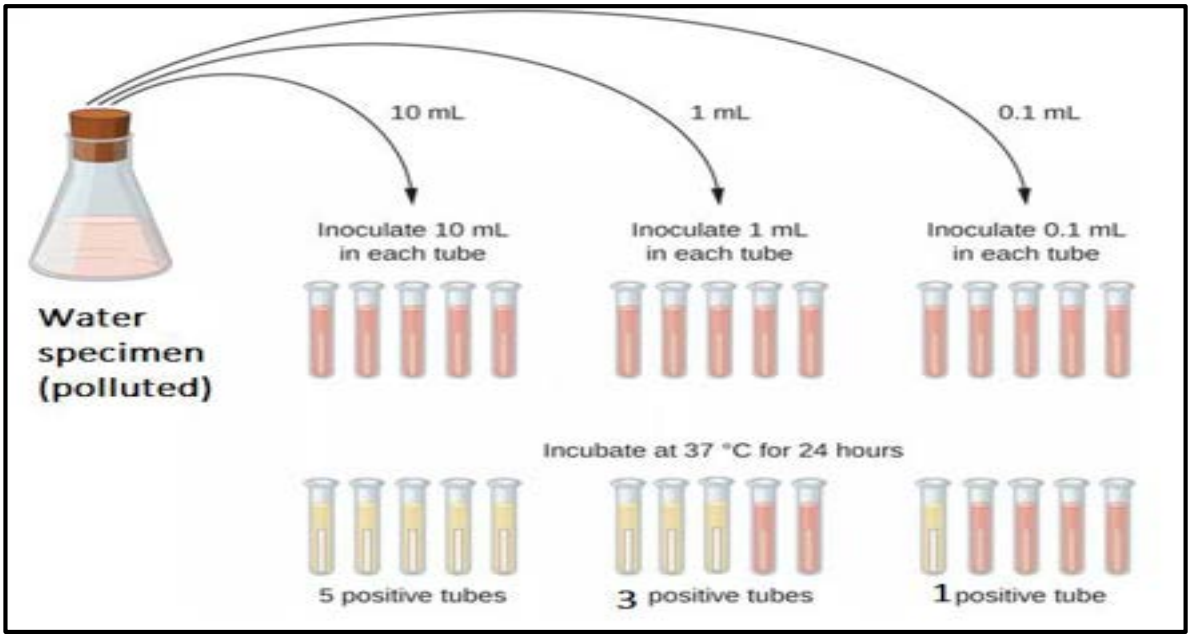
Multiple-tube method is used for estimation of presumptive coliform count expressed as Most Probable Number (MPN) of coliform organisms in 100 mL of water. Positive presumptive test is then subjected to confirmation test to find out presence of Thermo-

tolerant *E.coli*.

4.2.9. Multiple-tube method

Media used:

- For presumptive coliform count
  - ○MacConkey purple broth
  - ○Lauryl tryptose (Lactose) broth
  - ○Lactose broth
- ▪For detection of Thermotolerant *E. coli*:
  - •Brilliant green lactose bile broth
  - •Lauryl tryptose mannitol broth with tryptophan

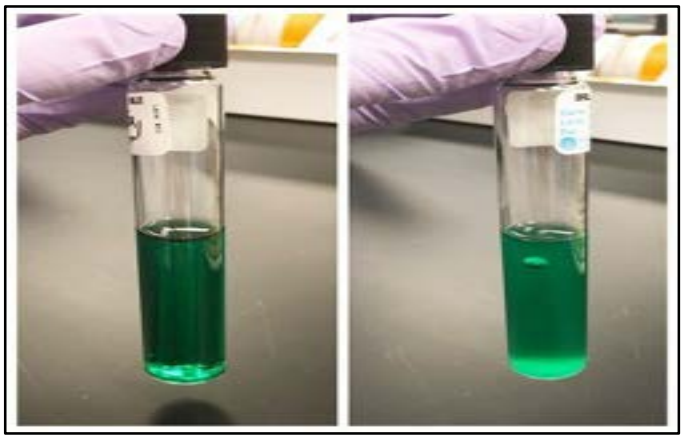


4.2.10. Differential coliform count- Eijkman test method:

- To confirm the presence of thermotolerant *E. coli*
- Method: subculture of positive tubes of multiple tube method on a lactose-

containing medium e.g. Brilliant green bile (BGB) broth

- Incubation at 44 ± 0.5ºC
- Detection of lactose fermentation with acid (color change) and gas production.



4.2.11. Membrane filtration method:

- For testing clean and treated water, especially in dialysis and transplant units
- **Principle:**
  - Filtration of a Known volume of water through a cellulose membrane
  - Bacteria retained on the surface of the membrane filter
- Filter transferred into a petri dish containing a suitable medium (MacConkey agar or Membrane lauryl sulfate broth or Lactose agar with tergitol 7) and incubated @ 37°C for Coliform and 44°C for thermotolerant E. coli for 18-24 hours.
  - Characteristic colonies of coliforms/ thermotolerant coliforms can be counted directly.
- **Interpretation:**

Presumptive coliform count per 100 ml=

$$\frac{\text{No. of colonies counted at 37°C X 100}}{\text{No. of ml of water sample filtered}}$$

Thermotolerant coliform count per 100 ml=

$$\frac{\text{No. of colonies counted at 44°C X 100}}{\text{No. of ml of water sample filtered}}$$

4.2.12. Confirmatory test:

Coliform:

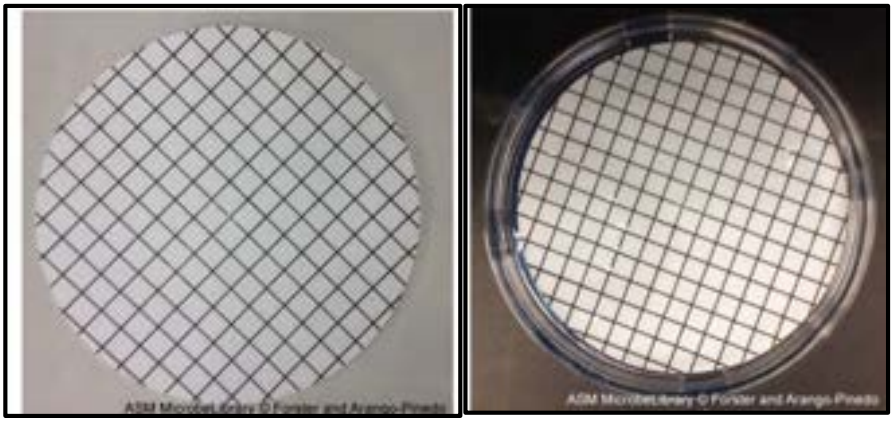
- Sub cultured into tubes of suitable media (lactose peptone water with Durham's tube) and incubated at 37°C for 48 hours.
- Any gas production in the tubes indicates the presence of coliform bacteria.

For thermotolerant coliform:

- Sub cultured to a tube of lactose peptone water and incubated at 44 °C for 24 hours:

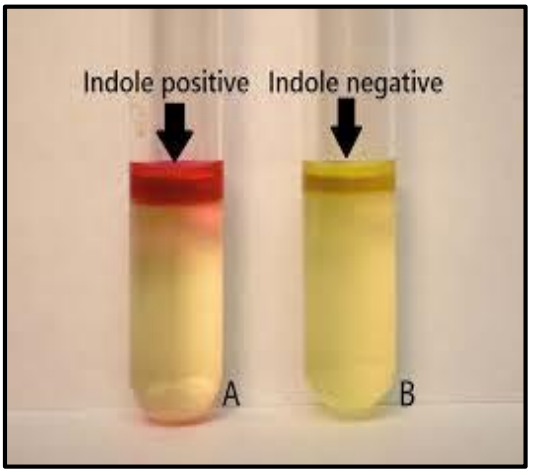
• Production of gas

• Positive indole test



A. Nitrocellulose membrane (47 mm diameter and a pore size of 0.45 µm)
-used to detect total coliform and fecal coliform bacteria from samples of water.

B. Placement of membrane onto agar following vacuum filtration.



4.2.13. Method for isolation of *Pseudomonas:*

- Membrane filtration method: A measured (100 ml) volume of the sample is filtered through a membrane filter (≤0.45 microns) to retain bacteria. The filter is then placed on a solid selective and differential medium such as Mac-Conkey agar or CN agar (containing Cetyltrimethylammonium bromide and Nalidixic acid) and is incubated @ 37°C for 48 hours.
- *P. aeruginosa* produces pale non-fermenting colonies on Mac-Conkey agar and blue-green or brown colonies on CN agar.
- Confirmation of isolates is done by subculturing to milk agar supplemented with cetyl-trimethylammonium bromide to demonstrate hydrolysis of casein.
- The result is calculated as the colony count per 100 mL of water.
- **Biofilm or slime layer** producing *Pseudomonas* growing in the water system is suspected. Swabs can be taken from the outlets, placed into Maximum recovery diluent (peptone saline diluent), and processed.

4.2.14. Method for isolation of *Legionella*:

Routine water surveillance for *Legionella* -not recommended

- Should be performed at least annually or more often for certain high-risk settings such as transplant units.
- **Sampling:** Centrifugation or membrane filtration, or combination of both.
- Recovery in the presence of other bacterial species present in the sample can be improved by heating, usually at 50^0C for 30 minutes and by treating with acid.
- **Culture media :** BYCE with antibiotic supplements/ Wadowsky-Yee-Okuda medium (WYO) can be used for isolation of legionellae from water. Albumin can be added to increase the recovery of *L. micdadei* .

Plates are inoculated with a portion of sample (generally 0.1–0.2 ml) by the spread plate technique and incubated at 36 °C, preferably in a humidified candle jar for 10 days.

4.2.15. Water testing in Dialysis units:

Microbiological contamination of dialysis water systems poses a major problem. Therefore, the purity of water must be ensured periodically.

Method:

- Membrane filtration method – method of choice as it permits large volumes of water to be assayed.
- Detection of Endotoxin – should be performed periodically
- Newer methods :
    - Luminometer
    - Chromogenic media
    - Molecular methods – real time PCR , FISH
    - Immunological method – ELISA , Lateral flow assay

TABLE- 5: Indicator organisms of fecal pollution of water

Indicator organisms	Interpretation
Coliform (other than *E. coli*)	Remote contamination- fecal (presumptive)
Fecal (thermotolerant) *E. coli*	1.Confirms recent fecal contamination of water 2.Most sensitive indicator
Fecal streptococci	Confirms remote fecal pollution
C. perfringens	Remote contamination
Pseudomonas aeruginosa	Least reliable indicator
Bacteriophages	Phage specific for *E. coli* - indicate fecal pollution of water

TABLE-6: Microbiological limits - drinking water

Organism	Colony count	Interpretation	Action
Organisms detected in drinking water (Multiple tube method)			
Coliform	0 MPN/100mL	Excellent	No action is required
	1-3 MPN/100mL	Satisfactory	Re-sample
	4-10 MPN/100mL	Suspicious	Re-sample and review water disinfection process
	>10 MPN/100mL	Unsatisfactory	Review of infection control measures Stop using the water for drinking purposes and hospital use Consider disinfection of the water distribution system
Fecal *E.coli*	>0 MPN/100mL	Satisfactory	No action is required

	>0 MPN/100mL	Unsatisfactory	Review of the control measures Stop using the water for drinking purposes and hospital use Disinfection of the water distribution system

TABLE-7: Microbiological limits - Hospital water distribution System

Legionella	<100 cfu/L	Satisfactory	No action is required
	>100 to <1000 cfu/L	System under Review	Re-sample and review control programme
	>1000 cfu/L	Unsatisfactory	Re-sample, if only a minority (1-3 no.) of samples are positive If most samples are positive, the system may be colonized. Disinfection of the system should be considered
Pseudomonas spp.	0 in 100ml	Satisfactory	No action is required
	1-10 in 100ml	Undesirable	Re-test and refer to those responsible for the Water Safety Plan to determine what actions may be required.
	>10 in 100ml	Unsatisfactory	Investigate cause and put corrective actions in place. Re-sample after 3 weeks
Aerobic colony count (cooling tower)	$<10^4$ per ml	Satisfactory	No action is required
	$>10^4$ to $<10^5$ per ml	Undesirable	Re-test, risk assessment and review the control measures

	>10^5 per ml	Unsatisfactory	Investigate cause and put corrective actions in place.

TABLE-8: Microbiological limits - Dialysis fluid or water used for dialysis

Dialysis fluid or water used for dialysis			
Aerobic colony count	<10 in 100 ml	Satisfactory	No action is required
	≥10 in 100 ml	Unsatisfactory	Investigate cause and put corrective action in place
Endotoxin (EU/ ml)	<0.03	Satisfactory	No action is required
	≥0.03	Unsatisfactory	Investigate cause and put corrective action in place

4.2.16. Biofilm detection in the water distribution system

Biofilm comprises of a group of microorganisms embedded within a slimy extracellular polymeric substance (EPS). Biofilms in drinking water pipe networks is quite common and can cause a wide range of water quality and operational problems.

- Once formed, biofilms are very hard to remove and the slimy outer layer become resistant to disinfection and heat.
- This leads to a sludging effect where biofilm mass continuously secretes matter and microorganisms into the passing water causing a steady level of contamination.
- Various organisms such as *Pseudomonas, Legionella* etc. can produce biofilm in water pipelines. Viruses and protozoa such as *Cryptosporidium* can be trapped in the biofilm

- Biofilm on the pipelines may be : Complete film or Small patches on pipe surfaces (more common)

Methods of Biofilm detection

Sample collection:

- **Swabbing:** Cotton swabs moistened with sterile water. Swabs should be rotated to cover the entire surface
- **Scraping off with a sterile scraper**: Thicker layers of biofilm
- **Alternate method-** immersing stainless steel or glass surface on test site for 3-5days and subsequently detecting biofilm on these surfaces.
- Note: Sample should be collected from the water-air interface as maximum growth of biofilm occurs at that area.

Detection methods:

- Tissue culture plate (TCP)
- Tube method (TM)
- Congo Red Agar method (CRA)
- Modified CRA method (MCRA)
- Bioluminescent assay
- Piezoelectric sensors
- Fluorescent microscopic examination
- 16S rRNA gene sequencing

Biofilm monitoring devices:

Specialized monitoring devices can be built into the water systems especially in cooling water systems to monitor biofilm detection. It incorporates studs of known surface area, which can be aseptically removed for subsequent detection of the biofilm growing on them.

Prevention of biofilm formation in water system:

A good water system design keeps microorganisms in the planktonic state; whereas

a poor design e.g. water flow too slow or in the wrong direction or allowing stagnation leads to biofilms formation.

Biofilm formation in water system can be prevented by

- Regular flushing of taps
- Regular removal, cleaning or descaling or replacement of water outlets and thermal mixing valves
- Heating the water distribution system by high pressure steaming or hot water circulation (>800ºC)
- Chemical treatment with chlorine or ozone
- Mechanical scrubbing of the surfaces
- Replacement or removal of pipework as a last resort

4.3. SURFACE SAMPLING:

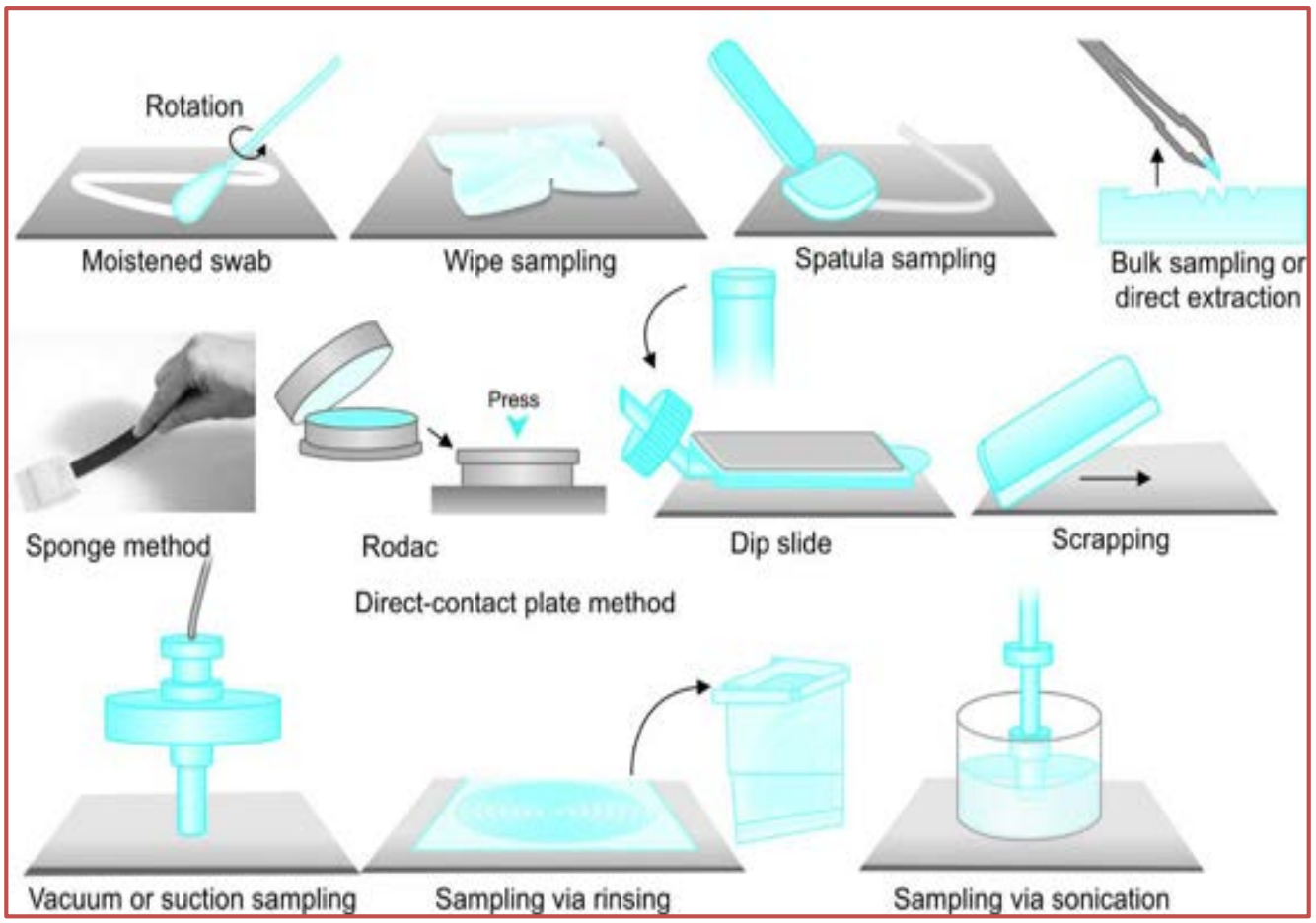
Surfaces may become contaminated in several ways e.g. microorganisms settling out from the environment or from the direct touch by an operator.

4.3.1. Objectives of surface sampling:

- To determine the efficiency of routine cleaning procedures in removing contamination.
- Therefore, sampling should be performed before and after cleaning to determine the effectiveness of the cleaning procedure.
- Other indications: To determine
    1. Potential environmental reservoirs of pathogens.
    2. Survival of microorganisms on surfaces
    3. The sources of the environmental contamination.

However, Routine environmental-surface sampling (e.g., surveillance cultures) in health-care settings is neither cost-effective nor warranted according to CDC.

Methods of sampling: moistened swab method most used.

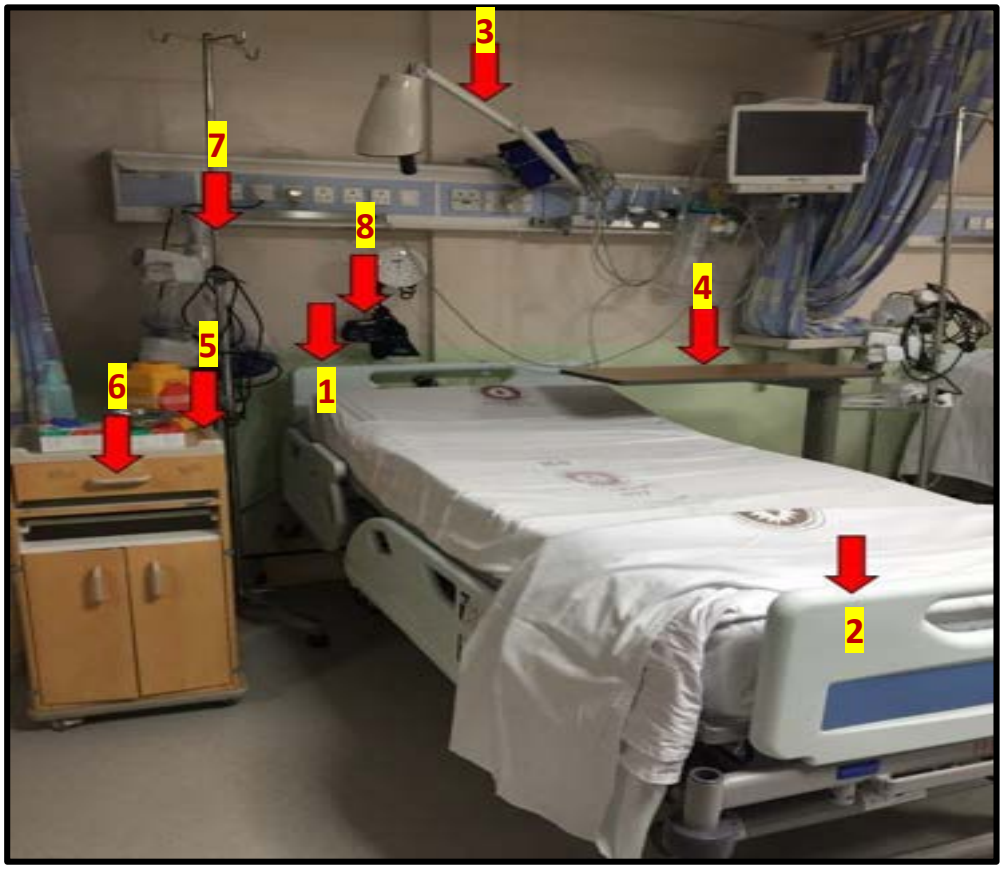


METHODS	INTERPRETATION
Moistened swab (25 cm^2), sponge (>100 cm^2), wipe (>100 cm^2), spatula	Per measured area
Direct immersion (Solid/liquid)	Per item
Bulk sampling or direct extraction method (>20 cm^2)	Assess microbial load in deeper area
Replicative Organisms Direct Agar Contact (RODAC)/Dip slide method	Direct quantitative results

Method	Suitable for appropriate surface(s)	Procedural notes	Points of interpretation
Sample/rinse (Moistened swab/rinse)	Non-absorbent surfaces, corners, crevices, devices, and instruments	Assay multiple measures areas or devices with separate swabs	Report results per measured areas or if assaying an object, per the entire sample site
Sample/rinse (Moistened sponge/rinse)	Large areas and housekeeping surfaces (e.g., floors or walls)	Vigorously rub a sterile sponge over the surface	Report results per measured area
Sample/rinse (Moistened wipe/rinse)	Large areas and housekeeping surfaces (e.g., countertops)	Use a sterile wipe	Report results per measured area
Direct immersion	Small items capable of being immersed	Use membrane filtration if rinse volume is large and anticipated microbiological concentration is low	Report results per item
Containment	Interior surfaces of containers, tubes, or bottles	Use membrane filtration if rinse volume is large	Evaluate both the types and numbers of microorganisms
RODAC (Replicate Organism Direct Agar Contact)	Previously cleaned and sanitized flat, non-absorbent surfaces; not suitable for irregular surfaces	Overgrowth occurs if used on heavily contaminated surfaces; use neutralizers in the agar if surface disinfectant residuals are present	Provides direct, quantitative results; use a minimum of 15 plates per an average hospital room

4.3.2. High touch surfaces include, but are not limited to:

1. Bed Rails
2. Bed Frames
3. Moveable Lamps
4. Tray Table
5. Bedside Table
6. Handles
7. Iv Poles
8. Blood-Pressure Cuff



4.3.3. Interpretation of colonies from surface sampling:

No internationally accepted standards for surface microbiological monitoring. Several studies use different cutoff values depending upon the methodology and

analytical parameters used. Two widely acceptable criteria for microbiological threshold values are:

1. Giovinazzo et al 2018 and
2. ISPESL and French Guideline, 2016

As we are following most used method i.e. moistened swab method, interpretation will follow Giovinazzo et al 2018 recommendations –

- Surface bacterial load of < 2.5 – 5 CFU /cm^2- Acceptable
- Index organisms that must be absent or < 1CFU /cm^2 are *Staphylococcus aureus* (including MSSA and MRSA), *Aspergillus* species, *Pseudomonas* species and Enterobacterales.

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VI. ANNEXURE

TABLE-1: Volumes and numbers of tubes required for different type of water samples in multiple-tube method

Sample type	Number of tubes for sample volume						
	50ml Double Strength	10ml Double Strength	1ml Single Strength	0.1ml Single Strength	0.01ml Single Strength	0.001ml Single Strength	0.0001ml Single Strength
	1	5					
Polluted water		5	5	5			
Heavily polluted water (>1800 MPN/100ml)	-	-	5	5	5	5	5

TABLE-2: MPN values per 100ml of sample (McCrady statistical table)

For non-polluted / treated water samples		
No. of tubes giving a positive reaction out of		MPN per 100ml of water
1 of 50ml	5 of 10ml	
0	0	<1
0	1	1
0	2	2
0	3	4
0	4	5
0	5	7
1	0	2
1	1	3
1	2	6
1	3	9
1	4	16
1	5	>18

For polluted / untreated water samples			
No. of tubes giving a positive reaction out of			MPN per 100ml of water
5 of 10ml	5 of 1ml	5 of 0.1 ml	
0	0	0	<2
0	1	0	2
0	2	0	4
1	0	0	2
1	0	1	4
1	1	0	4
1	1	1	6
2	0	0	5
2	0	1	7
2	1	0	7
2	1	1	9
2	2	0	9
2	3	0	12
3	0	0	8
3	0	1	11
3	1	0	11
3	1	1	14
3	2	0	14
3	2	1	17
3	3	0	17
4	0	0	13
4	0	1	17
4	1	0	17
4	1	1	21
4	1	2	26
4	2	0	22
4	2	1	26
4	3	0	27
4	3	1	33
4	4	0	34
5	0	0	23
5	0	1	31
5	0	2	43

5	1	0	33
5	1	1	46
5	1	2	63
5	2	0	49
5	2	1	70
5	2	2	94
5	3	0	79
5	3	1	110
5	3	2	140
5	3	3	180
5	4	0	130
5	4	1	170
5	4	2	220
5	4	3	230
5	4	4	350
5	5	0	240
5	5	1	350
5	5	2	540
5	5	2	540
5	5	3	920
5	5	4	1,600
5	5	5	>1,800

Table-3: Example of multiplying factors for determination of the MNP for different dilutions of heavily polluted water sample

Example	No. of Tubes giving a positive reaction out of					Coded result chosen	Multiplying factor for MNP
	5 of 1 ml	5 of 0.1ml	5 of 0.01ml	5 of 0.001ml	5 of 0.0001ml		
1	5	5	2	0	0	5-2-0	100
2	5	5	4	1	0	5-4-1	100
3	5	3	0	0	0	5-3-0	10
4	5	5	5	3	1	5-3-1	1,000

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