

State of Kuwait
Ministry of Health

**Guidelines of Microbiological Environmental
Sampling in Healthcare Settings**

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1. INTRODUCTION

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. In this guideline, we advocate the targeted instead of routine environmental culturing because rates of healthcare-associated infection had not been associated with levels of general microbial contamination of air or environmental surfaces, and because meaningful standards for permissible levels of microbial contamination of environmental surfaces or air did not exist. However, targeted microbiologic sampling for defined purposes and not routine sampling should be conducted in accordance with defined protocols that include:

- a) A written, defined, multidisciplinary protocol for sample collection and culturing;
- b) Analysis and interpretation of results using scientifically determined or anticipatory baseline values for comparison; and
- c) Expected actions based on the results obtained.

Infection control, in conjunction with laboratories (Aerobiology, Public Health and Hospitals), should assess the health-care facility's capability to conduct sampling and determine when expert consultation and/or services are needed.

Effective sampling of surfaces depends on the selection of appropriate sampling and assay techniques. If sampling is conducted as part of an epidemiologic investigation of a disease outbreak, identification of isolates to species level is mandatory, and characterization beyond the species level is preferred.

2. INDICATIONS OF ENVIRONMENTAL SAMPLING

Microbiologic sampling of air, water, and inanimate surfaces (i.e., environmental sampling) is indicated for only the following situations:

1. To support an investigation of an outbreak of disease or infections when environmental reservoirs or fomites are implicated epidemiologically in disease transmission.
 - It is important that such culturing be supported by epidemiologic data.
 - Environmental sampling, as with all laboratories testing, should not be conducted if there is no plan for interpreting and acting on the results obtained.

- Linking microorganisms from environmental samples with clinical isolates by molecular epidemiology is crucial whenever it is possible to do so.

2. Limit microbiologic sampling for quality assurance purposes:

- Biologic monitoring of sterilization processes;
- Microbiologic air sampling after construction of critical care areas : operation theatre (OT), intensive care unit (ICU) and protective environment room (PE) for allogeneic HSCT recipients;
- Periodic culturing of water and dialysate in hemodialysis units (at least once a month and during outbreaks);
- Periodic culturing and during outbreaks of drinking water, tap water, dental unit, pharmacy, swimming and hydrotherapy pool water;
- Certain equipment in health-care settings (e.g., biological safety cabinets) may also be monitored with air flow and particulate sampling as part of adherence to a certification program.
- For air handling unit, it is inappropriate to carry out microbiological investigations on cooling coils as they do not contribute significantly to the microbial quality of air delivered by the system. It is also inappropriate to carry out microbiological investigations on humidifiers since they should generate humidity via steam, in which case they pose a negligible risk. Humidifiers that aerosolise recirculated water (spinning disk humidifiers) pose too high a hazard and should not be used. The air handling unit should be properly constructed, finished and functioning. The humidifier and cooling coil in air handling units should be disinfected at least six-monthly. It is considered that physical cleaning, rather than disinfection with microbiological monitoring, is more appropriate.

3. For research purposes.

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

4. 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 the release of an agent of bioterrorism in an indoor environmental setting.

3. PRE SAMPLING INSPECTION

Several preliminary concerns must be addressed when designing a microbiologic environmental sampling strategy, which should meet with the four Circumstances of environmental sampling (mentioned earlier). Pre sampling inspection should be performed prior to sampling to ensure that area is well prepared for sampling. This is usually performed by the head nurse of the sampling area in cooperation with hotel services department. It should be supervised by Infection control personnel.

A. The inspector should evaluate and assure that these criteria's are met prior to each sampling event, to identify the conditions that may affect or interfere with the proposed testing:

- All new or reestablishment work is completed.
- The ventilation system had been running continuously for at least 24 hours following the completion of structural work, and cleaning.
- All engineering commissioning procedures has been completed.
- All fixed and portable equipments placed in their places.
- Floors, all surfaces and equipment are finished from cleaning prior the investigation by 24 hours. A Checklist for Monitoring Cleaning Prior to Sampling (appendix B) should be used by the head nurse of the sampling area to ensure proper cleaning.
- Ducting and air diffusion sites (inlet, outlet) are cleaned prior to sampling.
- Minimizing indoor traffic at all times when possible.
- The sampling personal should be completely familiar with the sampling protocol and type of the particular method to be used in different situations.

B. In case of microbiological out breaks by one or more microorganisms. The following should be considered:

- Avoid excessive cleaning.
- The decision to sample should be based on the extent and location of any suspected

contamination and the potential for the contaminant to migrate and the activities for which the facility is used.

- The decision to collect environmental samples should be made by the infection control committee with liaising with Aerobiology laboratory.
- All personnel who enter the contaminated area must follow the safety and infection control plan developed for that particular site.
- Shutting down the ventilation system serving the contaminated area may be necessary only in special cases to avoid dispersing certain contaminate (i.e. *Mucor species*, *Bacillus anthracis* etc).

4. OPERATION PROCESSES

When environmental sampling (air or surface) is needed, the corresponding Infection control department should inform the Air Microbiology Department verbally and using the request form (appendix C) to collect the samples and analysis it. Result will be send back to Infection control department in another result form (appendix D).

For water sampling on a routine basis, a defined annual schedule of all areas required (drinking water points, hydrotherapy, dental units, renal units) according to the considered frequency for each area should be prepared by the corresponding preventive medicine department. For water sampling as a part of outbreak investigation, the corresponding preventive medicine department is called by Infection control department to collect the samples, fill the request form and send it to Public Health Laboratory for analysis. All samples should be accompanied by an appropriate collection form (appendix E). Result will be send back to preventive medicine department which should act upon.

5. TYPES OF SAMPLING

The sampling method and number of samples collected will be influenced by the potential contamination circumstances. The first priority should be to collect samples from locations near the suspected release sources.

5.1. AIR SAMPLING

Sampling protocols should include:

1. Indoor (problem and non problem areas).
2. An outdoor reference samples.

5.1.1. NON CULTURAL SAMPLING: (Air borne and non viable particulates)

5.1.1.1. Indoor Air Sampling

- Air quality methodologies imply testing in representative areas to determine airborne particulates influences and access general indoor air quality parameters. Testing is conducted to access airborne particulates using laser particle counter or optical counters which quantifies particulates by size (0.3 to 10.0 microns).
- Results are comparatively evaluated to determine particulates concentration by size, the efficiencies of the air filtering systems and to identify areas with elevated particulate concerns (e.g. fungal influences are usually suspected when predominantly high concentration are noted between 1.0 - 10 microns).
- Other type of devices such as Allergen co – D is designed for a rapid collection and analysis for air borne particulates including bioaerosols (mold spore, pollen insect parts, skin cell fragments) or particulates such as (cellulose, fiber glass etc) and opaque particles such as (fly ash, oil droplets' etc). This device works using the inertial impaction principles similar to other spore trap devices. These air sampling cassettes designed for evaluating total airborne fungi and non viable particulates. Depending on site conditions, samples are collected at 15 liters/minutes over 5 minutes or 10 minutes sampling periods. Samples are analyzed using direct microscopy examination.

5.1.1.2. Out Door Air Sampling

A comparison of microbial species and densities in outdoor air versus indoor air has been used to help pin point fungal spore bursts. Fungal spore densities in outdoor air vary and the degree of variation based on many factors (season, climate changes, temperature, relative humidity). Seven

day volumetric spore trap “Burkard” is used as a reference device of choice and as a monitoring device for outdoor air borne contaminants method. (Fungal spore).

5.1.2. Cultural Sampling

The objective of this method is to assess the quantities as well as differentiation of viable (alive or / and capable of germination) fungi and bacteria, that can grow on surface of microbiologic media plates.

5.1.3. Equipment and Supplies

Impactors: Commercially available volumetric impactors are available and capable of viable air sampling. The properties and specification of the impactors (particle size cut point, operating flow rate, type of collection media i.e. Agar, etc) are considered.

A volumetric air sampler, Anderson N6 is considered in the following air sampling procedures.

5.1.4. Sampling strategy

Air sampling can provide information that should inform infection control professionals that the air quality is good enough for safe patient care, because the control measures are properly applied.

These factors should be considered before starting the air sampling:

1. Vacuum pump calibrated to sample 28.3L/M (Anderson two stages samplers N6).
2. The air sampler should be checked and cleaned by 70% alcohol before use following the manufacturer’s instruction.
3. The area being sampled should have been left vacant to avoid false-positive results due to recent area usage.
4. The doors must be kept closed prior to and during the air sampling period.
5. Staff should wear a proper disposable coveralls if available or sterile gowns and surgical mask. Shoe cover is acceptable. Hand should be washed and sterile non powdered gloves worn just before entering the room.
6. Using aseptic technique in handling all the sterile equipment should be followed (Agar sampler, swabs etc).

7. Establish laboratory time lines for sample collection, processing method and provision of results.
8. Ensure that adequate supplies are available.
9. Three minutes sampling time is a good general guideline; however sampling time should be adjusted is there if there is visible mold growth or heavy dust particulars. See table 1.
10. Choose the suitable medium for each type of microbial contaminants that is able to process the specimens.

Table (1) Sampling time adjustment according to environmental site and the load of dust particles

Environmental Site	Sampling Time
Ultra clean areas, operation theatre,	4 – 5 minutes (up to 10 minutes)
Normal offices, house holds	3 minutes
Outdoor reference	3 minute
Outdoor days heavy dusty	1-2 minute
Outdoor rainy days	3 – 4 minutes

5.1.5. Sampling procedure

- a) Place the sampler in the middle of the room. Average size of approximately 5 x 5 m².
- b) Connect vacuum pump to the 2 stage. Anderson unit without media plates.
- c) Turn on the pump and allow equilibrating for 1 – 2 minutes.
- d) Always use the same type of media plate in each stage upper and lower of the Anderson 2 stage, sampler unit, for each exposure i.e. (fungi and bacteria have separate exposures).
- e) When opening the Petri plates – place the cover in a specific place “ziploc bag” leave the cover in the bag until the sampling time completed and the plates removed from the Anderson, to prevent contamination then cover again.
- f) 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 drip.

- g) With a permanent marker, label each Petri dish from the bottom with following information's :
- specific location (room number)
 - date
 - facilities (IV lab, operation theatre etc)
 - duration of sampling (in minutes)
 - label upper plate and lower plate in the 2 stage Anderson Sampler (Respirable and Non respirable)
- h) Plates should be kept cool after collection and during transportation to laboratory to prevent growth of contaminants.
- i) Samples “Agar plates” are then incubated as soon as possible at 37°C for 24 – 48 hours, for the environmental bacteria. And at 27°C for 5 – 14 days for maximum recovery of fungi.

Note: Preliminary culture results are rarely available until after 24 hours incubation.

- j) After incubation, the total number and the type of colonies in each plate are counted, and analyzed for their species.
- k) The mean number of viable bacteria or fungi by cubic meter (m^3) of air can be calculated by knowing the air sample flow rate ($28.3 L/m^3$) and the sampling time, and the results will be written as colony forming unit per cubic meter of air (CFU/ m^3).
- l) Documentation of all the results should be done and sent to the infection control department as soon as possible.

5.2. SURFACE SAMPLING “ SWAB SAMPLES”

- Surface sampling is a non destructive methods which allows the determination of possible microbial contamination of suspected sites or and un transportable materials,
- Several methods can be applied for collecting environmental surface samples (i.e. tape – lift method and swab method)
- Swab method has the advantage of enabling a sample to be taken in a hard to reach area(s) without a removal of any material and it is a neutral, stable way to transport the sample.

-Swab sampling is performed using pre sterile swabs in various transport media that are commercially available.

5.2.1. Pre Sample collection

These factors should be considered before starting the environmental surface sampling:

- Background information (sampling objective (a) outbreak (b) renovation).
- Determine the target microorganism (s) in case of outbreaks.
- Location of surfaces to be sampled.
- Method of sample collection and the appropriate equipment or supplies for each task.
- Weather the sampling will be qualitative or/and quantitative.
- An estimate of maximum allowable microbial numbers or types on the sampled surfaces. (Clinical control points).
- If disinfectant residuals are expected on surfaces being samples, specific neutralizer, chemical should be used in both.
- Non sterile equipment used for sampling should be sanitized before sample collection (e.g. cooler, sample boxes, ice packs, sampling machine).
- Arrange all the supplies and equipments needed.
 - Sterile non powdered vinyl gloves (should be replaced as often as necessary).
 - A sterile non cotton or cotton swabs with transport media or dry swabs for moistened areas.
 - Sterile sponges for large surfaces.
 - Neutralizing buffer, sterile diluents, sterile water, sterile saline solution, and sterile peptone water to be used for moistening the swabs or sponges.
 - Alcohol.
 - The appropriate medium for each study case.

5.2.2 Sampling Procedure

1. Wear the appropriate clothing.
2. Wear the non powdered sterile gloves.

3. Remove a sterile, cotton swab from the package.
4. Moisten the swab with (sterile water, peptone water, or transport media).
5. Wipe the surface to be sampled by touching the cotton tip to the surface and rotating and rubbing back and forth for the deep irregular areas.
6. For the flat surfaces, the suggested sampling technique is to make a vertical S – strokes to cover an area of 10 x 10 cm². Figure (1)
7. Place the swab in its tube taking care not to touch the tip or the shaft of the swab.
8. Label tube containing the swab with date, specific location, and facility).
9. Transport the samples to laboratory for analysis as soon as possible, and samples should be kept in a refrigeration temperature (4°C). Do not freeze.
10. The appropriate methodology for testing micro organism should be followed to detect the organism(s) of concern (fungi / bacterial).
11. Documentation of the results sends to the health care setting personally.

5.3. WATER SAMPLING

5.3.1 Sampling methods for bacteriological testing

All samples should be accompanied by an appropriate collection form. When water samples are collected for analysis, care should be taken to ensure that there is no external contamination of the samples. Unless valid samples are collected, the results of the subsequent analysis may be misleading.

5.3.2 Sampling Procedure

5.3.2.1 Visual inspection

Visual inspection of the site should cover the following aspects:

- 1) Potential pollution sources (industrial, agricultural, domestic, animals)
- 2) Condition of water (clear, cloudy, color, smell, foam)
- 3) Map of Site (electronic or sketched, photograph if possible)
- 4) Brief written description of site.

5.3.2.2 Sampling and control

1. Sample all sites in triplicate.
2. Collect all samples in 100mL or 1L Whirlpak bags.
3. All samples must be clearly labelled by site, number of sample, and status as field blank (FB) or lab blank (LB). Use an indelible marker to label all bags before trying to take sample (wet plastic does not take ink well). In addition to the sample IDs, all samples should be dated.
4. If sampling a body of running water, point the mouth of the bag upstream and your hands downstream to avoid contamination.
5. If sampling from a water faucet, run the faucet for 1 minute before obtaining a sample.
6. Rinse the bag twice with the sample water prior to filling and closing.
7. Fill bag as full as possible. Half-filling the bottle leaves more room for oxygen which will promote degradation of your sample.
8. Collect data such as temperature and pH which affect the solubility of many ions. This is particularly important if an investigation is being carried out to determine the source of Legionella. Hot water should reach 50°C within 1 minute at outlets, whilst cold water should be 20°C or below after running the water for two minutes.

5.3.3 Preserving and Storing your Sample:

Storage before filtration should be at 4 °C for not more than 24 hours.

5.3.4 Sampling containers and its preparation

- Several types of bottle may be used for sampling, but glass bottles are best.
- These should have securely fitting stoppers or caps with nontoxic liners, and both bottles and stoppers should be sterilized.
- Each cap should have a metal sleeve clear of the screw thread to ensure that the risk of contaminating the water sample is minimized.
- Cotton wool plugs and paper caps should be avoided as they tend to fall off during and after sampling and increase the risk of contamination.
- The bottles should hold at least 200 ml of water.
- Whenever chlorine is used for disinfection, chlorine residual may be present in the water after sampling and will continue to act on any bacteria in the sample; the results of the microbiological analysis may

therefore not be indicative of the true bacteriological content of the water. To overcome this difficulty, it is common procedure to add sodium thiosulfate to the sample, which immediately inactivates any residual chlorine but does not affect the microorganisms that may be present.

- The sodium thiosulfate should be added to the sample bottles before they are sterilized. For 200-ml samples, four or five drops of aqueous sodium thiosulfate solution (100 g/liter) should be added to each clean sample bottle.
- The stopper is loosely inserted into the bottle, and a brown paper or aluminum foil cover is tied to the neck of the bottle to prevent dust from entering.

5.3.5. Types of water sampling

5.3.5.1 Sampling of Drinking Water

5.3.5.1.1 Location of sampling points

In selecting sampling points, each locality should be considered individually; however, the following general criteria are usually applicable:

- Sampling points should be selected such that the samples taken are representative of the water source, treatment plant, storage facilities, distribution network, points at which water is delivered to the consumer, and points of use..
- These points should include those that yield samples representative of the conditions at the most unfavorable sources or places in the supply system, particularly points of possible contamination such as unprotected sources, loops, reservoirs, low-pressure zones, ends of the system, etc.
- Sampling points should be uniformly distributed throughout a piped distribution system, taking population distribution into account; the number of sampling points should be proportional to the number of links or branches.
- Sampling points should be located in such a way that water can be sampled from reserve tanks and reservoirs, etc.
- In systems with more than one water source, the locations of the sampling points should take account of the number of inhabitants served by each source.
- There should be at least one sampling point directly after the clean-water outlet from each treatment plant.

5.3.5.1.2 Sampling frequency

The recommended minimum frequencies for these measurements for piped drinking water in the distribution system are once monthly.

The same frequency of sampling can be applied to piped tap water in healthcare facilities.

5.3.5.2 Renal unit water

Bacteriologic assays of renal unit water should be performed at least once a month and during outbreaks by using standard quantitative methods. In conjunction with microbiologic testing, Endotoxin testing should be performed. Renal unit water include the water used to prepare dialysis fluid (Ultra-pure) and water used to rinse and reprocess dialyzers.

If the sample is to be collected from a tap used solely for sampling ensure that this has been appropriately sanitized.

- a. Aseptically open a labeled sterile water bottle (usually 500 ml bottle containing neutralizer) and fill almost to the brim with water; replace the lid.

Note: If only small volumes of liquid are available for sampling, a smaller sterile plastic container can be used, as neutralizer is not essential for this sample type.

- b. Store the water between 2 and 8 C and return to the lab for examination ideally on the same day but always within 24 hours of collection.

5.3.5.3 Swimming and hydrotherapy pool water

The recommended minimum frequencies for these critical measurements minimum sample numbers for and hydrotherapy is weekly.

5.3.5.4 Dental unit water

Interest in the contamination of dental unit water lines (DUWLs) is growing amongst dental researchers. The recommended frequency of sampling is once monthly.

5.3.5.5 Pharmacy water

The recommended minimum frequency for the pharmacy where water can be used for preparation of medications is once weekly.

6. ISOLATION AND IDENTIFICATION OF MICROORGANISMS

MEDIA REQUIRED:

There are a number of different media types available depending on the sampling objective, unless the microorganism of specific type are suspected, the sampling medium should support growth of the widest variety of common fungi and bacteria, selective media to be used for certain type of contaminations.

- PDA (potato dextrose agar) for general mold identification.
- SDA (sabroud dextrose agar) also general mold identification and yeast.
- BA – (blood agar – tryptsoy agar + 5% sheep blood) for general bacterial culture.
- NA - (nutrient agar) used for sampling and cultivation of general bacteria.
- MH (Muller Hinton agar) for susceptibility testing.
- MAC (macConkey agar) selective medium for Gram-negative bacteria.

D -MICROSCOPIC EXAMINATION FOR ALL GROWN COLONIES, FUNGI OR BACTERIA:

- Gross appearance
- Morphological appearances by microscope
- Identification as will be described below.

I. Gram-positive bacteria

1. *Staphylococcus aureus*

A. Media used for isolation of *Staphylococcus aureus*:

Blood agar and Mannitol salt agar incubated in air at 37°C for 24hour. *Staphylococcus aureus* appear as yellow colonies on Mannitol salt agar and white creamy color on blood agar.

B. Identification:

- i. Gram-stain: Gram-positive cocci in clusters

- ii. Catalase test: All Staphylococci are Catalase positive.
- A loopful of growth is transferred from an agar plate culture to a glass of microscope slide. Evolution of bubbles on addition of a drop of 3% H₂O₂ signifies a positive result. *S. aureus* is used as a positive control and Streptococci as a negative control.
- iii. Coagulase: *S. aureus* is Coagulase positive
- Slide Coagulase: place one drop of normal saline solution on a clean microscope slide and emulsify in it 1-2 colonies of the tested organism. Add one drop of undiluted rabbit plasma warmed to room temperature and stir the adhering traces of plasma into the drop of bacterial suspension on the slide with a sterile loop. A positive reaction appears as coarse clumping visible to the naked eye within 5-10 seconds. While a negative reaction does not show any coarse clumping. With each patch put a known positive control (*S. aureus*) and negative control (*S. epidermidis*).
 - Tube Coagulase: prepare a 1:10 dilution of rabbit plasma in normal saline solution and place a 0.5ml of the diluted plasma in small tubes. Inoculate into the diluted plasma with 1-2 colonies of the strain under test. With each patch put a known positive control (*S. aureus*) and negative control (*S. epidermidis*). Then incubate the tubes at 37°C in air and examine after 4 hours incubation. Check for a clot or a stiff gel formation. A clot formation indicate a positive reaction (*S. aureus*). If a clot is not present, keep the tubes overnight at room temperature and check for the presence of a clot next day. A clot formation indicates a positive reaction (i.e. *S. aureus*) and absence of the clot indicate a negative reaction (i.e. *S. epidermidis*).

If Coagulase test is not available , then use DNase test.

- Deoxyribonuclease test (DNase): heavily spot inoculation several colonies of the test organism on DNase test medium containing the

metachromatic dye toluidine blue O. After 24 hr incubation at air at 35°C, the medium under and around the inoculums turns from azure blue to pink, indicating the hydrolysis of the DNA. The content of the toluidine blue in the medium should not exceed 0.005% because the blue color imparted to the agar by higher concentrations may mask detection of DNase activity.

- iv. Susceptibility testing to Methicillin: by disk diffusion method and using oxacillin 1µg disk on Mueller-Hinton agar following CLSI methodology. A suspension of the bacterium is prepared in Normal saline (0.9%) to a turbidity equivalent to 0.5 McFarland standards and then streaked on Mueller-Hinton agar. Then oxacillin disk is applied on the inoculated plate. Another plate is inoculated with a control *S. aureus* strain (*S. aureus* ATCC 25923) and oxacillin disc. Both plates will be incubated overnight in air at 35°C. The interpretation will be read according the zone diameter as follows: ≥ 13 mm sensitive, 11-12 mm intermediate, ≤ 10 mm resistant.

2. Coagulase negative staphylococci

Catalase positive

Coagulase negative

DNase negative

Susceptibility testing: as section iv

3. *Micrococcus* spp.:

A. Media used for isolation: blood agar

B. Identification:

- Pigment production (white to yellow pigmentation)
- Colony morphology
- Gram stain: Gram positive cocci in clusters, pairs, tetrads or cubes
- Catalase test : positive
- Oxidase test: positive

4. Other Gram-positive cocci: e.g. *Kocuria* and *Kytococcus* spp.

A. Media used for isolation: blood agar

B. Identification

- Colony morphology
- Gram stain: Gram-positive cocci in tetrads
- Pigment production: *Kocuria* spp. produce pale crème to orange, yellow, or pink to red pigmentation. *Kytococcus* spp. produce creamy white to yellow pigment.

5. Gram positive bacilli e.g. *Bacillus* spp.

A. Media used for isolation: blood agar

B. Identification:

- Colony morphology: irregular, raised, rhizoid, dry, and rootlike outgrowth from the colony margin.
- Gram stain: Gram positive-bacilli with spore formation

II. Gram-negative bacilli

e.g. *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacteriaceae* e.g. *E. coli*, *Klebsiella pneumoniae*, *Serratia* spp.

A. Media used to isolate Gram negative bacilli: MacConkey

B. Identification: via API 20E (for *Enterobacteriaceae*) and API 20NE for non-fermenters. Methodology: according to the manufacturer's instructions.

III. *Legionella pneumophila*

A minimum of 200 ml of water should be collected (after a swab sample) and 100ml is filter concentrated using a 47-mm, 0.2- μ -pore size polycarbonate filter. A reducing agent (i.e., sodium thiosulfate [Na₂S₂O₃]) needs to be added to neutralize residual chlorine or other halogen in the collected sample.

A. Media used for isolation :

Buffered Charcoal-Yeast Extract agar (BCYE) buffered with ACES [N-(2-acetamido)-2-aminoethane sulfonic acid] (10 mg/ml), and supplemented with L-cysteine (400mg/ml), ferric pyrophosphate (250 µg/ml), α-ketoglutaric acid (1 mg/ml) and activated charcoal (pH 6.9). It should also include antifungal agent cycloheximide. The plates will be incubated in 5-10%CO₂ incubator at 35-37°C and examined every 2 days for 1 week.

B. Identification:

- i. Gram stain: thin Gram-negative bacilli
- ii. Colony morphology: colonies are variable in size, glistening, convex, circular colonies
- iii. L-cysteine requirement: when subcultured under unsupplemented 5% sheep blood agar or L-cysteine deficient (BCYE) media or MacConkey agar, *Legionella pneumophila* will not grow in these media.
- iv. Direct fluorescent antibody testing.

NB. In case of outbreak investigation, send all isolates to Microbiology Laboratory of the involved hospital.

7. Interpretation and standard of results

7.1 Air Sampling

Hospital environments that require sampling include: OT, ICU and PE. Acceptable bacterial and fungal counts are listed in the following table (2) and (3).

Table (2): Environmental monitoring limits for controlled areas and devices in operation

Grade of controlled area / device*	Finger dabs (cfu/hand)	Active air sample (cfu/m ³)	Active air sample (cfu/m ³)	Contact plate (55mm) (cfu/plate)	Settle plates (90mm) (cfu/4hrs)
A	<1	<1	<1	<1	<1
B	N/A	10	10	5	5
C	N/A	100	100	25	50
D	N/A	200	200	50	100

*Grade A: The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a laminar air flow work station.

Grade B: For aseptic preparation and filling, this is the background environment for the grade A zone.

Grade C and D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

Table (3): Testing requirements and interpretation of results air quality (as determined by active air sampling)

Area	Hazard / Indicator	Result	Interpretation	Action
ICU	Total bacterial	counts < 50 cfu/m ³	Satisfactory	N/A
		counts > 50 cfu/m ³	Unsatisfactory	Take ICU out of use, clean thoroughly and re-test
	<i>Aspergillus</i>	Counts < 5 cfu/m ³	Satisfactory	N/A
		counts > 5 cfu/m ³	Unsatisfactory	Take ICU out of use, clean thoroughly and re-test
PE	Total bacterial	Counts < 35 cfu/m ³	Satisfactory	N/A
		counts > 35 cfu/m ³	Unsatisfactory	Take room out of use, clean thoroughly and re-test
	<i>Aspergillus</i>	no single <i>Aspergillus</i>	Satisfactory	N/A
		counts > 0.1 cfu/m ³	Unsatisfactory	Take room out of use, clean thoroughly and re-test
Conventionally (empty) ventilated theatre	Total bacterial	Counts < 35 cfu/m ³	Satisfactory	N/A
		counts > 35 cfu/m ³	Unsatisfactory	Take theatre out of use, clean thoroughly and re-test
	<i>Aspergillus</i>	no single <i>Aspergillus</i>	Satisfactory	N/A
		counts > 0.1 cfu/m ³	Unsatisfactory	Take theatre out of use, clean thoroughly and re-test
Ultra-clean theatre (air leaving final diffuser)	Total bacterial	≤0.5 cfu/m ³	Satisfactory	N/A
		counts > 0.5 cfu/m ³	Unsatisfactory	Take theatre out of use, clean thoroughly and re-test
	<i>Aspergillus</i>	no single <i>Aspergillus</i>	Satisfactory	N/A
		counts > 0.1 cfu/m ³	Unsatisfactory	Take theatre out of use, clean thoroughly and re-test

7.2 Surface Sampling

When interpreting the results of the sampling, the expected degree of microbial contamination associated with the various categories of surfaces in the Spaulding classification must be considered.

Aerobic colony count (ACC)

The total ACC is the total number of aerobic organisms from a sampled area. It can be quantified and provides a general measure of bacterial load. The internationally recognized figure of < 5 cfu/cm² could be used as a starting cut point.

A visual pass

For a surface being graded as 'clean' is based on the absence of visual soiling, presence of moisture, staining or poor surface condition (derived from ICNA guidelines).

ATP pass

An ATP pass rate is a bioluminescence result of <500 RLU.

MRSA pass

An MRSA pass rate results from the absence of detection of MRSA on a surface.

Presence of an 'indicator' organism

Possible indicator organisms are *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), *Clostridium difficile*, multiply resistant Gram-negative bacilli, vancomycin resistant enterococci and *Salmonella* spp. Also organisms associated with a significant infection risk in a clinical area, or associated with a serious infection incident or outbreak, e.g. *Aspergillus* spp in units housing immunocompromised patients.

There should be 1 cfu/cm² of the indicator organism(s) present in the clinical environment.

The identification of an indicator organism should generate immediate attention towards cleaning/disinfection practices and frequencies. Repeat sampling is mandatory. Risk assessment would determine a hygiene review, additional cleaning, or even the closure of a clinical area for deep cleaning if thought appropriate.

7.3 Water Sampling

a. Renal unit water

The renal unit water should be tested to determine whether it meets the minimum requirements for microbiological contamination at least once per month.

Table (4): Testing requirements and interpretation of results for renal dialysis fluid

Hazard / Hygiene Indicator	Timing / Frequency of Testing	Result	Interpretation	Action
<i>Dialysis fluid and post reverse osmosis fluid:</i>	Monthly (or more frequently if necessary)			
Aerobic Colony Count	Monthly (or more frequently if necessary)	>50 / ml	UNSATISFACTORY	Take out of use, investigate cause and put corrective action in place
		0 - <50 / ml	SATISFACTORY	N/A
Endotoxin /ml		>0.25 EU/ml	UNSATISFACTORY	Take out of use, investigate cause and put corrective action in place
		<0.25 EU/ml	SATISFACTORY	N/A
<i>Ultrapure fluid:</i>	Monthly (or more frequently if necessary)			
Aerobic Colony Count		>1 in 10 ml	UNSATISFACTORY	Investigate cause and put corrective action in place
		<1 in 10 ml	SATISFACTORY	N/A
Endotoxin /ml		>0.03 EU/ml	UNSATISFACTORY	Investigate cause and put corrective action in place
		<0.03 EU/ml	SATISFACTORY	N/A

b. Drinking/tap water

Tables 5 indicate testing requirements and interpretations of results for a variety of common water sample types from the hospital environment. For Legionella, it should be <100cfu/L.

c. Water in dental unit

Dental unit water should be sampled on a monthly basis. Organized dentistry has suggested that the standard for water from dental units should be less than 200 CFU/mL.

d. Swimming and hydrotherapy pool water

Refer to table 5

Table (5): Testing requirements and interpretation of results for drinking, tap, swimming and hydrotherapy pool water samples

Hazard / Hygiene Indicator	Timing / Frequency of Testing	Result	Interpretation	Action
<i>Escherichia coli</i>	Weekly for hydrotherapy and swimming pool and monthly when in use for drinking water, and tap water (not first thing in the morning before it is used)	>0 in 10 ml	UNSATISFACTORY	Investigate immediately and take repeat sample
		0 in 10 ml	SATISFACTORY	N/A
Coliform bacteria (Total Coliforms)		> 0 in 10 ml	UNSATISFACTORY	Investigate immediately and take repeat sample
		0 in 10 ml	SATISFACTORY	N/A
<i>Pseudomonas aeruginosa</i>		> 0 in 10 ml	UNSATISFACTORY	Investigate and take repeat sample. If the same then close pool and seek advice on remedial actions required
		0 in 10 ml	SATISFACTORY	N/A
Aerobic Colony Count		> 100 / 10 ml	UNSATISFACTORY	Take repeat sample And investigate
		0 - <100 / 10 ml	SATISFACTORY	N/A

e. Water in pharmacy

Water sampled from the pharmacy can be taken from the drain fluid, tap water or from distiller.

Table (6) Testing requirements and interpretation of results for pharmacy samples

Hazard / Hygiene Indicator	Timing / Frequency of Testing	Result	Interpretation	Action
Tap water – Aerobic Colony Count	weekly	Any abnormal change		Investigate cause. Alert/action limits determined locally. Also should be determined by trend analysis & characteristics, significance & source of isolates.
Tap water- Coliform bacteria (Total coliforms)		> 0 in 10 ml	Unsatisfactory	Note: it is not useful to take samples for Aerobic Colony Count unless to determine whether a disinfection has been successfully achieved
	0 in 10 ml	Satisfactory		

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VI. APPENDICES

Appendix A. Preparation of area prior to sampling

I. General principles

1. All new or refurbishment work has been completed;
2. All engineering commissioning procedures have been completed;
3. Ducting and air diffuser plates have been cleaned.

II. Cleaning and disinfection

1. Wash hands before and after cleaning.
2. Gloves and a plastic apron must be worn
3. Wet cleaning is recommended and drying is essential.
4. High dusting:- All surfaces above shoulder height should be dusted with a damp mop to prevent dust from being dispersed.
 - Mops must never be shaken.
 - To prevent missing spots, work should proceed either clockwise or anti clockwise from the starting point or in a straight line from left to right using one-directional stroke.
5. The areas should be cleaned first using a neutral detergent and warm water.
6. After rinsing, use 5.25-6.15% sodium hypochlorite in a dilution 1:100 which provides 525-615 ppm chlorine to decontaminate surfaces.
7. Areas that should be cleaned include : floor, wall, door knobs, ceiling, duct opening, duct covers, windows and equipment.
8. Clean all equipment in the area according to manufacturer's instructions.
9. Ducting should be cleaned and air diffuser plates (outlets and inlets) should be put down and cleaned by cleaners for 48 hr before putting it back.
10. Used the gloves, apron / towels should be dispose in to yellow waste bag.
11. Keep area dry
12. These instructions should be repeated every shift (3x a day) for at least three days.
13. Keep the ventilation system running continuously for 24 hours (Temp < 24 °C) throughout the cleaning process and environmental sampling.
14. Close area at least 24 hours prior to environmental screening.

Appendix B. Environmental Checklist for Monitoring Cleaning Prior to Sampling

Date:	
Unit:	
Room Number:	

Evaluate the following priority sites for each patient room:

High-touch Room Surfaces*	Cleaned	Not Cleaned	Not Present in Room
Bed rails / controls			
Tray table			
IV pole (grab area)			
Call box / button			
Telephone			
Bedside table handle			
Chair			
Room sink			
Room light switch			
Room inner door knob			
Bathroom inner door knob / plate			
Bathroom light switch			
Bathroom handrails by toilet			
Bathroom sink			
Toilet seat			
Toilet flush handle			
Toilet bedpan cleaner			

Evaluate the following additional sites if these equipment are present in the room:

High-touch Room Surfaces*	Cleaned	Not Cleaned	Not Present in Room
IV pump control			
Multi-module monitor controls			
Multi-module monitor touch screen			
Multi-module monitor cables			
Ventilator control panel			

Mark the monitoring method used:

- Direct observation Fluorescent gel
 Swab cultures ATP system Agar slide culture

*Sites most frequently contaminated and touched by patients and/or healthcare workers

Head nurse signature _____

Appendix D. Result form

State of Kuwait

Ministry of Health

Environmental Sampling Result Form

Date:.....

Hospital Name:

Place of sampling:.....

I. Air samples results

Total number of samples:.....

Location room/place	Isolated microorganism	Result (cfu/m ³)	Interpretation	Action
	Total bacteria			
	<i>Aspergillus</i>			
	Total bacteria			
	<i>Aspergillus</i>			
	Total bacteria			
	<i>Aspergillus</i>			
	Total bacteria			
	<i>Aspergillus</i>			
	Total bacteria			
	<i>Aspergillus</i>			
	Total bacteria			
	<i>Aspergillus</i>			
	Total bacteria			
	<i>Aspergillus</i>			
	Total bacteria			
	<i>Aspergillus</i>			

Appendix E. Water sampling request and result form

Hospital /center : Place of sampling:.....

Source:.....Sample number:

Date of sampling :..... /..... /.....Time :.....

Date of receiving the sample :..... /..... /.....Time :.....

Type of sample: Renal unit: Dialysis fluid Renal unit: Ultrapure fluid Pharmacy
 Drinking water Tap water Dental unit
 Swimming pool Hydrotherapy

Required test :.....

Extra information :.....

Name and title of sampler :.....Doctor signature :.....

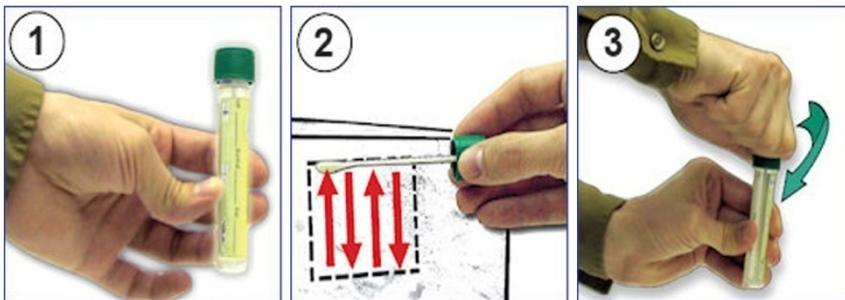
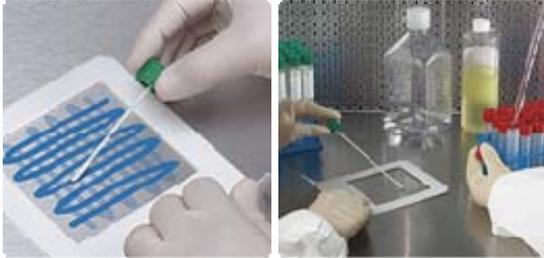
Test	Result	Interpretation
Free Chlorine (PPM) / PH		
Aerobic Colony Count		
Coliform bacteria		
Pseudomonas aeruginosa		
Escherichia coli		
Endotoxin /ml		
Others		

Laboratory tests were done by:Date:

Recommendations of Preventive medicine department :

.....Doctor signature :.....Date:.....

Appendix F. Figure (1) The suggested sampling technique for the flat surfaces



Appendix G. Table (7) Gram-negative bacteria associated with water and moist environment

Organism	Implicated contaminated environmental vehicle
<i>Pseudomonas aeruginosa</i>	Potable (tap) water\ Distilled water Antiseptic solutions Contaminated with tap water, Sinks, hydrotherapy pools, whirlpools and whirlpool spas, water baths, lithotripsy therapy tanks, Dialysis water, Eyewash stations, Flower vases, Endoscopes with residual moisture in the channels
<i>Burkholderia cepacia</i>	Distilled water Contaminated solutions and disinfectants Dialysis machines Nebulizers Water baths Intrinsically-contaminated mouthwash Ventilator temperature probes
<i>Stenotrophomonas maltophilia,</i> <i>Sphingomonas spp.</i>	Distilled water Contaminated solutions and disinfectants Dialysis machines Nebulizers Water Ventilator temperature probes
<i>Ralstonia pickettii</i>	Fentanyl solutions Chlorhexidine Distilled water Contaminated respiratory therapy solution
<i>Serratia marcescens</i>	Potable water Contaminated antiseptics (i.e., benzalkonium chloride and chlorhexidine) Contaminated disinfectants (i.e., quaternary ammonium compounds and glutaraldehyde)
<i>Acinetobacter spp.</i>	Medical equipment that collects moisture (e.g. mechanical ventilators cool mist humidifiers, vaporizers, and mist tents) Room humidifiers Environmental surfaces
<i>Enterobacter spp.</i>	Humidifier water Intravenous fluids Unsterilized cotton swabs Ventilators Rubber piping on a suctioning machine Blood gas analyzers

Appendix H. Table (8) Air borne fungi associated with the transmission of infection, and their implicated environments at Kuwait health care settings.

Microorganism	Implicated Environment
<i>Aspergillus spp.</i>	Cooling coils Floor dust Dust in duct work Evaporative air cooler HVAC fiber-glass insulation Cooling system & coils fans Ventilated systems Air filters and their frames Window air conditioning Air exhaust system False ceiling Fibrous insulations for ceilings Acoustic ceiling tiles Damp wood building materials Opening doors to construction sites Open window Hospital vacuum cleaner Elevator Walls Ornamental plants Food
<i>Mucor spp.</i>	Fans dust Air filters False ceiling Ventilation system house dust HVAC system dust, Dust in duct work They are rapid growers
<i>Cryptococcus spp.</i>	Improperly functioning ventilation system. Air filters Floor dust
<i>Fusarium spp.</i>	Often associated with humidifiers edge of the bath or shower stall or even on the ceiling in bathrooms. Filters, fans, duct work dust Floor dust Humidifier water
<i>Cladosporium spp.</i>	Carpet and floor dust, walls, moist building materials, metal surfaces, duct work dust, evaporative air coolers, HVAC fiberglass insulation

<i>Pencillium spp.</i>	On wallpaper, latex painted surfaces, dust, air conditioners. Evaporative air coolers Air conditioners, Humidifier water painted surfaces HVAC ducts Filters, fans
<i>Acronium spp.</i>	Humidifier water, fiber-glass insulation
<i>Alternaria spp.</i>	Cooling coils, filters, paint, drywall paper, ceiling tiles and wood it can be found in house dust, carpet, damp areas around showers and window frames, and anywhere condensation occurs.
<i>Aureobasidium</i>	Found in humid areas such as bathrooms, kitchens, poorly maintained HVAC systems, and window frames. Moist Building materials, latex painted surfaces, filters
<i>Epicoecum spp.</i>	Fiberglass-insulation
<i>Stachybotrys spp.</i>	Moist Building materials, fans & humidifier water. Water-damaged cellulose rich materials, such as sheet rock, paper, ceiling tiles, insulation backing, gypsum board, and wallpaper.
<i>Chaetomium spp.</i>	HVAC Fiber-glass insulation filters, dust in duct work
<i>Rhizopus spp.</i>	Floor dust, Fans, filters, dust in duct work
<i>Ulocladium spp.</i>	Humidifier water, Floor dust , filters
<i>Trichoderma spp.</i>	Grow indoors on water-damaged building materials, Fans, filters, duct work dust
<i>Eurotium spp</i>	Gypsum based finishes
<i>Scopulariopsis spp.</i>	Floor dust, Filters
<i>Paecilomyces spp.</i>	Humidifier water
<i>Phoma spp.</i>	Humidifier water, dust, filters, paint mildew
<i>Rhizopus spp.</i>	Dust in duct work Filters, floor dust fans

Appendix I

Water sampling from three basic source types

1. Water from a tap in a distribution system or from a fixed pump outlet, etc.
2. Water from a watercourse (river, lake, etc.) or a tank
3. Water from a dug well, etc., where sampling is more difficult than from an open watercourse.

1. Sampling from a tap or pump outlet

- A. Clean the tap
- B. Open the tap
- C. Sterilize the tap
- D. Open the tap before Sampling
- E. Open the sterilized bottle
- F. Fill the bottle
- G. Stopper or cap the bottle

2. Sampling from a watercourse or reservoir

- A. Open the sterilized bottle as described in section. 1.
- B. Fill the bottle

3 Sampling from dug wells and similar sources

- A. Prepare the bottle
- B. Attach the bottle to the string
- C. Lower the bottle
- D. Fill the bottle
- E. Raise the bottle

1. Sampling from a tap or pump outlet

A. Clean the tap

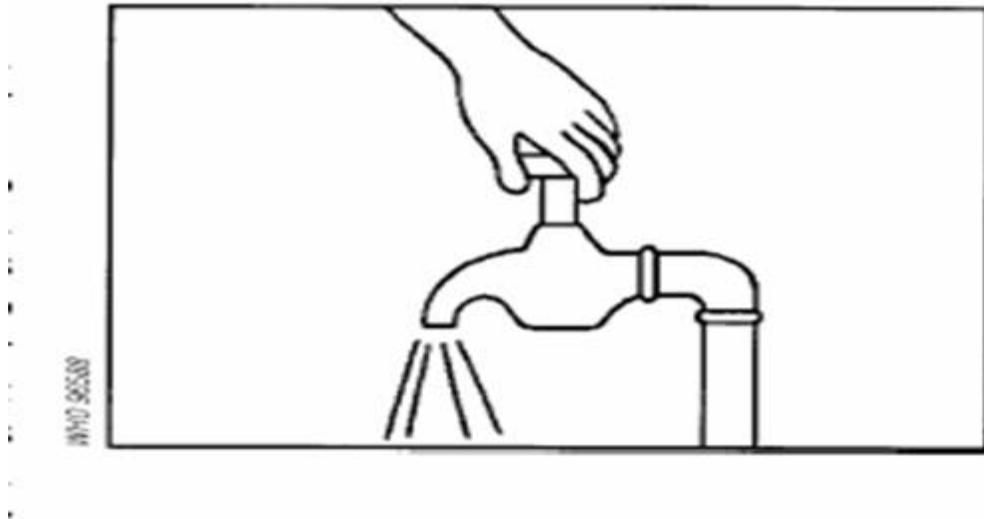
Remove from the tap any attachments that may cause splashing. Using a clean cloth, Wipe the outlet to remove any dirt.



B. Open the tap

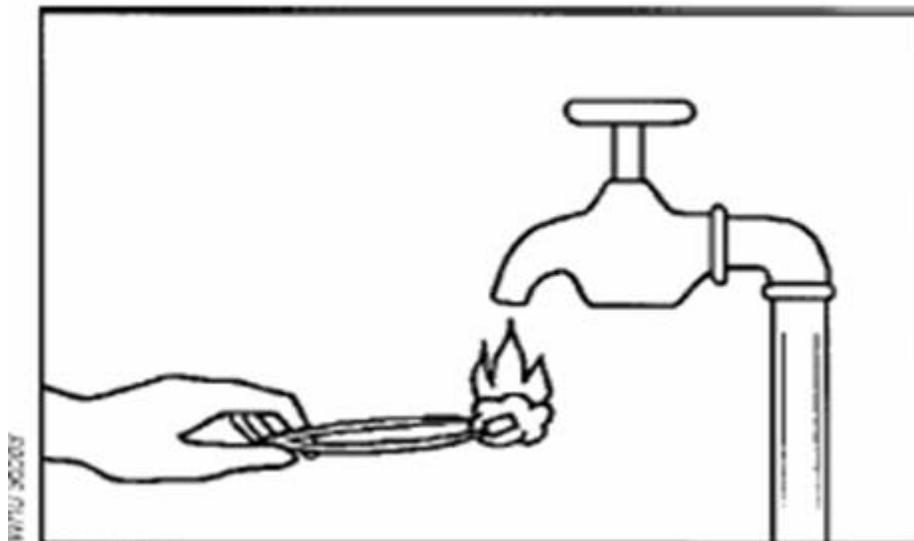
Turn on the tap at maximum flow and let the water run for 1–2 minutes.

Note: Some investigators do not continue to stages C and D but take the sample at this stage; in this case, the tap should not be adjusted or turned off, but left to run at maximum flow. The results obtained in this way will provide information on the quality of the water as consumed. If the procedure is continued to stages C and D, however, the results represent the quality of the water excluding contamination by the tap.



C. Sterilize the tap

Sterilize the tap for a minute with the flame from a gas burner, cigarette lighter, or an ignited alcohol-soaked cotton-wool swab.



D. Open the tap before Sampling

Carefully turn on the tap and allow the water to flow for 1–2 minutes at a medium flow rate. Do not adjust the flow after it has been set.



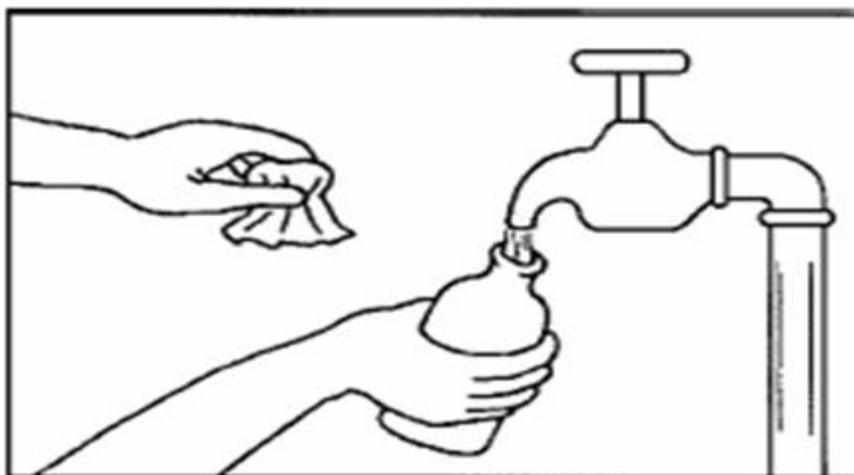
E. Open the sterilized bottle

Take out a bottle and carefully unscrew the cap or pull out the stopper.

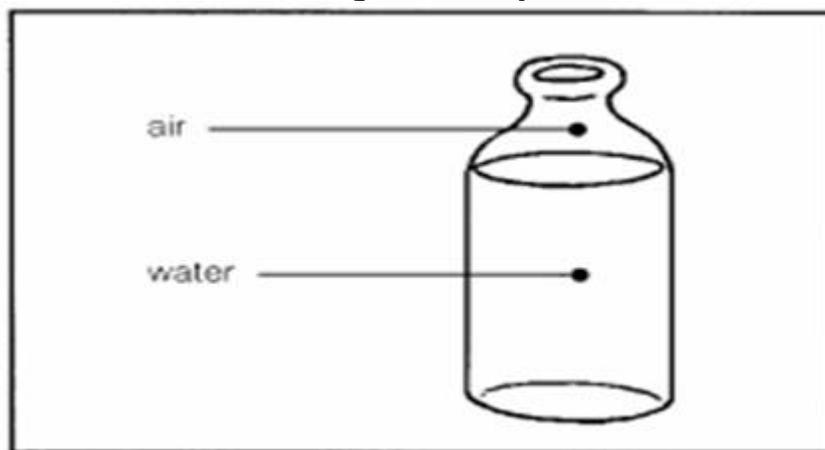


F. Fill the bottle

While holding the cap and protective cover face downwards (to prevent entry of dust, which may contaminate the sample), immediately hold the bottle under the water jet, and fill.



A small air space should be left to make shaking before analysis easier.



G. Stopper or cap the bottle

Place the stopper in the bottle or screw on the cap and fix the brown paper protective cover in place with the string. A small air space should be left to make shaking before analysis easier.

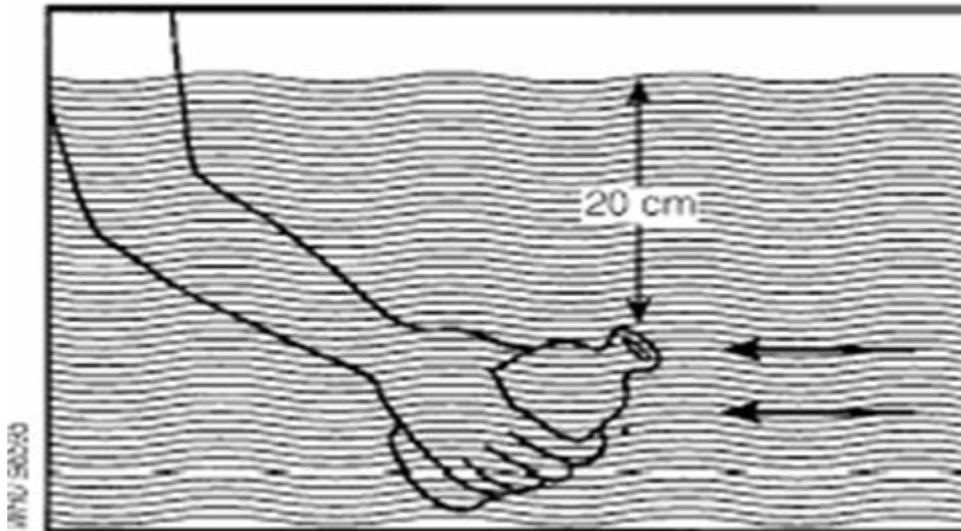


2. Sampling from a watercourse or reservoir

A. Open the sterilized bottle as described in section.1.

B. Fill the bottle

Holding the bottle by the lower part, submerge it to a depth of about 20cm, with the mouth facing slightly upwards. If there is a current, the bottle mouth should face towards the current. The bottle should then be capped or stoppered as described previously.



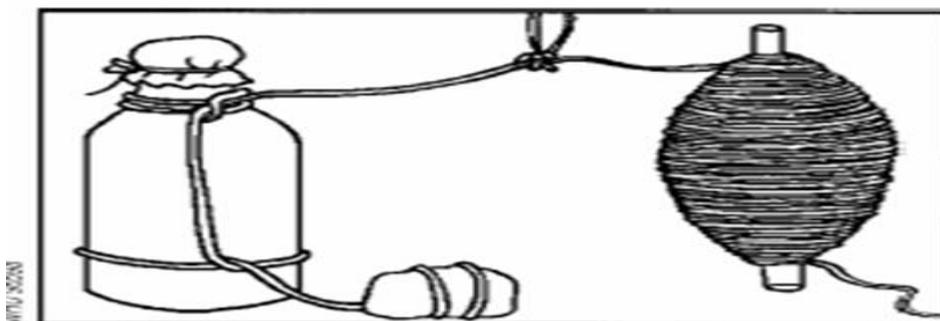
3 Sampling from dug wells and similar sources

A. Prepare the bottle

With a piece of string, attach a clean weight to the sampling bottle.

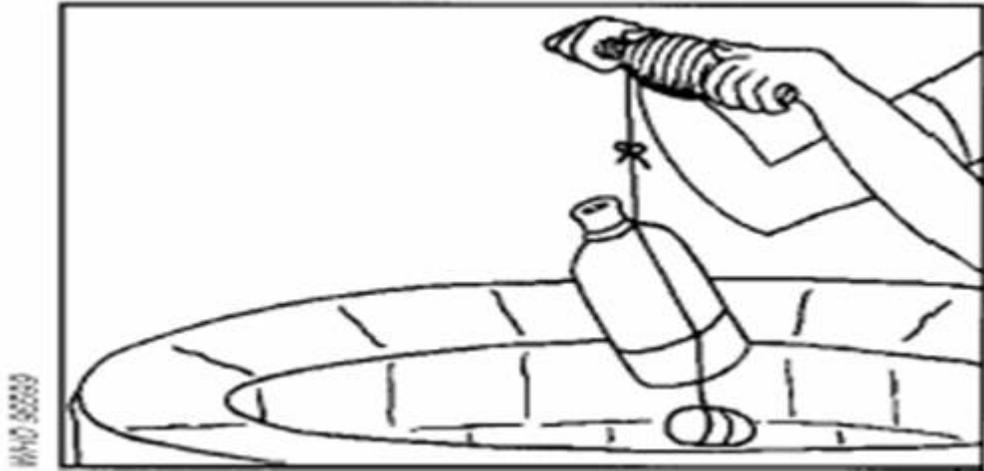
B. Attach the bottle to the String

Take a 20-m length of clean string rolled around a stick and tie it to the bottle string. Open the bottle as described in section



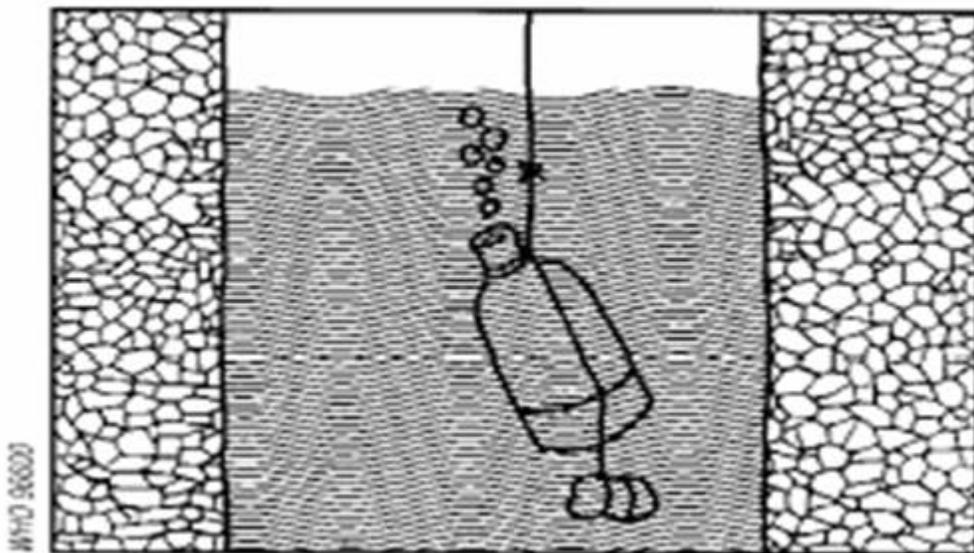
.C. Lower the bottle

Lower the bottle, weighed down by the weight, into the well, unwinding the string slowly. Do not allow the bottle to touch the sides of the well.



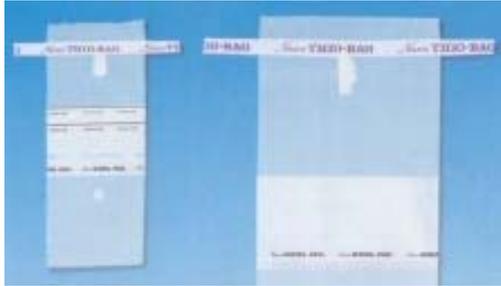
D. Fill the bottle

Immerse the bottle completely in the water and lower it well below the surface without hitting the bottom or disturbing any sediment.



E. Raise the bottle

Appendix J. Thio-Bag EPA Approved for Potable Water Collection



Specially designed for sampling chlorinated water, the Thio-Bag is economical and convenient to use. This single service, sterilized, lightweight, unbreakable container is always ready for immediate use.

It will save...

- **Technician Time**
- **Replacing Sample Containers**
- **Utility Costs**
- **Storage Space**

Used by many states and EPA approved, this strong transparent bag holds a nontoxic, nonnutritive tablet containing 10 mg of active sodium thiosulfate to neutralize chlorine at the time of collection. As an added feature, Thio-Bags have:

- **A Convenient Write-On Strip**
- **Markings for 100 ml Measurements**

These markings help the technician collect the correct amount of water as required by the testing procedure (fill lines are approximate). The write on strip will accept virtually any writing instrument and minimizes ink smearing on the surface. The 4-oz., 100ml size bags are packaged 25 in a sealed bag, four bags per box, for easy use.

Stand-Up Thio-Bags

The job of collecting water samples just got easier Whirl-Pak Stand-Up Thio-Bag! A special gusset on the bottom of the bag flattens out when water is put in bag, allowing it to stand up completely by itself. No rack or other holder is needed to keep the bag upright. The bag contains 10 mg of active sodium thiosulfate to neutralize chlorine in the sample.

