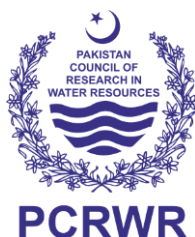


FIELD TRAINING MANUAL FOR MICROBIAL TESTING & TREATMENT OF WATER



Pakistan Council of Research in Water Resources (PCRWR)
2026



FIELD TRAINING MANUAL FOR MICROBIAL TESTING & TREATMENT OF WATER

**Pakistan Council of Research in Water Resources (PCRWR)
2026**

Table of Contents

Part 1.	Water Quality Contamination	2
1.1	Introduction	2
1.2	Impacts of Unsafe Water	3
1.3	Drinking Water Quality Monitoring.....	3
1.4	Key Water Quality Monitoring Parameters	4
1.5	Purpose of the Training Manual	5
Part 2.	Water Quality Sampling for Microbiological Examination	8
2.1	Guidelines for Sampling	8
2.2	Sampling Sites	9
2.3	Sampling Methods for Bacteriological Testing	13
2.3.1	Preparations for Water Quality Sampling	13
2.3.2	Sampling from different water sources.....	16
Part 3.	Test Procedures for Microbial Contamination	21
3.1	Introduction	21
3.2	Indicator Bacteria Used in Drinking Water Monitoring	22
3.2.1	Total Coliforms.....	22
3.2.2	Fecal Coliforms	22
3.2.3	Escherichia coli (<i>E. coli</i>).....	22
3.3	Sampling for testing of Indicator bacteria	23
3.4	Test Procedures	25
Part 4.	Water Quality Decontamination	34
4.1	Key Methods of Decontamination	34
4.2	Safe Drinking Water During Emergencies.....	34
4.3	Emergency Water Treatment Hierarchy	35
4.3.1	Boiling (Most Reliable Method)	36
4.3.2	Water Filtration	36
4.3.3	Solar Water Disinfection (SODIS).....	36
4.3.4	Bio-Sand Filtration System	37

4.3.5	Water Treatment Systems	42
4.3.6	Chlorination.....	44
4.4	Post-Disaster Water Quality Monitoring and Disinfection	45
Part 5.	Application of Chlorine to Water Supplies	47
5.1	Introduction	47
5.2	Chlorine Chemistry and Forms of Residual Chlorine	47
5.3	Chlorine Dose and Chlorine Demand.....	48
5.4	Factors Affecting Chlorination Efficiency	50
5.5	Mode of Action of Chlorine on Microorganisms	51
5.6	Chlorination throughout the Water Supply Chain	52
5.6.1	Chlorination at the Source	52
5.6.2	Chlorination During Storage and Distribution	53
5.6.3	Chlorination at Household Level	53
5.7	Household and Emergency Water Chlorination	53
5.8	Multi-Barrier Approach to Water Safety	54
5.9	Determination of Free Residual Chlorine in Drinking Water	55

List of Figures

Figure 1:	Major Sources and Pathways of Drinking Water Contamination.....	2
Figure 2:	High-Risk Sampling Locations	10
Figure 3:	Uniform Distribution of Sampling Points.....	10
Figure 4:	Representative System-Wide Sampling.....	11
Figure 5:	Reservoir and Storage Tank Sampling	11
Figure 6:	Sampling in Multi-Source Supply Systems	11
Figure 7:	Treatment Plant Outlet Sampling	12
Figure 8:	Classification of Sampling Sites.....	12
Figure 9:	Pre-sterilized Sampling Bottle.....	13
Figure 10:	Sampling from a Water Course or Reservoirs	18
Figure 11:	Key Indicator Bacteria for Water Testing	22
Figure 12:	Flow Sheet for Bacteriological Testing.....	25
Figure 13:	Mixing of Media with Sample; Pouring into Quanti tray	31
Figure 14:	Sealing for incubation	31
Figure 15:	<i>Count yellow wells as positive for “Total Coliforms”</i>	32
Figure 16:	<i>Blue Florescence under UV as positive for E-coli</i>	32
Figure 17:	Solar water disinfection.....	37
Figure 18:	Bio-Sand Filter	38
Figure 19:	Construction of Bio-sand Filter.....	41
Figure 20:	PCRWR Solar powered water filtration system.....	42
Figure 21:	PCRWR hand operated water filtration system.....	43
Figure 22:	PCRWR Mobile water treatment units	43
Figure 23:	Laboratory Determination of Chlorine Demand and Optimum Chlorine Dose	49
Figure 24:	chematic Illustration of Chlorine Disinfection Showing HOCl Formation, Cell Penetration, and Destruction of Microbial Cells	52
Figure 25:	Determination of Free Residual Chlorine Using the DPD Color Comparator Method.....	57

List of Tables

Table 1:	Key water quality parameters and their importance in evaluating drinking water safety.....	5
Table 2:	Recommended minimum sample numbers for faecal indicator testing in distribution systems	8
Table 3:	Minimum number of samples for un-piped drinking water	9
Table 4:	Major microorganism groups prevalent in water	21
Table 5:	Material for construction of biosand filters	39
Table 6:	possible options for emergency water treatments	44

Foreword

Access to safe drinking water is a fundamental human need and a prerequisite for protecting public health, particularly during emergencies and disasters. Pakistan is among the countries most vulnerable to the impacts of climate change and has experienced recurrent floods, droughts, heatwaves, and other extreme weather events over the past three decades. The catastrophic floods of 2022 affected more than 33 million people, causing widespread damage to water supply infrastructure and severely compromising drinking water quality across large parts of the country. More recently, intense monsoon rains and flood events in 2025 once again disrupted water supply systems, contaminated drinking water sources, and highlighted the vulnerability of communities to waterborne disease outbreaks. These events underscore the critical importance of strengthening emergency preparedness, water quality monitoring, and safe drinking water interventions to protect public health during and after disasters.

Contaminated drinking water remains one of the primary pathways for the transmission of diseases such as diarrhea, cholera, typhoid fever, hepatitis, and dysentery. Following disasters, water sources are frequently polluted by floodwaters, sewage, animal waste, agricultural runoff, and other contaminants, making rapid water quality assessment and disinfection essential. The Pakistan Council of Research in Water Resources (PCRWR), as the national focal organization for water quality monitoring and drinking water research, has played a leading role in assessing water quality, strengthening surveillance systems, developing water treatment technologies, and supporting emergency response efforts throughout the country. Drawing upon this experience, the present manual has been developed to provide practical guidance on emergency drinking water quality monitoring, microbiological testing, chlorination, household and community-level water treatment, and safe water management practices.

This manual is intended for field personnel, laboratory staff, public health professionals, water supply operators, local government authorities, humanitarian organizations, emergency responders, and community workers engaged in drinking water quality management and emergency response activities. By enhancing water quality monitoring, treatment, and disinfection practices, this manual will contribute to safeguarding public health, reducing the burden of waterborne diseases, and building resilience against future disasters and climate-related challenges.

Dr. Hifza Rasheed

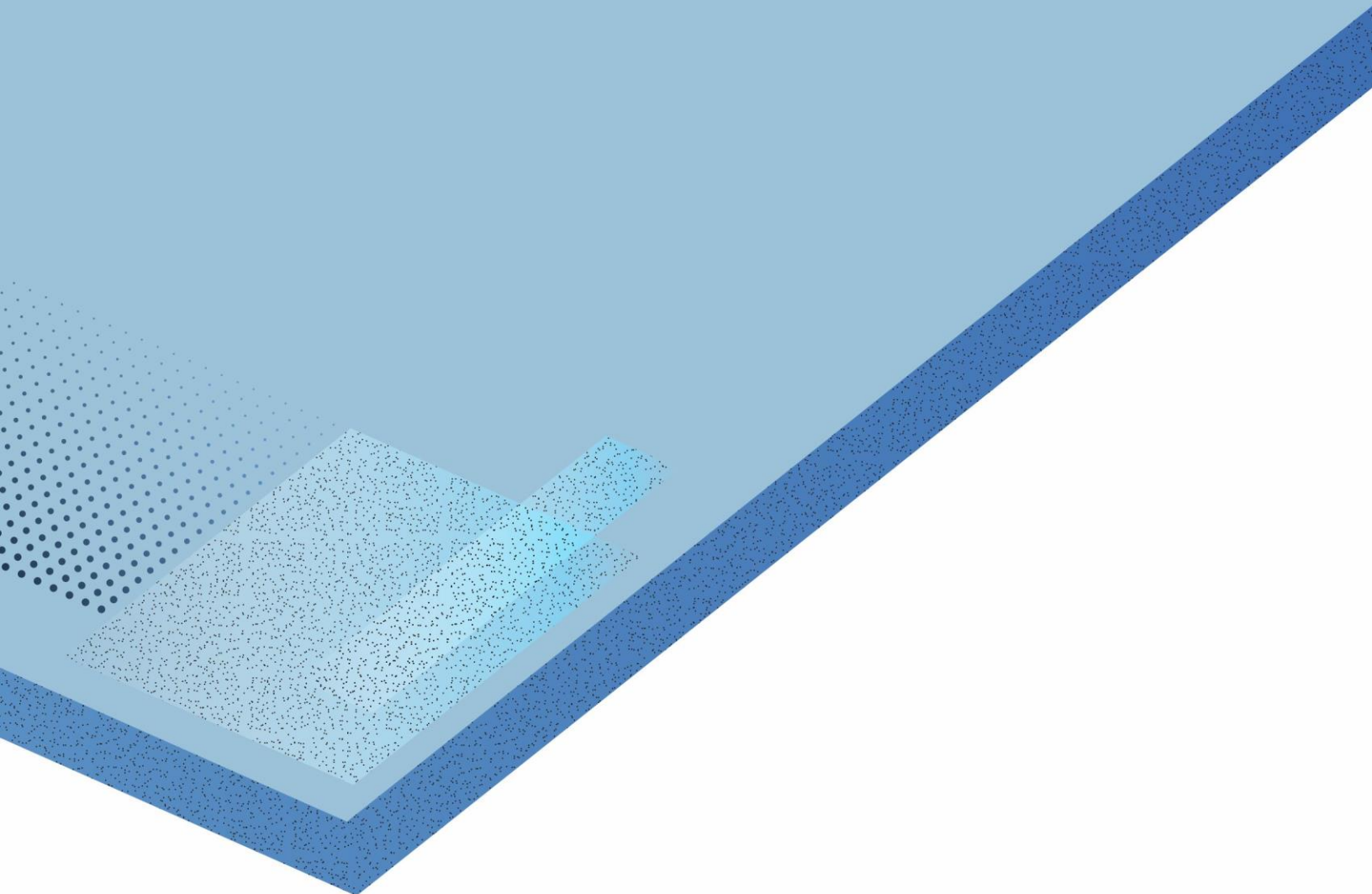
Director General (WQ)

Pakistan Council of Research in Water Resources (PCRWR)

Islamabad, Pakistan

PART-1

WATER QUALITY CONTAMINATION



Part 1. Water Quality Contamination

1.1 Introduction

Access to safe drinking water is fundamental to public health, well-being, and sustainable development. However, water that appears clean and clear is not always safe for consumption. Water sources are frequently exposed to a variety of contaminants that can compromise their quality and pose significant risks to human health. Water quality hazards generally fall into three categories: Microbial, Chemical, and Physical Contamination.

Microbial contamination is one of the most common and serious threats to drinking water safety. Disease-causing microorganisms, including bacteria, viruses, and parasites, can enter water through human or animal fecal waste and may cause waterborne illnesses such as diarrhea, cholera, typhoid fever, dysentery, and other gastrointestinal infections. Several factors contribute to water becoming unsafe for human consumption.

Chemical contamination can occur when pollutants from industrial activities, agricultural practices, mining operations, or natural sources enter water supplies. Common contaminants include pesticides, fertilizers, heavy metals, arsenic, and fluoride. Long-term exposure to these substances can result in adverse health effects, including developmental disorders, organ damage, and certain types of cancer.

Physical contamination, such as sediments, silt, suspended solids, and other particulate matter, can reduce water quality by affecting its appearance, interfering with treatment processes, and creating conditions that allow microorganisms to survive and multiply.

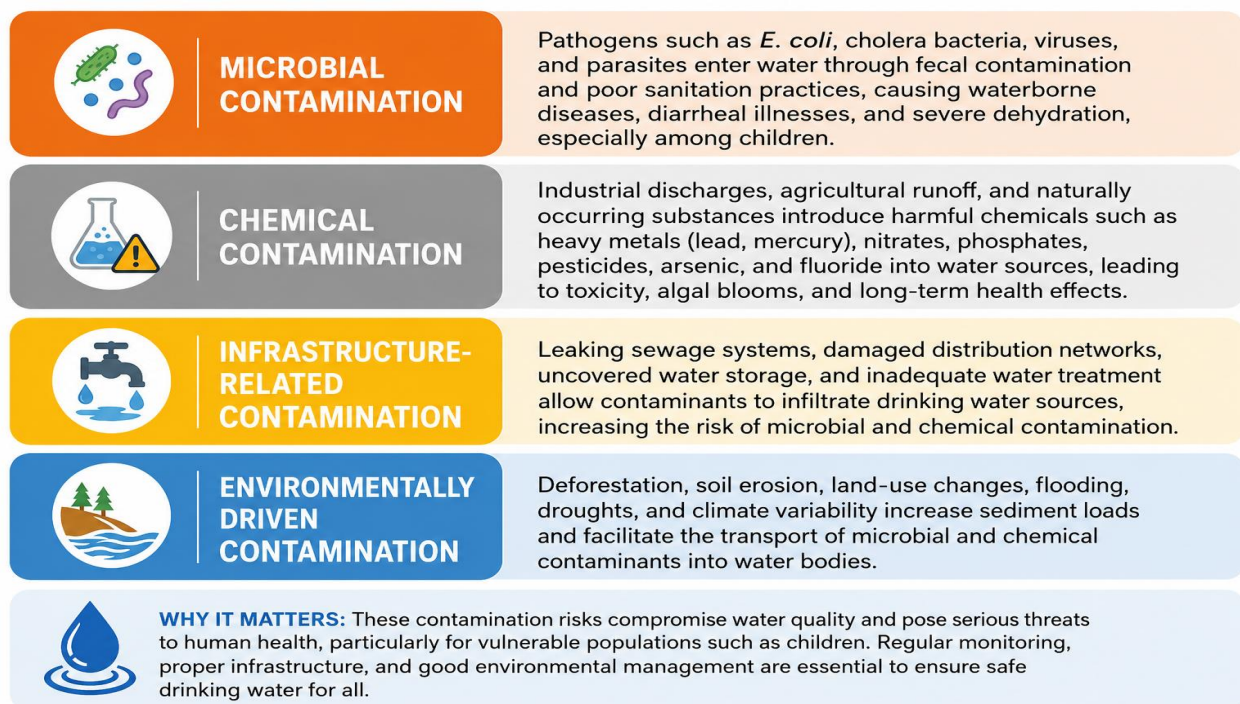


Figure 1: Major Sources and Pathways of Drinking Water Contamination

Key sources of contamination include untreated sewage, agricultural runoff, industrial discharges, inadequate sanitation, poorly maintained water supply infrastructure, and improper waste disposal practices. Environmental changes, including deforestation, land-use changes, flooding, droughts, and other climate-related events, can further increase the vulnerability of water sources to contamination.

Regular monitoring and testing of drinking water are essential to identify potential hazards, assess water quality, and ensure compliance with established standards. Effective water quality surveillance not only helps prevent waterborne diseases but also supports community health, economic productivity, and sustainable development. For this reason, microbial water quality testing is a critical component of drinking water safety management and public health protection.

1.2 Impacts of Unsafe Water

Unsafe drinking water has far-reaching consequences that extend beyond individual health, affecting communities, economies, and the environment. The impacts can be immediate or long-term and often disproportionately affect vulnerable populations, particularly children and low-income communities.

1. **Acute Health Impacts:** Exposure to contaminated water can cause a range of waterborne diseases, including diarrhea, cholera, typhoid fever, dysentery, and parasitic infections, leading to illness, dehydration, and, in severe cases, death.
2. **Chronic Health Consequences:** Prolonged exposure to contaminated water and recurrent infections can contribute to malnutrition, anaemia, impaired physical growth, developmental delays, and chronic diseases, including certain types of cancer associated with chemical contaminants.
3. **Economic Burden:** Unsafe water increases healthcare expenditures, reduces workforce productivity, and results in lost income due to illness, caregiving responsibilities, and school absenteeism.
4. **Environmental Degradation:** Chemical pollutants, excess nutrients, and untreated wastewater can degrade water quality, disrupt aquatic ecosystems, promote harmful algal blooms, and reduce biodiversity.
5. **Social and Developmental Impacts:** Poor water quality undermines livelihoods, compromises educational outcomes, exacerbates poverty, and hinders broader social and economic development by placing additional burdens on households and communities.

1.3 Drinking Water Quality Monitoring

The provision of safe drinking water is a fundamental responsibility of the State and a cornerstone of public health protection. Ensuring that water supplied to communities is safe for human consumption requires systematic and continuous monitoring of water quality throughout the supply chain, from source to consumer.

Water quality monitoring is a critical component of Water Safety Management. It involves the routine assessment, measurement, and evaluation of physical, chemical,

and microbiological parameters to verify that water treatment processes, distribution systems, and water supplies comply with established standards and regulatory requirements. Effective monitoring provides the evidence needed to safeguard public health, support decision-making, and ensure the sustainable delivery of safe drinking water.

The key objectives of water quality monitoring include:

1. Characterizing and tracking water quality

- Establish baseline water quality conditions and monitor changes over time to ensure that water remains safe and fit for human consumption.

2. Identifying risks and threats to water safety

- Detect existing or emerging sources of microbial, chemical, or physical contamination that may compromise drinking water quality and pose risks to public health.

3. Supporting Prevention and control measures

- Generate data to inform the design and implementation of preventive actions, including source protection, pollution control, water treatment improvements, and risk management interventions.

4. Evaluating the effectiveness of water safety measures

- Assess the performance of water treatment processes, distribution systems, and regulatory programs in maintaining compliance with water quality standards and protecting public health.

5. Facilitating timely response to water quality incidents

- Provide early warning of contamination events and enable rapid corrective actions during emergencies such as outbreaks, chemical spills, infrastructure failures, or flood-related contamination.

6. Supporting evidence-based decision-making

- Supply reliable information for planning, policy development, resource allocation, and continuous improvement of water supply services.

Through these functions, water quality monitoring serves as an essential tool for protecting public health, ensuring regulatory compliance, and maintaining public confidence in drinking water supplies.

1.4 Key Water Quality Monitoring Parameters

Water quality monitoring involves the assessment of a range of parameters that provide information on the safety, acceptability, and suitability of water for human consumption. These parameters are broadly categorized into physico-chemical and microbiological parameters. Together, they provide a comprehensive understanding of water quality and help identify potential risks to public health (Table 1).

Table 1: Key water quality parameters and their importance in evaluating drinking water safety

CATEGORY	SUB-CATEGORY	PARAMETERS	SIGNIFICANCE
Physico-Chemical Parameters	Physical and Aesthetic Characteristics	Electrical Conductivity (EC), Color, Odor, Taste, pH, Turbidity	Influence the appearance, acceptability, and general quality of drinking water. These parameters can also indicate the presence of contamination or treatment deficiencies.
	Major Chemical Constituents	Calcium, Magnesium, Hardness, Bicarbonate, Carbonate, Alkalinity, Sodium, Potassium, Sulphate, Nitrate, Total Dissolved Solids (TDS)	Affect the chemical composition, stability, and suitability of water for drinking and domestic use. Some constituents may indicate pollution or geochemical influences.
	Trace and Ultra-Trace Elements	Iron, Fluoride, Arsenic, Total Chlorine, Free Residual Chlorine, and other trace metals	Present in low concentrations but can significantly affect water safety, treatment efficiency, and human health when concentrations exceed recommended limits.
Microbiological Parameters	Indicators of Microbial Contamination	Total Coliforms, Fecal Coliforms (Thermotolerant Coliforms), <i>Escherichia coli</i> (<i>E. coli</i>), Total Plate Count (Heterotrophic Plate Count)	Used to assess the microbiological safety of drinking water and detect contamination from human or animal fecal sources, which may indicate the presence of disease-causing pathogens.

Regular monitoring of these parameters is essential to verify compliance with drinking water quality standards, evaluate treatment performance, identify contamination risks, and ensure the provision of safe drinking water to communities. Among these, microbiological parameters are of particular significance due to their direct association with waterborne diseases, making microbial examination a fundamental element of drinking water quality monitoring and surveillance.

1.5 Purpose of the Training Manual

This training manual has been developed to provide practical and technical guidance to field and laboratory personnel involved in drinking water quality monitoring, testing, and disinfection. It aims to strengthen the knowledge and skills required to assess water quality, identify potential contamination risks, and implement appropriate corrective measures to ensure the provision of safe drinking water.

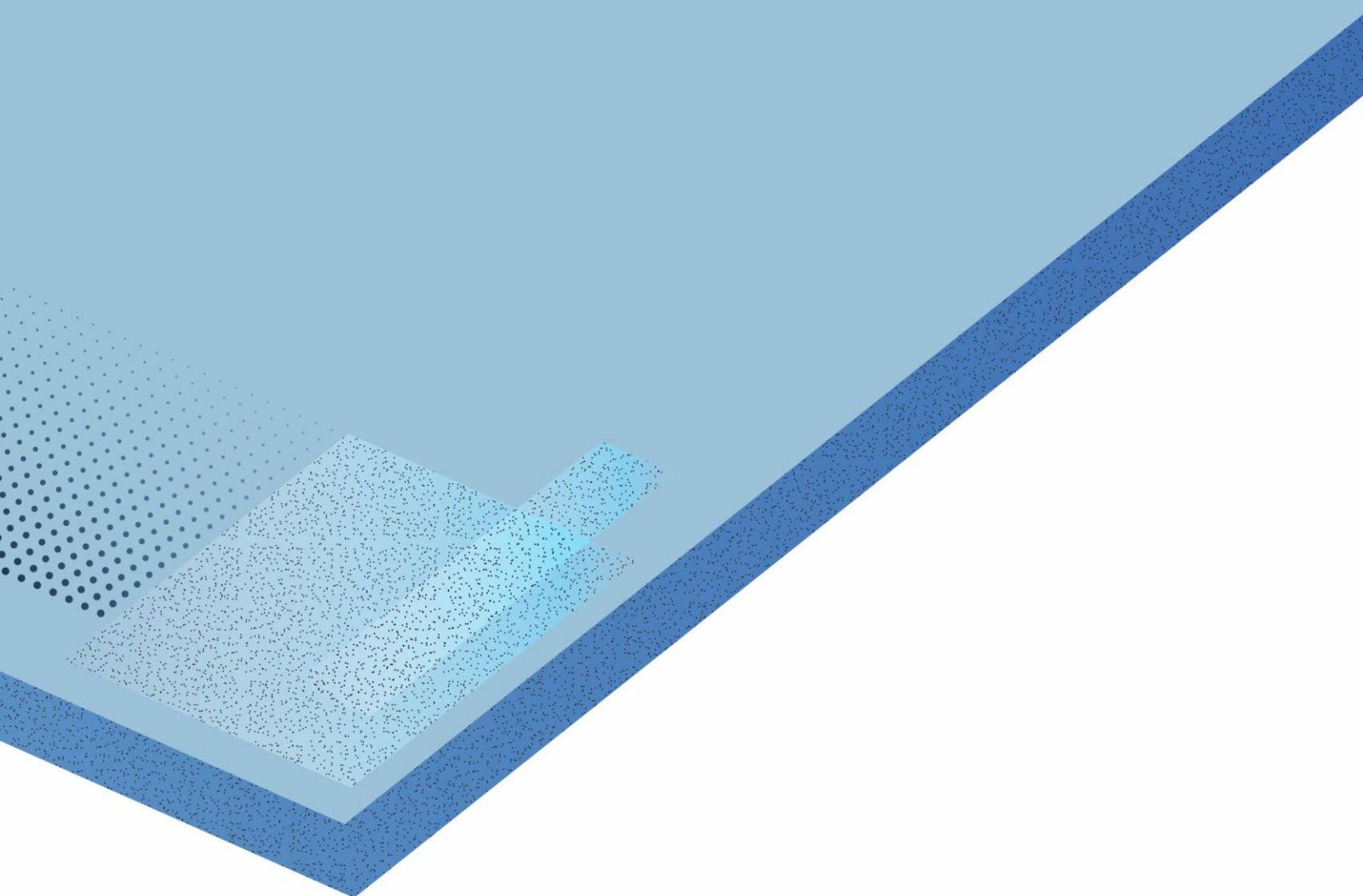
The manual covers the principles and procedures for monitoring key physico-chemical and microbiological water quality parameters, as well as essential water treatment and disinfection practices, including chlorination and decontamination techniques. Emphasis is placed on standardized methods, quality assurance, and step-by-step field and laboratory procedures that can be applied in routine monitoring programs and emergency response situations.

By promoting consistent and reliable testing practices, this manual seeks to enhance the technical capacity of personnel working in small-scale laboratories and field settings. It serves as both a training resource and a practical reference guide to support accurate water quality assessment, effective water safety management, regulatory compliance, and the protection of public health.

Ultimately, the manual contributes to strengthening water quality surveillance systems and ensuring that communities have access to safe, reliable, and sustainable drinking water supplies.

PART-2

WATER QUALITY SAMPLING FOR MICROBIOLOGICAL EXAMINATION



Part 2. Water Quality Sampling for Microbiological Examination

2.1 Guidelines for Sampling

A critical part of monitoring water quality is samples collection. It is important that samples represent the water source and that sampling procedures are reproducible and consistent. Samples must be taken from locations that 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. Sampling keeps basic importance to generate reliable test data. Water sampling requirements are as following:

Sampling frequency

The recommended minimum sample numbers for piped drinking water in the distribution system are shown in the Table-2¹ whereas minimum frequency of sampling and analysis of un-piped water supplies is given as in Table-3:

Table 2: Recommended minimum sample numbers for faecal indicator testing in distribution systems²

Type of water supply and population	Total number of samples per year
Point sources	Progressive sampling of all sources over 3- to 5-year cycles (maximum)
Piped supplies	
< 5000	12
5000–100 000	12 per 5000 population
> 100 000–500 000	12 per 10 000 population plus an additional 120 samples
> 500 000	12 per 50 000 population plus an additional 600 samples

¹ Edition, F., 2011. *Guidelines for drinking-water quality*. WHO chronicle, 38(4), pp.104-8.

² Parameters such as chlorine, turbidity and pH should be tested more frequently as part of operational and verification monitoring.

Table 3: Minimum number of samples for un-piped drinking water³

Source and mode of supply	Minimum frequency of sampling and analysis		Remarks
	Bacteriological	Physical/chemical	
Open wells for community supply	Sanitary protection measures; bacteriological testing only if situation demands	Once initially for community wells	Pollution usually expected to occur
Covered dug wells and shallow tube wells with hand-pumps	Sanitary protection measures; bacteriological testing only if situation demands	Once initially, thereafter as situation demands	Situations requiring testing: change in environmental conditions, outbreak of waterborne disease, or increase in incidence of waterborne diseases
Deep tube wells with hand-pumps	Once initially, thereafter as situation demands	Once initially, thereafter as situation demands	Situations requiring testing: change in environmental conditions, outbreak of waterborne disease, or increase in incidence of waterborne diseases
Protected springs	Once initially, thereafter as situation demands	Periodically for residual chlorine if water is chlorinated	Situations requiring testing: change in environmental conditions, outbreak of waterborne disease, or increase in incidence of waterborne diseases
Community rainwater collection systems	Sanitary protection measures; bacteriological testing only if situation demands	Not needed	

2.2 Sampling Sites

a) Location of sampling points

Samples must be taken from locations that 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. In selecting sampling points, each locality should be considered individually; however, the following general criteria are usually applicable:

³ Edition, F., 2011. *Guidelines for drinking-water quality*. WHO chronicle, 38(4), pp.104-8.

- Sampling points should be selected such that the samples taken are representative of the different sources from which water is obtained by the public or enters the system (Figure 2).

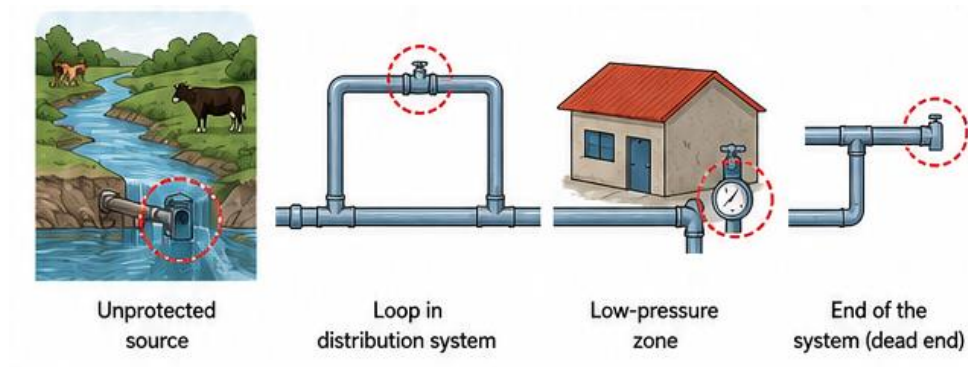


Figure 2: High-Risk Sampling Locations

- 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. as indicated in Figure 3.

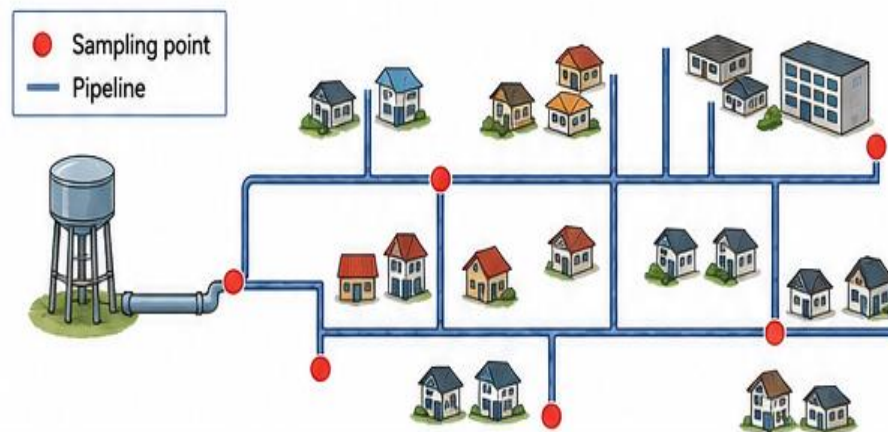


Figure 3: Uniform Distribution of Sampling Points

- 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 (Figure 4).

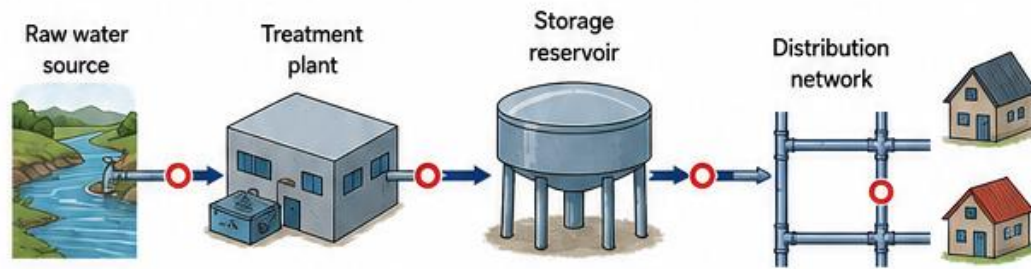


Figure 4: Representative System-Wide Sampling

- The points chosen should generally yield samples that are representative of the system as a whole and of its main components (Figure 5). Sampling points should be located in such a way that water can be sampled from reserve tanks and reservoirs, etc.



Figure 5: Reservoir and Storage Tank Sampling

- 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 (Figure 6).

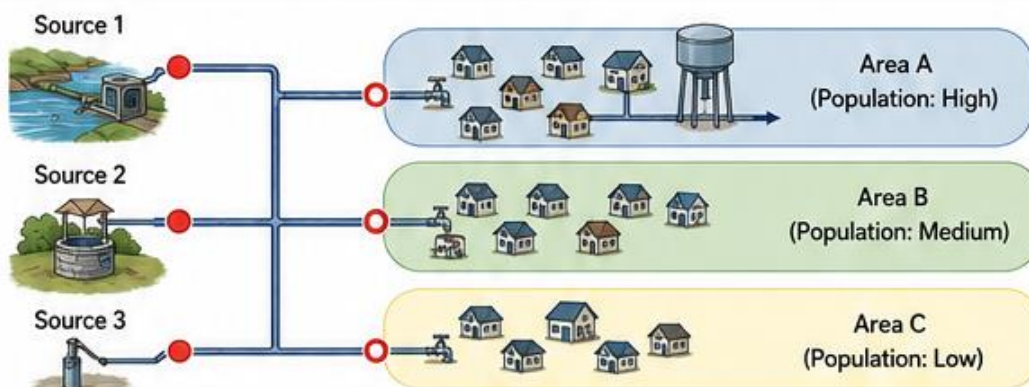


Figure 6: Sampling in Multi-Source Supply Systems

- There should be at least one sampling point directly after the clean-water outlet from each treatment plant (Figure 7).



Figure 7: Treatment Plant Outlet Sampling

- Sampling sites in a piped distribution network may be classified as following and as shown in Figure 8.
 - a) fixed and agreed with the supply agency;
 - b) fixed, but not agreed with the supply agency; or
 - c) Random or variable.



Figure 8: Classification of Sampling Sites

Each type of sampling site has certain advantages and disadvantages. Fixed sites agreed with the supplier are essential when legal action is to be used as a means of ensuring improvement; otherwise, the supply agency may object to a sample result on the grounds that water quality may have deteriorated in the household, beyond the area of responsibility of the supplier.

b) Common sampling sources

Water can be divided into three basic types for the purpose of sampling:

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

2.3 Sampling Methods for Bacteriological Testing

2.3.1 Preparations for Water Quality Sampling

a) Sampling Containers

Appropriate sampling containers are essential for obtaining representative water samples and ensuring the accuracy, reliability, and integrity of microbiological test results. For microbiological examination, only sterile sampling bottles should be used to prevent contamination during sample collection, transportation, and storage. When sampling chlorinated water supplies, the bottles should contain an appropriate amount of sodium thiosulfate to neutralize residual chlorine and prevent continued disinfection after collection, which may otherwise affect the microbial population and compromise analytical results. Pre-sterilized sampling bottles with a capacity of approximately 200 mL, made of disposable sterile plastic or autoclavable glass, are recommended for microbiological water sampling. Prior to use, all bottles should be inspected for physical defects such as cracks, damaged caps, or leakage that could affect sample integrity. For commercially supplied pre-sterilized bottles, a certificate of sterilization should be obtained from the manufacturer or supplier to verify compliance with sterility requirements.



Figure 9: Pre-sterilized Sampling Bottle

- Pre-sterilized sampling bottles of 100-250 ml capacity made of pre-sterilized disposable/autoclavable plastic, or good quality soda / borosilicate glass are used to collect the sample.
- Prior to sampling all bottles should be checked for physically defects i.e. leakage etc.

b) Dechlorination of Water Samples

When water samples are collected from chlorinated supplies, residual chlorine may remain present in the sample and continue to inactivate microorganisms during storage and transportation. As a result, the microbiological analysis may not accurately reflect the true bacteriological quality of the water at the time of sampling. To prevent this, a dechlorinating agent, sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$), is added to the sample container before sample collection. Sodium thiosulfate immediately neutralizes residual chlorine without affecting the microorganisms present in the water, thereby preserving the integrity of the sample for microbiological analysis. Following steps to be followed for dechlorination purpose:

- For a 200 mL sample bottle, add 4–5 drops of sodium thiosulfate solution (100 g/L) to each clean sampling bottle before sterilization.
- After addition of sodium thiosulfate, sterilize the bottle in an autoclave at 121°C for 15–20 minutes.
- Where an autoclave is not available, a pressure cooker or portable sterilizer may be used, although a longer sterilization time of 30–45 minutes may be required.
- For commercially pre-sterilized bottles, sodium thiosulfate may be added aseptically at the time of sampling if it has not already been incorporated by the manufacturer.

c) Importance of Dechlorination

Dechlorination is essential when sampling chlorinated drinking water because it:

- Prevents continued bactericidal action after sample collection.
- Preserves microorganisms present at the time of sampling.
- Ensures accurate and representative microbiological test results.
- Improves the reliability of laboratory analyses and water quality assessments.

Key points for preventing contamination during microbiological sampling

Contamination is a real problem during water sampling. Most of the risks can be overcome by introducing basic aseptic techniques into the sampling procedure. The following points suggest the most obvious ways to reduce the chance of contamination:

- Contamination must be avoided during the sampling procedure as 92% error comes from sampling.
- Sampling points may often introduce contamination and therefore disinfection or flushing may be required to obtain a representative sample.
- Never touch the neck of the bottle, or inside the lid. When filling the bottle, the lid should not be placed on a surface, but remain in the hand.
- The sample bottle should not be rinsed out prior to filling.
- The flow rate of the tap should not be changed during sampling as this may dislodge bacterial films inside the tap.

- A small air gap should be left in the bottle.
- Once the bottle is filled and the lid replaced the sample should be placed in a cool box for transfer to the laboratory/testing point in the field.
- If accidental contamination is suspected then the sample should be discarded and taken again using a fresh container.

d) Sample Labels for chemical and microbiological samples

Correct labeling and accurate records are essential during water sampling due to the large number of people who will be processing the sample. If this practice is not followed carefully, then samples can be incorrectly reported or lost completely. Labeling is done with permanent ink. The label should contain the following information;

- Sample code
- The date and time of sampling,
- The exact location of a sampling point,
- The type of water to be tested (e.g. filtered, treated)

e) Sample transportation

Samples for microbial testing should immediately be transferred to dark storage between 2 and 8 °C. The most suitable containers for this purpose would be ice boxes.

- The boxes should be used exclusively for the transport of water samples, and should be regularly cleaned and disinfected.
- Ideally, samples should be analyzed within 6 hours, and during this time should be stored in cool dark conditions.
- The longer the delay between sampling and analysis, the more likely that changes will occur in the sample, which may no longer be representative.

f) Reception of samples by the laboratory

It is good practice for a member of the laboratory staff to sign for the receipt of samples and to make the following checks at the same time:

- All of the necessary details are recorded on the labels of sample bottles.
- The samples are contained in appropriate bottles.
- Samples have arrived in time for subsequent analysis to provide a reliable picture of water quality at the time of sampling.
- Samples have been treated with any necessary preservatives.
- Samples have been stored at appropriate temperatures, maintained throughout transport.

Samples should be logged into the laboratory system as soon as these arrive and (generally) transferred to a refrigerator at about 4 °C. If someone other than laboratory staff is to receive the samples, they should be instructed to transfer them directly to a

refrigerator, noting the time and the condition of the samples, and then inform laboratory staff accordingly. Where sample storage times and/or conditions have been such as to make it unlikely that analyses will yield reliable results, laboratory staff should decline to accept the affected samples. Supervisory staff should lend their support to the decision to reject samples on this basis.

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.

2.3.2 Sampling from different water sources

a) Sampling from distribution network or pump outlet

The water samples from the distribution network are collected from the source of supply (as closely as possible) to minimize the effects of pollution in the distribution system and from consumers end to evaluate the actual quality of water being used. All water sample containers are filled slowly to avoid turbulence and air bubbles after flushing the system for sufficient time. Un-rusted taps are selected for collection of water samples. These taps are properly cleaned and allowed to flow for a few minutes before collecting the sample.

Step One: Clean the tap

Choose a sample tap that represents the water in **distribution** system. Avoid poor sample sites such as swivel faucets, hot and cold mixing faucets (with a single lever), leaky or spraying faucets, drinking fountains, janitorial sinks and faucets below or near ground level. Using a clean cloth, wipe the outlet to remove any dirt.



Step Two: Open the tap

Remove any attachments from the faucet, including **aerators**, screens, washers, hoses, and water filters. Open the tap for 5 minutes to flush out the standing water.



Step Three. Sterilize the tap

Sterilize the tap for a minute with the flame from a gas burner, cigarette lighter, or an ignited alcohol- or spirit-soaked cotton-wool swab or spray a small quantity of spirit on surface of tap and flame it with match stick and let it cool down.



Step Four: Open the tap before Sampling

Spray some spirit on your hands and then open the tap again to turn the water down to a thin stream (about the width of a pencil) and let it run for one minute. *Do not adjust the flow after it has been set.*



Step Five: Open the sterilized bottle

Take out a bottle and carefully unscrew the cap or pull out the stopper. To avoid contamination while taking the sample, hold the bottle near the bottom with one hand, hold the top of the cap with the other, and then unscrew the cap. *Do not place the cap on the ground.*



Step Six: 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. Be careful not to let the bottle touch the sample tap. Fill the bottle to the neck (leave 1" from tap) but do not allow it to overflow. Remove the bottle from the water flow. A small air space should be left to make shaking before analysis easier.



Step Seven: Stopper or cap the bottle

Place the stopper on the bottle or screw on the cap and tightly close it, as shown below;



Step Eight: Record the Information

Complete the questionnaire/Performa. If there was anything unusual about the sample collection, note it down. Label the bottle with permanent marker and deliver the sample to laboratory or to a designated drop-off location for the lab as soon as possible. Lab analysis must begin within 6 hours of sample collection.

b) Sampling from a Watercourse or Reservoir

Open the sterilized bottle as described in Step 5. Holding the bottle by the lower part, submerge it to a depth of about 20 cm, with the mouth facing slightly upwards. If there is a current, the bottle mouth should face towards the current. Fill the bottle and bottle should then be capped or Stoppard as described previously (Figure 10).

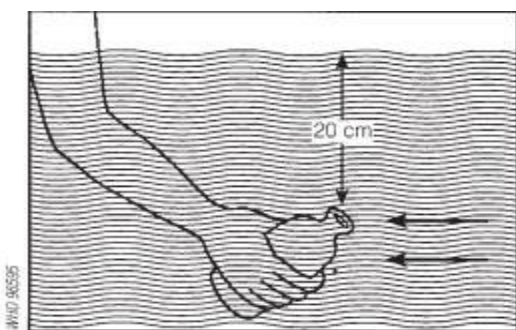


Figure 10: Sampling from a Water Course or Reservoirs

c) Sampling from Tube wells

Water samples from tube wells are collected after purging the well by continuous pumping for a minimum of 10 minutes to remove stagnant water from the system. This procedure ensures that the collected sample is representative of the aquifer groundwater quality.

Sampling from Dug wells

Step One: Prepare the bottle

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

Step Two: 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 2.3.2.

Step Three: 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.

Step Four: 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.

Step Five: Raise the bottle

Cap the bottle tightly and proceed further.

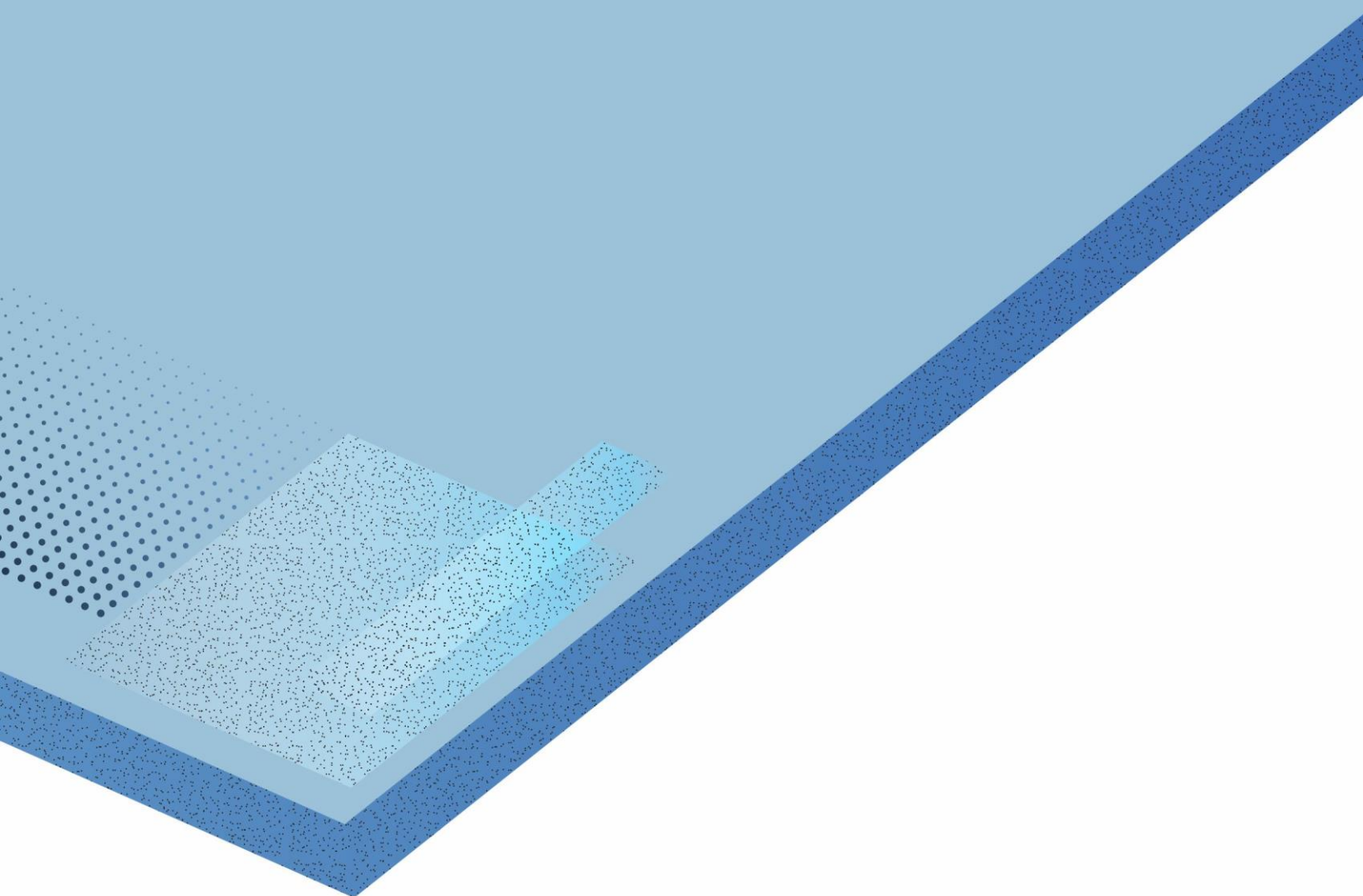
Water samples are collected from hand pumps or dug wells after purging of the hand pump or well. The purging is carried out by making one stroke for every foot of depth (A hand pump or dug well having 30 feet of depth, needs 30 strokes for its purging).

Water samples should be transported in dedicated, clean, and disinfected ice boxes and maintained at 2–8°C in dark conditions, with analysis performed preferably within 4 hours of collection to minimize changes that may affect sample representativeness.



PART-3

TEST PROCEDURES FOR MICROBIAL CONTAMINATION

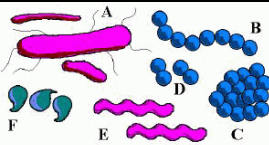
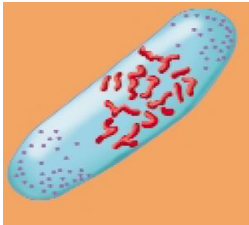
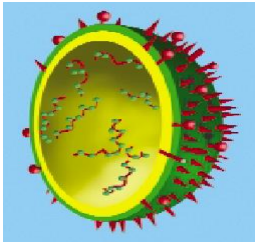


Part 3. Test Procedures for Microbial Contamination

3.1 Introduction

Microorganisms are microscopic living organisms that are present in all natural environments, including water, soil, air, plants, animals, and humans. While many microorganisms are harmless or beneficial, some can cause disease and pose significant risks to public health when present in drinking water. The major groups of microorganisms relevant to water quality are listed as Table-4:

Table 4: Major microorganism groups prevalent in water

Microorganism Group	Typical Size	Shape under microscope	Significance in Water Quality
Bacteria	0.5–5 μm		Some species cause waterborne diseases; indicator bacteria are routinely used to assess water safety.
Protozoa	>5 μm		Certain species, such as <i>Entamoeba histolytica</i> , can cause gastrointestinal infections.
Fungi and Algae	>5 μm		May affect water quality and aesthetics but are generally not used as indicators of fecal contamination.
Viruses	20–100 nm		Can cause diseases such as hepatitis, poliomyelitis, and gastroenteritis; however, routine detection is complex and not commonly performed in basic water quality monitoring programs.

Microbiological examination provides one of the most sensitive indicators of drinking water contamination and public health risk. Because testing for every disease-causing microorganism is impractical, routine water quality monitoring relies on the detection of **indicator bacteria**. These organisms are easier to detect, occur in greater numbers than pathogens, and provide evidence of possible fecal contamination and treatment failure.

The presence of indicator bacteria suggests that disease-causing microorganisms may also be present and that the water may be unsafe for consumption.

3.2 Indicator Bacteria Used in Drinking Water Monitoring

There are hundreds of bacteria which can be tested in water. However, considering time, resources and urgency, indicator organisms are preferred. Indicator bacteria are microorganisms used to assess the microbiological safety of drinking water and the effectiveness of water treatment and distribution systems.

3.2.1 Total Coliforms

Total coliforms are a group of bacteria commonly found in soil, vegetation, surface water, and the intestinal tracts of humans and animals. Although most total coliforms are not harmful, their presence in treated drinking water may indicate inadequate treatment, contamination, or deterioration within the distribution system.

Total coliforms are broadly divided into:

- **Fecal Coliforms** (including *Escherichia coli* (*E. coli*))
- **Non-Fecal Coliforms** (e.g., *Citrobacter*, *Enterobacter*, *Klebsiella*, and *Serratia*)

3.2.2 Fecal Coliforms

Fecal coliforms are a subgroup of total coliform bacteria that originate primarily from the intestinal tracts of warm-blooded animals. Their presence indicates recent fecal contamination and a higher likelihood that pathogens may be present in the water.

3.2.3 *Escherichia coli* (*E. coli*)

E. coli is the preferred and most specific indicator of fecal contamination. It normally inhabits the intestines of humans and other warm-blooded animals and is rarely found in uncontaminated water. Detection of *E. coli* in drinking water is considered strong evidence of recent fecal pollution and requires immediate investigation and corrective action (Figure 11).

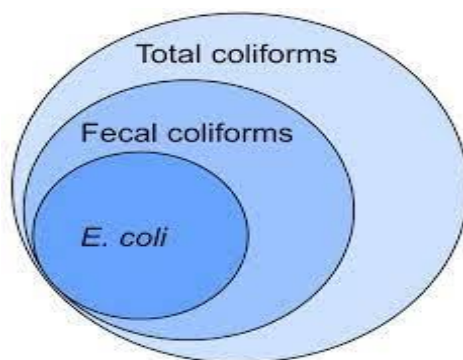


Figure 11: Key Indicator Bacteria for Water Testing

Fecal contamination of drinking water may introduce pathogenic microorganisms capable of causing diseases such as:

- Diarrhea
- Dysentery
- Typhoid fever
- Cholera
- Hepatitis
- Gastroenteritis

The detection of coliform bacteria, particularly *E. coli*, indicates that water may have been contaminated by human or animal waste and may therefore contain disease-causing microorganisms.

3.3 Sampling for testing of Indicator bacteria

Detailed sampling procedure from different water sources is described in section 2.3. However, key points are explicitly listed here to refresh the procedure. Accurate microbiological results depend largely on proper sample collection, handling, and transportation. Errors introduced during sampling can compromise the reliability of laboratory results and lead to incorrect conclusions about water safety.

To ensure representative and reliable results:

- Use sterile sampling containers.
- Avoid contamination during sample collection.
- Transport samples under appropriate conditions.
- Analyze samples within the recommended holding time.
- Follow standard operating procedures for sample collection, preservation, and testing.

Careful adherence to sampling procedures is essential for generating reliable microbiological data and making informed decisions regarding drinking water safety.

i. Sampling Instruction:

- 1) Never touch the neck of the bottle, or inside the lid. When filling the bottle, the lid should not be placed on a surface, but remain in the hand.
- 2) The sample bottle should not be rinsed out prior to filling.
- 3) The flow rate of the tap should not be changed during sampling as this may dislodge bacterial films inside the tap.
- 4) A small air gap should be left in the bottle.
- 5) Once the bottle is filled and the lid replaced the sample should be placed in an ice box for transfer to the laboratory/testing point in field.
- 6) If accidental contamination is suspected then the sample should be discarded and taken again using a fresh container.

Note: 92% error comes from sampling, 7% errors from wrong sample transportation and 1% from laboratory practices.

A) Sampling from Tap

Generalized sampling from different water sources is described in section 2.3, whereas key steps for water sampling for indicator bacteria are reiterated below for ease of reference.

Step 1: Spray spirit on your hands up to arms for disinfection purpose.



Step 2: Remove any attachments from the tap such as pipes, filters etc.

Open the tap for 5 minutes to flush out the standing water, close the tap and clean with tissue paper.

Spray a small quantity of spirit on surface of tap and flame it with match stick and let it cool down.



Step 3: After flaming, open the tap again to turn the water down to a thin stream (about the width of a pencil) and let it run for one minute.



Step 4: To avoid contamination while taking the sample, hold the bottle near the bottom with one hand, hold the top of the cap with the other, and then unscrew the cap. Do not place the cap on the ground. Sampling will be more reliable if performed near flame.



Step 5: Hold the bottle under the stream of water, being careful not to let the bottle touch the sample tap. Fill the bottle to the neck (leave 1" from top) but do not allow it to overflow. Remove the bottle from the water flow and replace the cap.



Step 6: Label the bottle with permanent marker and kept in insulated ice box having sterilized coolants (under controlled condition of 4°C). Sample should be delivered to laboratory as soon as possible.



3.4 Test Procedures

Method-I: Bacteriological Testing Using Presence–Absence Test Kit

a) Scope and Application

The Presence–Absence Test Kit is a simple, rapid, and cost-effective tool for the preliminary assessment of microbiological water quality. The test is designed to detect the presence of bacteria associated with fecal contamination by identifying the production of hydrogen sulphide gas, a metabolic by-product of certain microorganisms commonly found in contaminated water.



Figure 12: PA Test Method Kit

The test provides a practical screening method for determining whether bacterial contamination is present or absent in a water sample. Due to its simplicity and minimal equipment requirements, it is particularly useful in rural, remote, and resource-limited settings where access to advanced laboratory facilities may be limited. The kit is commonly used for:

- Routine drinking water quality monitoring.
- Surveillance of wells, hand pumps, springs, rivers, ponds, and storage reservoirs.
- Community-based water quality monitoring programs.
- Household water quality assessment.
- Emergency response and rapid field investigations.

- Educational and awareness activities related to water safety.

b) Principle of the Test

The Presence–Absence test is based on the ability of certain bacteria associated with fecal contamination to produce hydrogen sulphide gas during growth. When these bacteria are present in the sample, they react with the culture medium contained in the test vial, resulting in the formation of a black color. The appearance of black coloration indicates bacterial contamination and suggests that the water may be unsafe for drinking. The absence of black coloration indicates that indicator bacteria were not detected under the test conditions.

c) Direct Sampling for PA Kit

Where feasible, water samples may be collected directly into the Presence–Absence test vial provided with the kit. Care should be taken to avoid contamination of the vial, cap, or culture medium during sample collection.

d) Sampling from Wells and Hand Pumps

For tube wells, dug wells, storage tanks, and other sources where direct sampling is impractical, the water sample should first be collected in a sterile sampling bottle. A measured volume of 20 mL of the collected water is then transferred aseptically into the Presence–Absence test vial containing the prepared culture medium.

e) Aseptic Inoculation Procedure

The transfer of a defined volume of water into a test vial containing microbiological culture medium is known as inoculation. Key steps include:

1. Conduct inoculation in a clean, dust-free environment.
2. If available, use a laminar flow hood for microbiological work.
3. Where a laminar flow hood is not available, designate a clean workspace exclusively for sample inoculation.
4. Disinfect the work surface with 70–90% ethanol or another suitable disinfectant before starting work.
5. Use a spirit lamp, alcohol lamp, or gas burner to create a sterile working zone and minimize airborne contamination.
6. Avoid touching the inside of the vial cap, bottle neck, or any sterile surfaces during inoculation.

f) Incubation

After inoculation:

1. Tightly close the test vial.
2. Gently shake the vial to ensure complete mixing of the sample and culture medium.
3. Place the vial in an incubator maintained at $35 \pm 0.5^{\circ}\text{C}$.
Incubation conditions are as follows:

Condition	Incubation Requirement
Laboratory incubation	35°C for 24 hours
Field incubation with no electricity	Store the vial at a location where the ambient temperature remains above 25°C for 48 hours
Vehicle-supported field testing	Portable incubators may be powered using a vehicle battery and maintained at 35°C for 24 hours

Where laboratory facilities are accessible, inoculated vials may be transported to the laboratory for incubation and analysis.

g) Interpretation of Results

After incubation, examine the test vial and record the observations.

Observation	Interpretation
Black coloration develops in the medium	Presence of indicator bacteria. The sample is considered bacteriologically contaminated and the water should be regarded as unsafe for drinking without appropriate treatment.
No black coloration develops	Indicator bacteria were not detected under the test conditions. The sample is considered bacteriologically satisfactory at the time of testing.

Results of the testing kits are interpreted on the basis of color change of kits:

- If the color of the kits changes from yellow to black, then sample is considered unsafe and water sample is considered unfit for the drinking purpose.
- If the color of the kit remains yellow after incubation, then the water sample is considered as safe and considered as fit for the drinking purpose.

h) Limitations of the Test

The Presence–Absence (H₂S) test is intended as a screening tool for detecting potential bacterial contamination. It does not identify specific pathogens or quantify bacterial concentrations. Positive results should be followed by confirmatory laboratory testing where required.

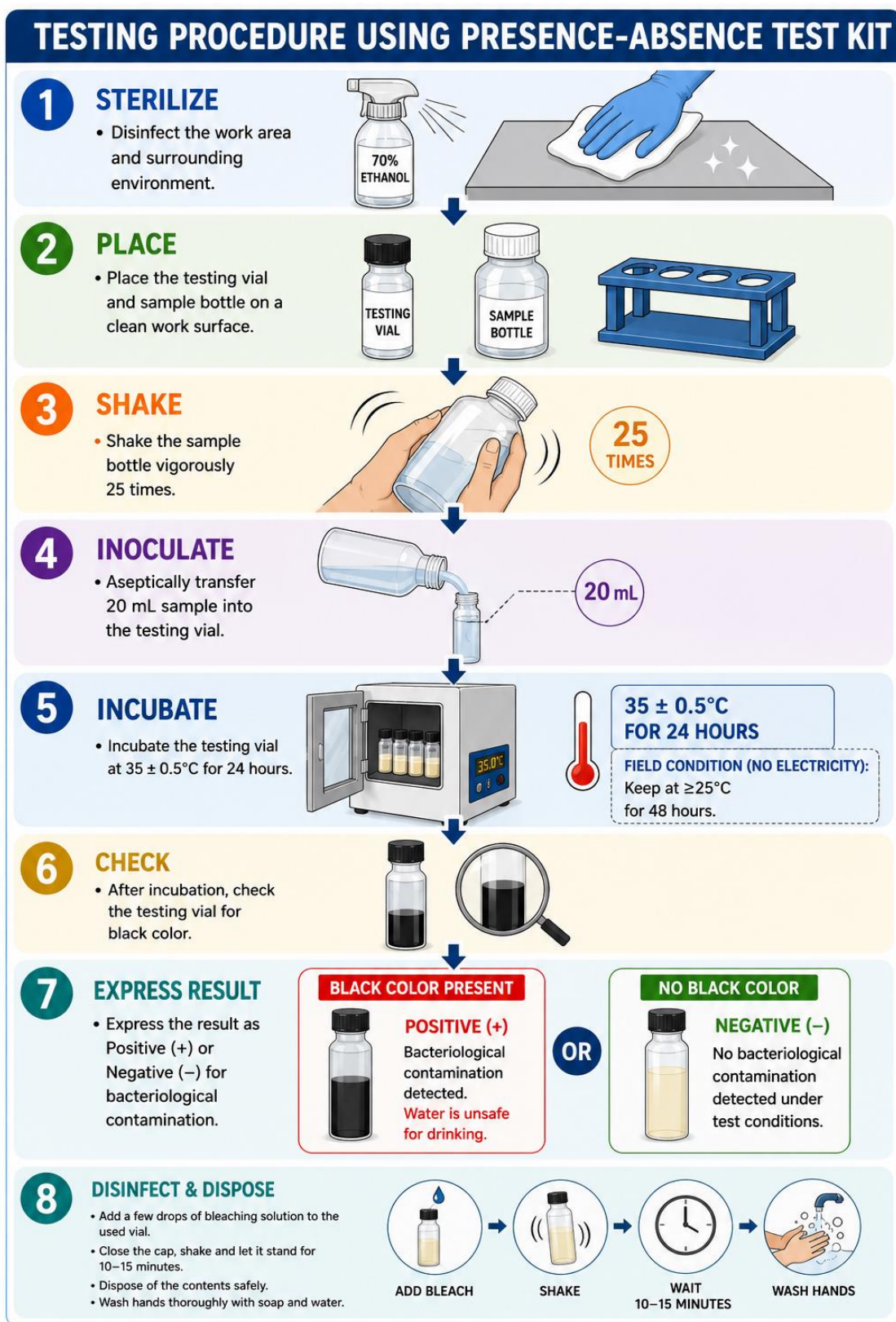
i) Disposal of Used Test Vials

All used test vials and culture media should be disinfected before disposal.

1. Add a few drops of bleaching solution (sodium hypochlorite) to the used test vial.
2. Securely close the cap and gently shake the vial.
3. Allow the disinfectant to remain in contact with the contents for 10–15 minutes.
4. Dispose of the contents according to local laboratory waste management procedures.
5. Wash reusable materials, if applicable.
6. Thoroughly wash hands with soap and clean water after completing the procedure.

Proper disinfection and disposal of used microbiological media are essential for laboratory safety and environmental protection.

Key steps of use of PA Test kit as explained above are described as below:



Method-II: Determination of Total Coliform and *E-coli* by Colilert IDEXX Quanti-tray Method

1. Scope

This standard operating procedure describes the test method that simultaneously detects or quantifies both Total Coliforms and *Escherichia coli*, with results in 24 hours. It is recommended for the analysis of drinking and source water samples. Formulations are also available for the analysis of marine waters^{4, 5}.

2. Principle

- Two enzyme substrates are included in Colilert, a chromogen that reacts with the enzyme found in Total Coliforms (galactosidase) and a fluorogen that reacts with an enzyme found in *E-coli* (glucuronidase). After 24 hours incubation at 35 °C, a Total Coliform-positive reaction turns the medium yellow; and *E-coli* positive reaction causes the medium to fluorescence under a long wave ultraviolet light (366 nm).

3. Apparatus and glassware:

- Sterile, 100ml sampling bottles.
- Quanti-Tray Sealer
- Incubator at 35±0.5 °C
- Incubator at 44.5±0.2 °C
- Autoclave at 121±1 °C
- Biological Safety Cabinet
- Analytical Balance±0.001/0.2g

5. Reagents and Standards

- Colilert® reagent for 100 ml samples
- Quanti-Tray®/2000 containing 97 wells each

6. Procedure:

- Water samples (100 ml) is collected in sterilized sampling bottle before inoculation of sample and is shaken well before analysis.
- The entire sample is poured into a larger sterile container, mix properly, and proceeds for the analysis. 100 ml of sample is poured directly into the sterile

○ ⁴ APHA, AWWA and WEF. 2017. Method No. 9223. Standard Methods for the Examination of Water and Wastewater. 23rd Edition. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC.

○ ⁵ IDEXX Laboratories, Inc. Westbrook, ME 04092. Instruction manuals for use of: Colilert®, Quanti-Tray®/2000, and Quanti-Tray Sealer®.

100 ml mixing bottle (by filling to the 100 ml line) and one package of the colilert reagent is added.

- Colilert ampule is opened and contents of ampule are added into the sample.
- Mix thoroughly; making sure the Colilert reagent is completely dissolved (Figure 13).
- Allow bubbles to settle or dissipate. Failure to do this may result in the wells filling or sealing improperly.
- Sample/reagent mixture is transferred from the mixing bottle into a quanti-tray and then it is sealed in the IDEXX Sealer. Labeling is done by writing the sample code, date and time on the back of the tray (Figure 14).



Figure 13: Mixing of Media with Sample; Pouring into Quanti tray



Figure 14: Sealing for incubation

- Incubate at $35.0 \pm 0.5^{\circ}\text{C}$ for 24 hours for Total Coliforms and E-coli.321322
- After completion of the incubation period, trays are ready to get results.
- Count the number of small and large positive wells (Yellow wells) after incubation and refer to the MPN table at appendix A to find the most probable number for Total Coliform.
- E. coli results are obtained by placing the trays under a black box having UV light and counting the number of fluorescent wells. Refer to the MPN table to determine the *E. coli* concentration as per method leaflet/brochure.



Figure 15: Count yellow wells as positive for "Total Coliforms"

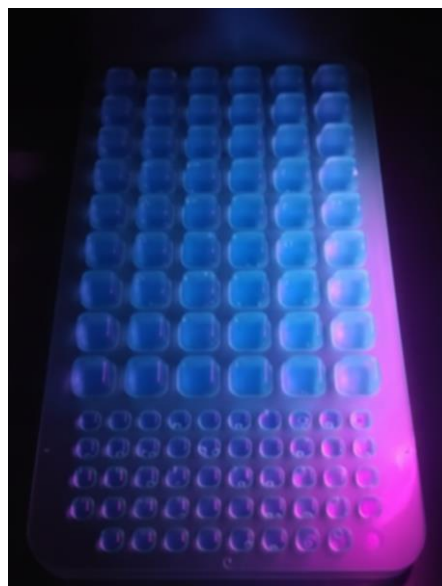


Figure 16: Blue Fluorescence under UV as positive for *E. coli*

7. Quality control:

Following positive and negative control organisms are tested as a quality control.

- Positive *Escherichia coli*
- Negative *Staphylococcus aureus*

8. Interpretation of results:

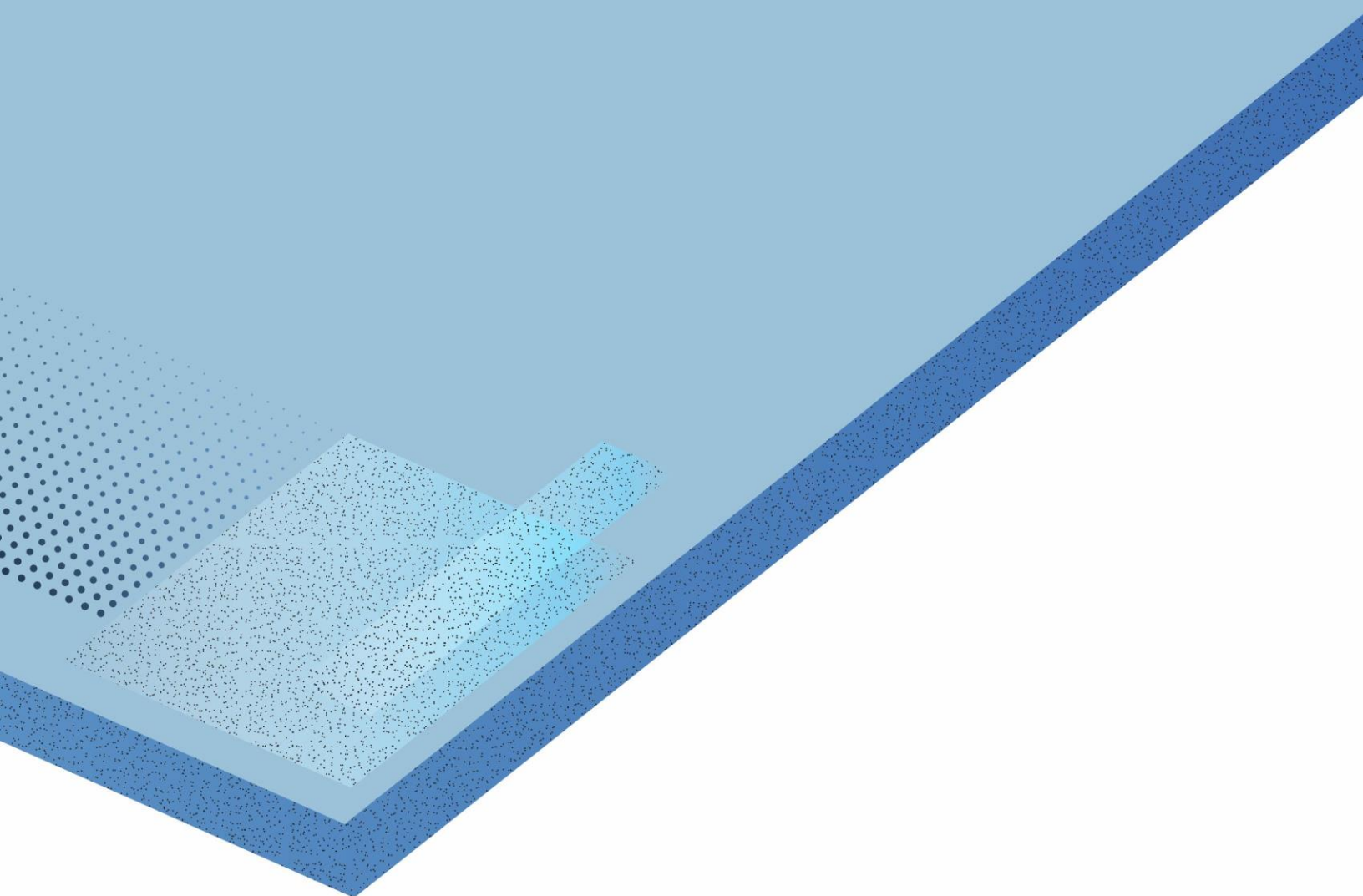
For Total Coliform and *E. coli* use the following Result Interpretation Table:

Appearance	Result
Colorless or slight tinge	Negative for Total Coliforms and <i>E. coli</i>
Yellow equal to or greater than the comparator (supplied by IDEXX)	Positive for Total Coliforms
Yellow equal to or greater than the comparator and fluorescence	Positive for <i>E. coli</i>

If a dilution was performed, after obtaining the initial MPN result from the table, multiply that result by the dilution level to obtain the final result (e.g., if a 1:10 dilution was employed, multiply the result from the MPN table by 10 to get the final result in MPN/100 ml).

PART-4

WATER QUALITY DECONTAMINATION



Part 4. Water Quality Decontamination

4.1 Key Methods of Decontamination

If your water source is found unsafe due to microbial contamination, it is utmost important to take remedial measures to decontaminate and to use it as safe water. The commonly adopted practices at household level are explained as below:

1. **Cleaning of Water Storage Tanks:** It is necessary to clean the underground and overhead water tanks after every 3 months. If water is supplied from water supply line, the connection of main line and distribution line should have no leakage and are properly air tight. Any leakage can be a source of bacteriological contamination. Covering the overhead and underground water tanks is also very important to avoid contamination.
2. **Boiling Method:** Microbiological Contamination can be treated by boiling the water up to 105 °C for 10 Minutes.
3. **Chlorination:** Chlorination is the most reliable disinfection treatment for drinking water. The permissible limit of Chlorine in drinking water is 0.2 to 0.5 mg/L as per National Drinking Water Quality Standards of Pakistan. Chlorine as calcium hypochlorite granules can be added in underground/overhead water storage tanks by calculating the total water storage capacity of the Tank. Chlorine tablets are also available in the market having the capacity to disinfect 20 or 30 liter water per tablet.
4. **Household Filters:** Household filters (comprising cartridge filter, GAC cartridge and UV lamp) available in the market may also be used for water filtration and decontamination however; these need regular replacement of filter cartridges.
5. **Ultraviolet (UV) Disinfection:** Microbiologically Contaminated water can be treated by using ultra violet radiation. Water treatment and disinfection using high energy UV Radiations is an environment friendly method. Special UV lamp Destroy Microorganisms including Bacteria, viruses and to some extent protozoans. UV Treatment does not affect the smell or taste of water.

4.2 Safe Drinking Water During Emergencies

During floods, earthquakes, storms, disease outbreaks, and other emergencies, drinking water sources may become contaminated with sewage, floodwater, animal waste, chemicals, and other pollutants. Consuming contaminated water can lead to serious waterborne diseases such as diarrhea, cholera, typhoid fever, hepatitis, and dysentery. Therefore, ensuring access to safe drinking water is one of the most important public health measures during emergency situations.

Floodwater often carries contaminants and disease-causing microorganisms into wells, boreholes, springs, and water distribution systems. Water from flooded wells should never be consumed without proper treatment. Following a flood event, affected wells and water sources should be thoroughly cleaned, disinfected, and tested for

bacteriological contamination before being returned to service. Guidance from local health authorities or water supply agencies should be sought whenever possible.

Individuals who consume contaminated water may develop symptoms of waterborne illness within hours or several days after exposure. Common symptoms include diarrhea, vomiting, nausea, fever, abdominal cramps, fatigue, and dehydration. Young children, older adults, pregnant women, and individuals with weakened immune systems are particularly vulnerable and should seek immediate medical attention if severe symptoms occur.

When the safety of drinking water is uncertain, only water that has been properly treated or disinfected should be used for drinking, cooking, preparing beverages, brushing teeth, making ice, washing fruits and vegetables, and other consumption-related purposes. Household water treatment methods such as boiling, chlorination, filtration, and solar disinfection can significantly reduce the risk of waterborne diseases and should be applied whenever safe water is not readily available.

Community awareness and timely public health interventions are essential for preventing disease outbreaks during emergencies. Public health personnel, community leaders, and water supply operators should regularly monitor water quality, conduct field testing for bacteriological contamination and residual chlorine, ensure adequate chlorination of water supplies, promote safe water storage and treatment practices, and disseminate educational materials in local languages. Effective communication and public awareness campaigns play a vital role in protecting communities from water-related health risks.

In addition to household-level treatment methods, larger-scale emergency water treatment systems may be deployed during disasters and humanitarian response operations to provide safe drinking water to affected populations. The Pakistan Council of Research in Water Resources (PCRWR) has developed and deployed several emergency water treatment solutions in flood-affected and water-stressed areas of Pakistan. These systems are designed to rapidly produce safe drinking water where conventional water supply infrastructure has been damaged, disrupted, or is unavailable.

4.3 Emergency Water Treatment Hierarchy

Access to safe drinking water at the household level is essential for preventing waterborne diseases and protecting public health. Where centralized treatment systems are unavailable or water quality is uncertain, a range of household water treatment methods can be used to improve the microbiological and physical quality of drinking water. The following methods are commonly recommended for making water safe for human consumption.

1. Boiling (most reliable if only microbial contamination)
2. Filtration combined
3. Solar Disinfection (SODIS)
4. Bio sand Filters
5. Water Treatment Systems
6. Chlorination (bleach or chlorine tablets)

4.3.1 Boiling (Most Reliable Method)

Boiling is the simplest and most effective method for destroying disease-causing microorganisms, including bacteria, viruses, and parasites. Following steps are involved in boiling:

1. If the water is cloudy, allow it to settle and filter it through a clean cloth.
2. Bring the water to a vigorous rolling boil.
3. Continue boiling for at least 1 minute.
4. At elevations above 2,000 meters (6,500 feet), boil for 3 minutes.
5. Allow the water to cool naturally.
6. Store the treated water in a clean, covered container.

Boiling is particularly effective against microorganisms such as *Giardia* and *Cryptosporidium*, which may be resistant to some chemical disinfectants.

4.3.2 Water Filtration

Filtration helps remove suspended particles and can reduce microbial contamination.

Common filtration methods include:

- Ceramic filters
- Biosand filters
- Household water filters
- Membrane filtration systems

For maximum protection, filtration should be followed by boiling or chlorination whenever possible.

4.3.3 Solar Water Disinfection (SODIS)

Where fuel, electricity, and chlorine are unavailable, solar disinfection may be used. Steps include:

1. Fill clean, transparent plastic bottles with clear water.
2. Place the bottles in direct sunlight.
3. Expose them for at least 6 hours on a sunny day.
4. If the weather is cloudy, expose them for up to 2 consecutive days.

Solar radiation and heat help reduce microbial contamination and provide an effective emergency treatment option (Key steps in Figure 17).

SOLAR WATER DISINFECTION (SODIS)

A simple and effective way to make water safe using sunlight

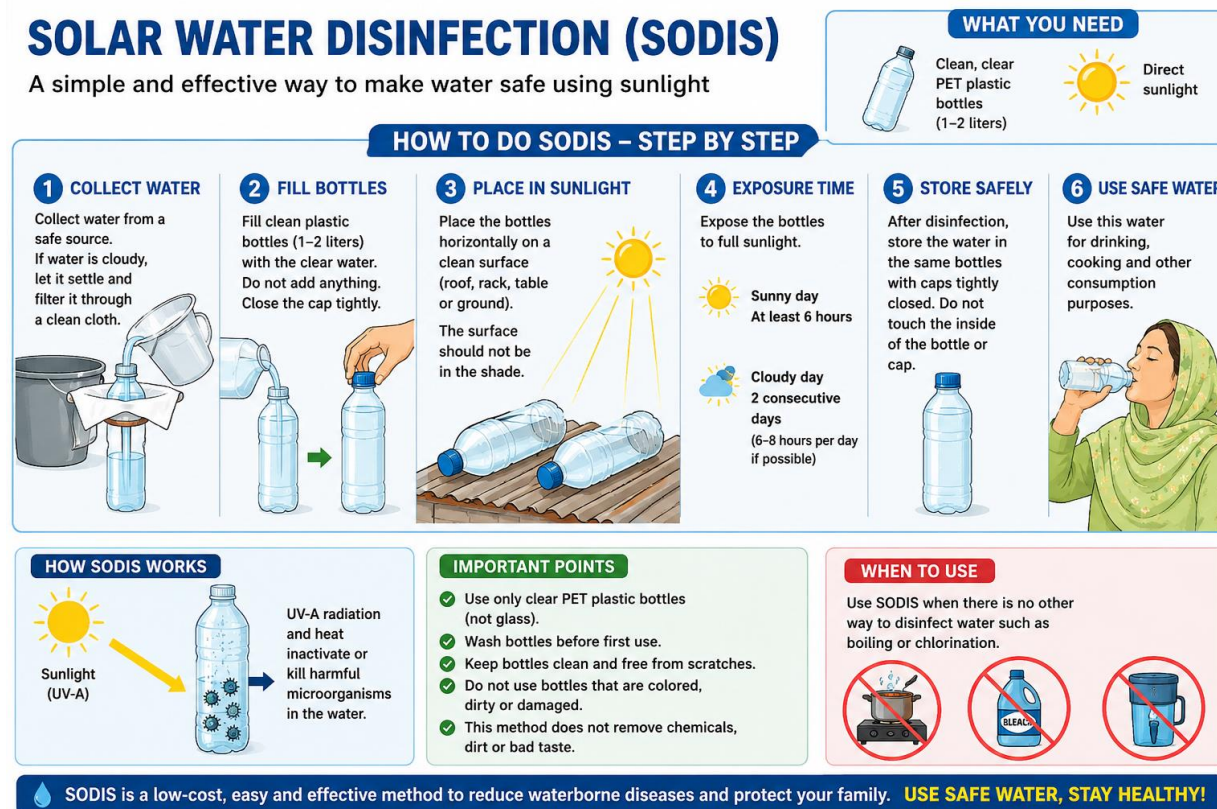


Figure 17: Solar water disinfection

Clear PET bottles may be safely used for Solar Water Disinfection (SODIS) when they are clean, transparent, and in good condition. Bottles that are scratched, damaged, discolored, or excessively aged should be replaced. Current evidence indicates that the health benefits of SODIS greatly outweigh any potential risks associated with chemical migration from PET bottles under normal use conditions.

For a PCRWR or public health manual, it is also worth noting that glass bottles are generally not recommended for SODIS because they are heavier, more fragile, and transmit less UV radiation than clear PET bottles.

4.3.4 Bio-Sand Filtration System

A biosand filter is a simple household water treatment technology that uses layers of sand and gravel to remove suspended solids, turbidity, and disease-causing microorganisms from water. Over time, a biologically active layer (called the Schmutzdecke or Biolayer) develops on the top surface of the sand, enhancing the removal of bacteria, viruses, protozoa, and organic matter. Bio-sand filters are particularly suitable for rural communities because they are inexpensive, easy to construct using locally available materials, and require little maintenance.



Figure 18: Bio-Sand Filter

How a Biosand Filter Works

Water treatment occurs through four main processes:

1. **Mechanical Straining:** Larger particles, sediments, and debris are trapped between sand grains.
2. **Predation and Biological Activity:** Beneficial microorganisms in the biolayer consume and destroy harmful pathogens.
3. **Adsorption:** Some microorganisms and organic matter attach to sand particles and are removed from the water.
4. **Natural Die-Off:** Pathogens trapped within the filter gradually die due to lack of nutrients and oxygen.

Construction Steps

Step 1: Prepare the Container

- Use a concrete, plastic, or fiberglass container approximately:
 - Height: 80–100 cm
 - Width: 25–35 cm

Step 2: Install Outlet Pipe

- Install a PVC outlet pipe near the base.
- Ensure the outlet creates a permanent standing water layer above the sand.

Step 3: Add Filter Media (Table 5)

Carefully place the washed filter media into the container in the following order (from bottom to top):

- i. Drainage gravel
- ii. Support gravel

- iii. Coarse sand
- iv. Fine filtration sand

Step 4: Install Diffuser Plate

- Place a perforated plastic or metal plate above the sand.
- This prevents disturbance of the biological layer when water is added.

Step 5: Commission the Filter

- Fill with clean water.
- Operate the filter daily for approximately 2–4 weeks to allow the biological layer to develop.
- During this period, the biological layer develops and treatment efficiency improves.
- Filtration efficiency improves as the biological layer matures.

Details of required material is given in Table-5 and indicated as Figure 19.

Table 5: Material for construction of biosand filters

Component/Layer	Recommended Material	Typical Size/Thickness	Purpose
Filter Body	Concrete, plastic, fiberglass, or HDPE container	Height: 80–100 cm; Diameter/Width: 25–35 cm	Houses the filter media and provides structural support.
Lid/Cover	Plastic, metal, or fiberglass	Fits securely on filter body	Prevents contamination from dust, insects, and debris.
Diffuser Plate	Perforated plastic, metal, or concrete plate	Placed above sand layer	Distributes incoming water evenly and prevents disturbance of the biological layer.
Standing Water Layer	Water retained above sand surface	5–10 cm depth	Maintains moisture required for biological activity.
Biological Layer (Schmutzdecke)	Naturally develops on sand surface	Forms naturally during operation (2–4 weeks)	Consumes and inactivates harmful microorganisms.
Fine Sand (Filtration Layer)	Clean, washed fine sand	50–60 cm	Main filtration layer that removes suspended particles and microorganisms.
Coarse Sand (Transition Layer)	Clean, washed coarse sand	5–10 cm	Supports the fine sand layer and prevents migration into gravel.

Component/Layer	Recommended Material	Typical Size/Thickness	Purpose
Support Gravel	Clean, washed gravel (6–12 mm)	5 cm	Prevents sand movement and provides support to upper layers.
Drainage Gravel	Clean, washed gravel (12–25 mm)	5 cm	Facilitates water drainage and protects the outlet system from clogging.
Outlet Pipe	PVC pipe and fittings	12–20 mm diameter	Collects and conveys filtered water from the filter.

BIOSAND FILTER

How it looks and its composition

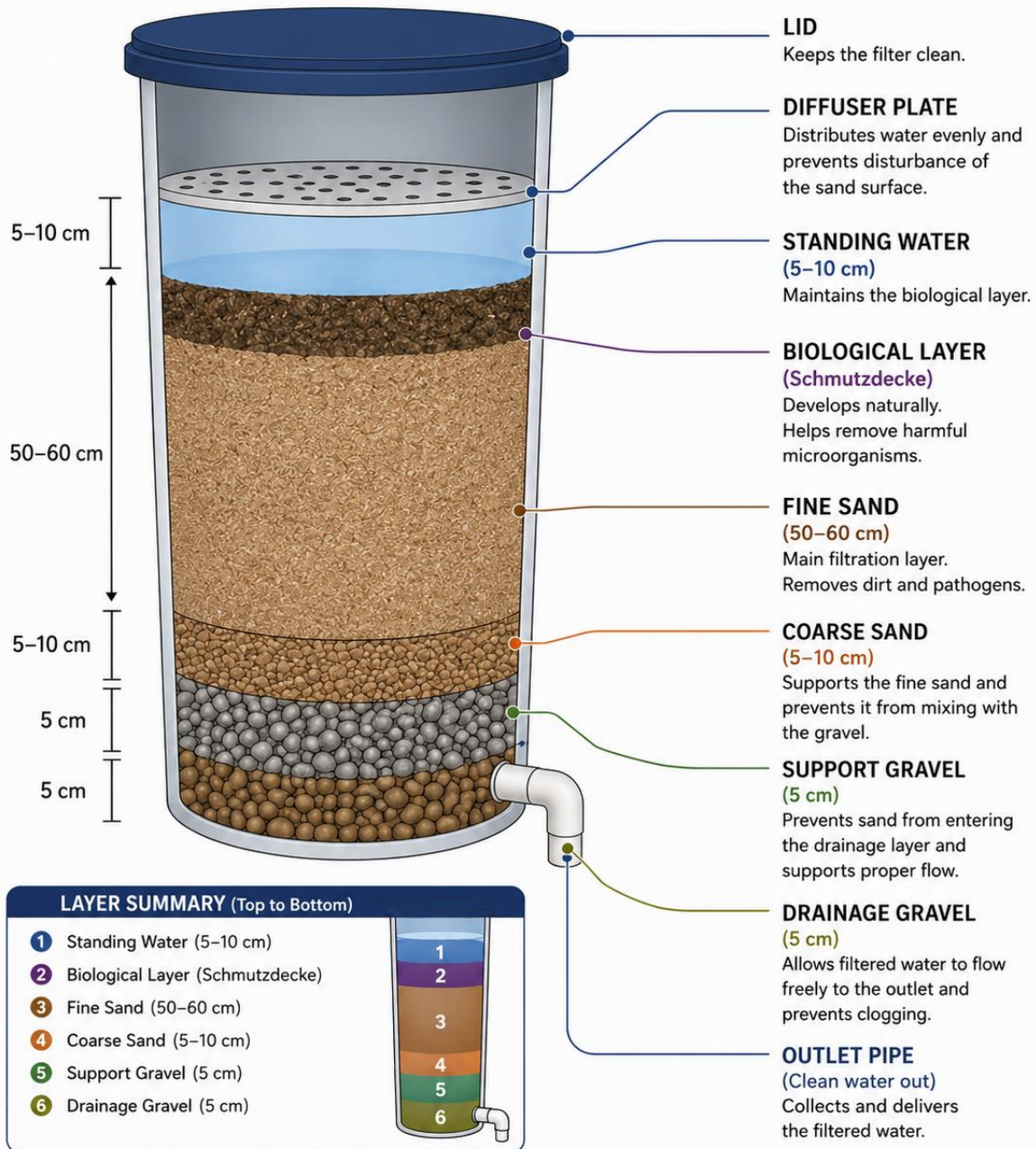


Figure 19: Construction of Bio-sand Filter

4.3.5 Water Treatment Systems

a). Solar-Powered Ultrafiltration Water Treatment Systems

Solar-powered ultrafiltration (UF) systems utilize renewable solar energy to operate advanced membrane filtration units capable of removing suspended solids, bacteria, protozoa, and many disease-causing microorganisms from contaminated water sources (Figure 20).



Figure 20: PCRWR Solar powered water filtration system

Key Features:

- Operate without grid electricity.
- Suitable for remote and disaster-affected areas.
- Can treat surface water, canal water, and floodwater.
- Capable of supplying safe drinking water to large communities.
- Environmentally friendly and cost-effective.

PCRWR has successfully deployed several solar-powered ultrafiltration plants in flood-affected areas of Sindh and Balochistan, with some systems capable of producing up to 20,000 gallons of safe drinking water per day.

b). Hand-Operated Water Treatment Systems

Hand-operated water treatment systems are designed for situations where electricity and fuel are unavailable. These systems utilize manual pumping mechanisms combined with filtration and disinfection technologies to produce safe drinking water (Figure 21).

Advantages:

- Simple operation and maintenance.
- Suitable for small communities and emergency shelters.
- Can function during power outages and disaster situations.
- Portable and easy to deploy.



Figure 21: PCRWR hand operated water filtration system

c). Mobile Water Treatment Units

Mobile water treatment units are mounted on vehicles or transportable platforms and can be rapidly deployed to emergency locations. These units are capable of treating water from rivers, ponds, canals, and floodwater sources (Figure 22).



Figure 22: PCRWR Mobile water treatment units

Advantages:

- Rapid deployment during disasters.
- Flexible operation in remote locations.
- Can serve multiple communities as required.
- Suitable for flood, drought, and emergency response operations.

Additional Information

The technologies described above have been developed and implemented by PCRWR in various regions of Pakistan to support emergency response and improve access to safe drinking water. Technical specifications, operational guidelines, training materials, and information on deployment requirements can be obtained directly from the Pakistan Council of Research in Water Resources (PCRWR). For further information on system design, installation, operation, maintenance, and suitability for specific applications, readers are encouraged to contact PCRWR or visit the relevant PCRWR regional offices.

4.3.6 Chlorination

Chlorination is one of the most effective and widely used methods for disinfecting drinking water. It involves the addition of chlorine or chlorine-based compounds to inactivate disease-causing microorganisms, including bacteria, viruses, and other pathogens. In addition to providing rapid disinfection, chlorination leaves a residual concentration in water that helps protect against recontamination during storage and distribution. Detailed information on the principles, procedures, dosage requirements, and safe application of chlorination is provided in Section V of this manual.

The selection of an appropriate emergency water treatment option depends on the scale of the emergency, population served, availability of resources, water quality conditions, and operational requirements. Table 6 provides general guidance on suitable water treatment approaches for different emergency situations and community needs.

Table 6: possible options for emergency water treatments

Situation	Recommended Option
Household emergency water treatment	Boiling, chlorination, SODIS, household filtration
Small communities or emergency shelters	Hand-operated treatment systems
Remote off-grid communities	Solar-powered ultrafiltration systems
Large-scale disaster response	Mobile water treatment units
Post-flood recovery	Water quality monitoring and chlorination programs

4.4 Post-Disaster Water Quality Monitoring and Disinfection

Following floods, earthquakes, and other emergencies, PCRWR and public health agencies conduct water quality surveillance to assess the safety of drinking water supplies.

Activities typically include:

- Microbiological testing for bacteriological contamination.
- Measurement of free and total residual chlorine.
- Assessment of physico-chemical water quality parameters.
- Chlorination and disinfection of contaminated water sources.
- Community awareness and hygiene promotion activities.
- Identification of safe and unsafe water sources.

Regular monitoring helps prevent outbreaks of waterborne diseases and ensures that affected populations receive safe drinking water.

Role of Public Health Professionals During Water Quality Emergencies

During water quality emergencies, public health professionals play a critical role in protecting communities from waterborne diseases and ensuring access to safe drinking water. They must be adequately trained, prepared, and equipped to respond rapidly to situations such as floods, disease outbreaks, infrastructure failures, and other emergencies that may compromise water quality.

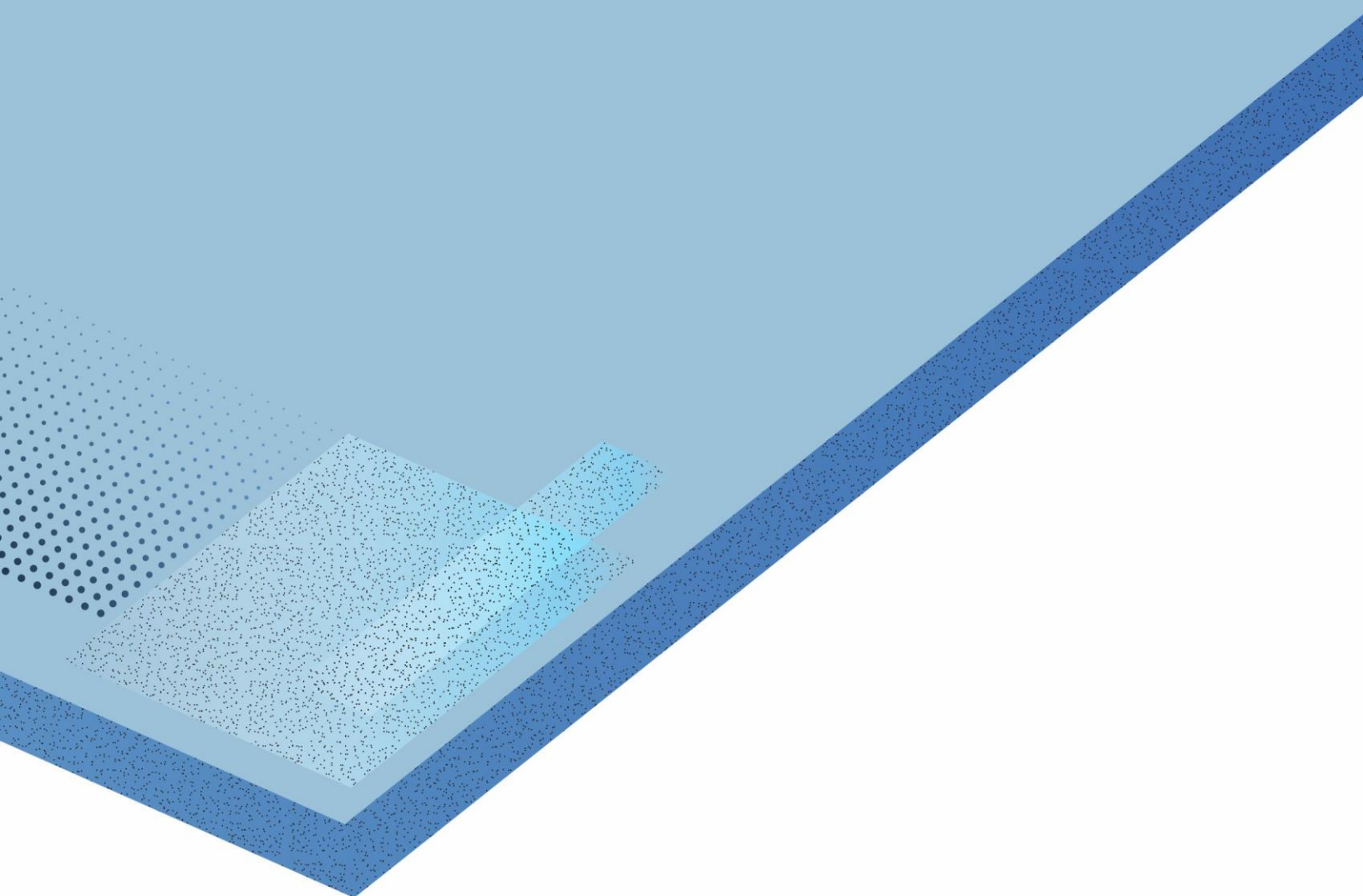
Public health personnel should possess practical knowledge of emergency water treatment and disinfection methods, particularly chlorination, and be capable of conducting field-based water quality assessments. Essential competencies include the ability to perform bacteriological testing, measure free and total residual chlorine, identify potential contamination risks, and recommend appropriate corrective actions to restore water safety.

In addition to technical response activities, community awareness and risk communication are vital components of emergency management. Public health professionals should actively educate communities on safe water handling, household water treatment methods, hygiene practices, and disease prevention measures. Information materials such as leaflets, posters, and awareness messages should be prepared and disseminated in national and local languages to ensure that critical health information is accessible to all affected populations.

Through effective surveillance, rapid response, water quality monitoring, and community engagement, public health professionals contribute significantly to preventing disease outbreaks and safeguarding public health during emergency situations.

PART-5

CHLORINATION AND CHLORINE DEMAND



Part 5. Application of Chlorine to Water Supplies

5.1 Introduction

Chlorination is the process of adding chlorine to water to destroy or inactivate disease-causing microorganisms, including bacteria, viruses, and some parasites. It is one of the most widely used, reliable, and cost-effective methods of drinking water disinfection and has played a major role in reducing waterborne diseases worldwide.

When chlorine is added to water, it reacts to form disinfecting compounds, primarily hypochlorous acid (HOCl) and hypochlorite ion (OCl⁻). These compounds penetrate microbial cells and disrupt essential biological functions, thereby rendering pathogens harmless. In addition to disinfection, chlorination provides residual protection that helps prevent recontamination of water during storage and distribution.

The main objectives of chlorination are to:

- Destroy disease-causing microorganisms and improve the microbiological quality of drinking water.
- Provide a disinfectant residual that protects water from recontamination during storage, transportation, and distribution.
- Control nuisance organisms that may contribute to taste, odor, and biological growth in water systems.
- Enhance public health protection by reducing the risk of waterborne disease outbreaks.

Common chlorine-based disinfectants used in water treatment include chlorine gas, sodium hypochlorite (liquid bleach), calcium hypochlorite (powder or granules), chlorine tablets, and chloramines.

Chlorination is highly effective against most bacteria and viruses. However, some protozoan parasites are more resistant and may require additional treatment processes.

Examples include:

- Cryptosporidium
- Giardia (partially resistant)

5.2 Chlorine Chemistry and Forms of Residual Chlorine

When chlorine is added to water, it forms a number of chemical species that influence disinfection performance. Understanding these forms is important for effective water treatment and monitoring.

Free Chlorine

Free chlorine is the portion of chlorine available for immediate disinfection and is considered the most effective form for controlling microorganisms. It consists mainly of:

- Dissolved chlorine gas (Cl₂)
- Hypochlorous acid (HOCl)
- Hypochlorite ion (OCl⁻)

The effectiveness of free chlorine depends strongly on pH. At lower pH values, hypochlorous acid (HOCl) predominates and provides stronger disinfection. As pH increases, a larger proportion converts to hypochlorite ion (OCl^-), which is less effective.

pH Range	Predominant Chlorine Species
2–7	HOCl predominates
Around 7.2	HOCl and OCl^- occur in nearly equal amounts
Above 7.5	OCl^- predominates

Because HOCl is a much stronger disinfectant than OCl^- , chlorination generally performs better at pH values below 8.

Combined Chlorine

Combined chlorine forms when free chlorine reacts with ammonia or nitrogen-containing compounds present in water. These compounds, commonly known as chloramines, provide weaker disinfection than free chlorine but remain in water for a longer period.

Examples include:

- Monochloramine
- Dichloramine
- Organic chloramines

Total Chlorine

Total chlorine represents the combined concentration of free chlorine and combined chlorine present in water.

Total Chlorine = Free Chlorine + Combined Chlorine

5.3 Chlorine Dose and Chlorine Demand

The chlorine dose refers to the total quantity of chlorine added to water for disinfection. The amount of chlorine required varies according to the quality of the water being treated. **Chlorine demand** is the amount of chlorine consumed by reactions with microorganisms and other substances present in water before a measurable residual chlorine concentration remains. The relationship can be expressed as:

Chlorine Dose = Chlorine Demand + Residual Chlorine

Step by step outline of calculation of chlorine demand is given as below in Figure-23

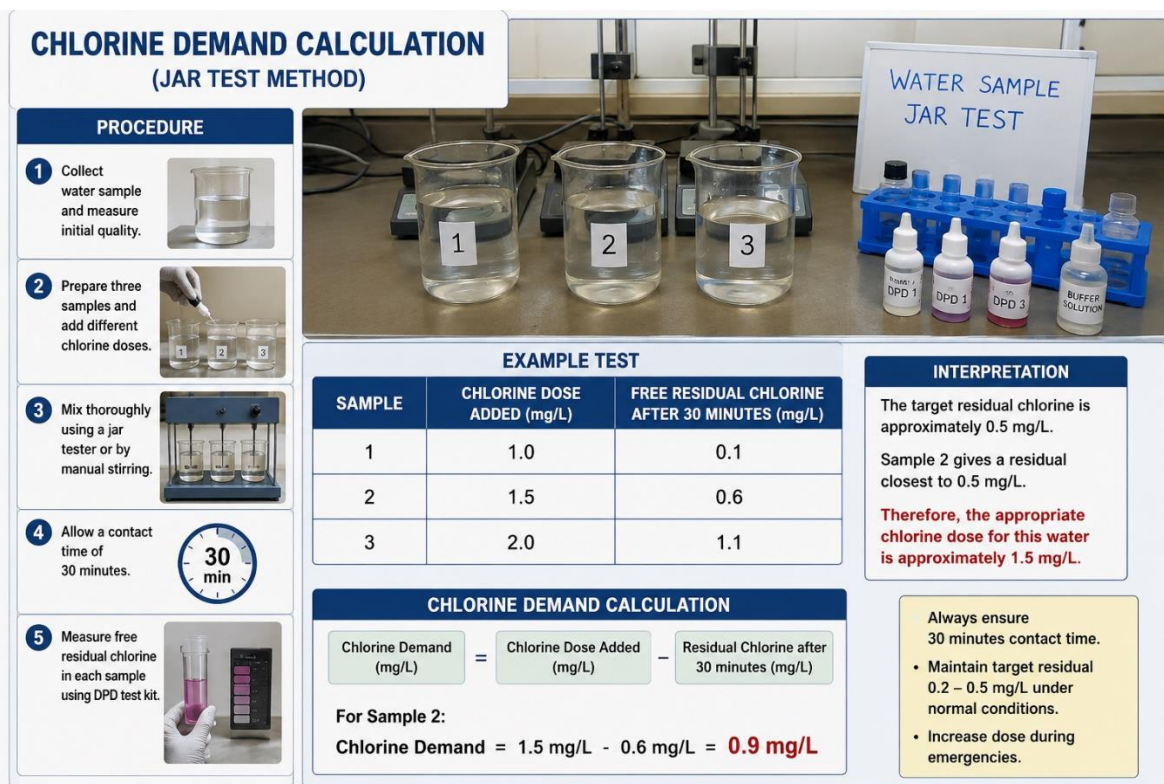


Figure 23: Laboratory Determination of Chlorine Demand and Optimum Chlorine Dose

Residual Chlorine and Operational Requirements

Residual chlorine is the amount of chlorine remaining in water after the required contact time has elapsed. The presence of residual chlorine indicates that sufficient chlorine has been added to satisfy chlorine demand and provide continuing protection against recontamination.

Recommended free residual chlorine concentrations after at least 30 minutes of contact time are:

Situation	Recommended Free Residual Chlorine
Normal conditions	0.2–0.5 mg/L
Routine operational target	Approximately 0.5 mg/L
Emergency situations	≥ 0.5 mg/L
Severe contamination events	Up to 2.0 mg/L

Residual chlorine concentrations exceeding 3.0 mg/L may adversely affect taste and may cause discomfort in some consumers.

Determining Chlorine Demand

Where water quality is unknown, a simple jar test can be used to estimate chlorine demand.

1. Measure and record water quality parameters such as pH and temperature.
2. Prepare several identical water samples.
3. Apply different chlorine doses to each sample.
4. Allow a contact period of 30 minutes.
5. Measure the free residual chlorine concentration in each sample.
6. Select the dose that achieves the target residual chlorine concentration, typically around 0.5 mg/L.

5.4 Factors Affecting Chlorination Efficiency

The effectiveness of chlorination depends on several water quality and operational factors. Understanding these factors is essential for determining the appropriate chlorine dose and ensuring effective disinfection.

Chlorine Dose

The chlorine dose refers to the total quantity of chlorine added to water for disinfection. The amount of chlorine required varies according to the quality of the water being treated. Several factors influence the required chlorine dose:

- **Turbidity:** Water containing suspended particles generally requires a higher chlorine dose because these particles can shield microorganisms from direct contact with chlorine and may also consume part of the disinfectant through chemical reactions.
- **Organic Matter:** Natural organic matter reacts readily with chlorine, reducing the amount available for pathogen inactivation and thereby increasing chlorine demand.
- **Microbial Contamination:** Water with higher concentrations of bacteria, viruses, and other microorganisms requires additional chlorine to achieve effective disinfection.
- **Chemical Constituents:** Substances such as iron, manganese, hydrogen sulfide, ammonia, and other reducing compounds consume chlorine before a residual concentration can be established.
- **Desired Residual Chlorine:** The applied chlorine dose must be sufficient not only to satisfy the chlorine demand of the water but also to maintain the recommended residual chlorine concentration for continued protection against recontamination.

Contact Time

Effective disinfection depends not only on the amount of chlorine added but also on the length of time chlorine remains in contact with the water. A minimum contact time of 30 minutes is generally recommended before water is consumed. Longer contact periods allow chlorine to more effectively inactivate microorganisms and establish a stable residual chlorine concentration.

pH

The efficiency of chlorination is strongly influenced by pH. At lower pH values, chlorine exists predominantly as hypochlorous acid (HOCl), which is a highly effective disinfectant. As pH increases, a larger proportion of chlorine converts to hypochlorite ion (OCl⁻), which has significantly lower disinfecting power. For optimum performance, chlorination is generally most effective at pH values below 8.0.

Temperature

Water temperature affects the rate of disinfection reactions. Higher temperatures generally increase the activity of chlorine and accelerate microbial inactivation, whereas lower temperatures slow the disinfection process and may require longer contact times to achieve the same level of treatment.

Turbidity

Turbidity is one of the most important factors affecting chlorination efficiency. Suspended particles and colloidal matter can protect microorganisms from exposure to chlorine and may increase chlorine demand. Therefore, highly turbid water should always be clarified through settling, coagulation, or filtration before chlorination. Effective removal of turbidity significantly improves disinfection performance and reduces chlorine requirements.

Type of Microorganism

The susceptibility of microorganisms to chlorine varies considerably. Chlorination is highly effective against most bacteria and viruses; however, certain protozoan parasites exhibit greater resistance. For example, *Cryptosporidium* is highly resistant to chlorination, while *Giardia* is moderately resistant. In such cases, additional treatment processes such as filtration may be necessary to ensure adequate pathogen removal.

5.5 Mode of Action of Chlorine on Microorganisms

Chlorine is the most widely used disinfectant in drinking water treatment due to its ability to effectively inactivate a broad spectrum of pathogenic microorganisms. When chlorine is added to water, it reacts to form hypochlorous acid (HOCl), which is the principal disinfecting agent responsible for microbial inactivation.

Hypochlorous acid is a powerful oxidizing agent that readily penetrates the cell walls and membranes of microorganisms. Because HOCl is electrically neutral, it can diffuse through the negatively charged outer surfaces of bacterial cells more efficiently than the hypochlorite ion (OCl⁻). Once inside the cell, HOCl oxidizes and damages essential cellular components, including proteins, enzymes, nucleic acids, and membrane lipids.

The antimicrobial action of chlorine occurs through several mechanisms:

- Oxidation and denaturation of cellular proteins and enzymes.
- Disruption of cell membrane permeability and integrity.
- Inactivation of metabolic and respiratory processes.
- Damage to genetic material (DNA and RNA).
- Interference with cellular reproduction and repair mechanisms.

These reactions result in the loss of cellular function, inhibition of microbial growth, and eventual death or inactivation of bacteria, viruses, and other pathogenic microorganisms.

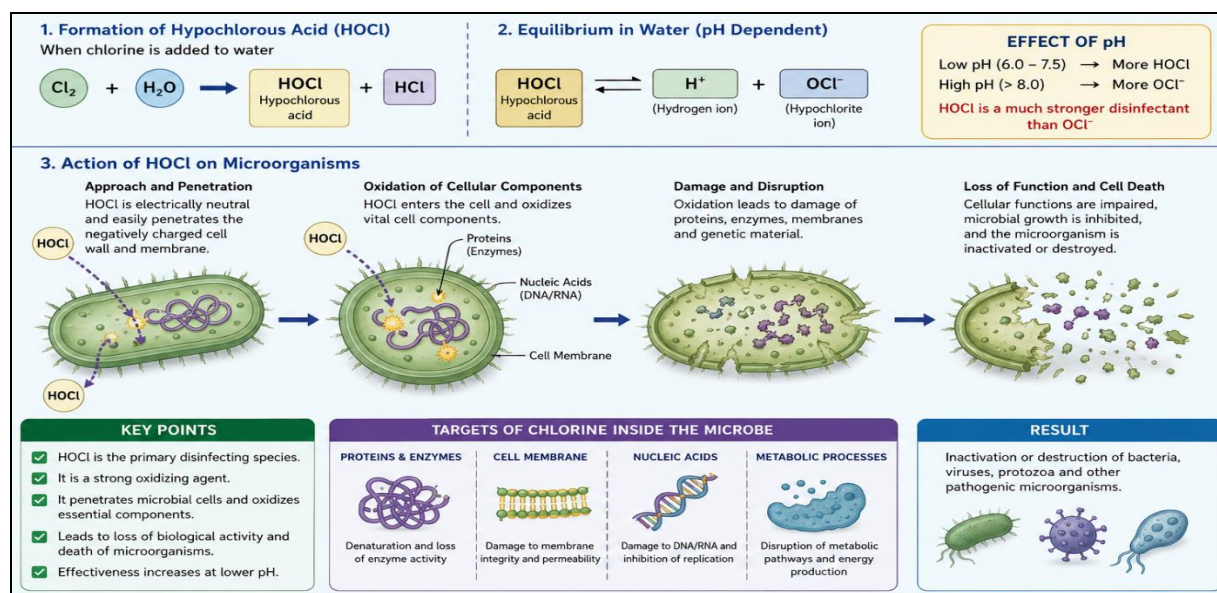


Figure 24: Schematic Illustration of Chlorine Disinfection Showing HOCl Formation, Cell Penetration, and Destruction of Microbial Cells

The effectiveness of chlorination is strongly influenced by pH. At lower pH values, a greater proportion of chlorine exists as hypochlorous acid (HOCl), which is significantly more effective as a disinfectant than hypochlorite ion (OCl⁻). Consequently, chlorination is generally more efficient under slightly acidic to neutral pH conditions.

In addition to providing rapid microbial inactivation, chlorine leaves a measurable residual concentration in the distribution system, offering continued protection against microbial recontamination during storage and distribution of drinking water.

5.6 Chlorination throughout the Water Supply Chain

Chlorination should not be viewed as a single treatment step applied only at the source. Its purpose is to maintain microbiological safety from the point of treatment to the point of consumption.

5.6.1 Chlorination at the Source

Water should be chlorinated immediately after treatment or collection to ensure that disease-causing microorganisms are inactivated before distribution.

Sources commonly requiring chlorination include:

- Surface water supplies
- Community reservoirs
- Water treatment plants
- Tanker-supplied water
- Emergency storage systems

5.6.2 Chlorination During Storage and Distribution

Even when water leaves a treatment facility in a safe condition, it may become contaminated during transportation or storage. Common causes of recontamination include:

- Leaking pipelines
- Unsanitary storage tanks
- Cross-connections with wastewater systems
- Poor handling practices
- Contaminated storage containers

Maintaining an adequate chlorine residual provides ongoing protection against such contamination.

5.6.3 Chlorination at Household Level

Household chlorination may be necessary where centralized treatment is unavailable, unreliable, or disrupted. It is particularly important during:

- Floods and natural disasters
- Disease outbreaks
- Emergency water supply operations
- Rural and remote water supply situations

Routine monitoring of residual chlorine should be carried out throughout the water supply system, including treatment facilities, reservoirs, distribution networks, water tankers, and household storage containers.

5.7 Household and Emergency Water Chlorination

When boiling is not feasible, chlorine-based disinfectants can be used to improve the microbiological safety of drinking water.

Before chlorination:

- Filter cloudy water through a clean cloth whenever possible.
- Allow suspended solids to settle before treatment.
- Use only approved chlorine products intended for water disinfection.

Recommended Dosages for Household Water Treatment

Disinfectant Type	Available Chlorine	Recommended Dosage
Diluted chlorine solution	Approximately 1%	10 drops per litre
Unscented household bleach	4–6% sodium hypochlorite	2 drops per litre
Concentrated bleach	7–10% sodium hypochlorite	1 drop per litre
Chlorine tablets (NaDCC)	Varies by product	Follow manufacturer's instructions
Unknown chlorine concentration	Unknown	10 drops per litre

For cloudy water, the recommended dosage should generally be doubled.

Chlorination Procedure

1. Filter or settle cloudy water before treatment.
2. Add the required amount of chlorine solution or tablet.
3. Mix thoroughly to ensure uniform distribution.
4. Allow the water to stand for at least 30 minutes.
5. Check for a slight chlorine odour before use.
6. If no chlorine odour is detected, repeat the dose and allow an additional 15 minutes of contact time.

Chlorine Tablets

Chlorine tablets provide a convenient and portable means of water disinfection, particularly during emergencies and disaster response operations. The tablets should always be used according to the manufacturer's instructions regarding water volume and contact time.

Calcium Hypochlorite (High-Test Chlorine)

Calcium hypochlorite is commonly used for treating larger quantities of water at community level.

A stock solution may be prepared by dissolving approximately 7 grams of 70% calcium hypochlorite in 7.5 litres of water. For disinfection, approximately 0.5 litre of this stock solution may be added to 50 litres of water, followed by thorough mixing and a minimum contact period of 30 minutes before use.

Safety Precautions

The following precautions should always be observed when handling chlorine products:

- Use only unscented bleach intended for disinfection.
- Do not use products containing detergents, perfumes, or colorants.
- Store chlorine products in a cool, dry, and well-ventilated area.
- Keep chlorine products out of reach of children.
- Avoid direct contact with concentrated chlorine solutions.
- Store treated water in clean, covered containers to prevent recontamination.

5.8 Multi-Barrier Approach to Water Safety

Chlorination is most effective when combined with other water safety measures, including:

- Source protection
- Filtration or sedimentation
- Proper storage and handling
- Regular water quality monitoring
- Maintenance of adequate residual chlorine throughout the distribution system

Maintaining residual chlorine from the source to the consumer provides a critical safeguard against waterborne diseases and is one of the most effective public health interventions for ensuring safe drinking water.

5.9 Determination of Free Residual Chlorine in Drinking Water

1. Purpose

The purpose of this SOP is to describe the procedure for determining Free Residual Chlorine (FRC) in drinking water samples using the DPD (N,N-diethyl-p-phenylenediamine) colorimetric method. Monitoring free residual chlorine is essential to verify the effectiveness of disinfection and ensure the microbiological safety of drinking water supplies.

2. Scope

This SOP applies to the analysis of free residual chlorine in:

- Drinking water supply systems
- Water treatment plants
- Distribution networks
- Storage reservoirs
- Consumer taps
- Emergency water supplies

3. Principle

Free chlorine present in water reacts with DPD reagent to produce a pink coloration. The intensity of the color is directly proportional to the concentration of free residual chlorine in the sample. The developed color is measured using a portable chlorine meter, colorimeter, spectrophotometer, or comparator and reported as mg/L (ppm) as Cl_2 .

4. Responsibilities

Laboratory Analyst / Field Technician

- Collect representative water samples.
- Perform testing according to this SOP.
- Record and report results accurately.
- Maintain testing equipment.

Laboratory Supervisor

- Ensure compliance with SOP requirements.
- Verify analytical results.
- Review quality control records.

5. Safety Precautions

- Wear laboratory coat, gloves, and safety glasses.
- Avoid direct contact with chemical reagents.
- Handle reagents according to Material Safety Data Sheets (MSDS).
- Dispose of used reagents and sample waste according to laboratory waste disposal procedures.

6. Equipment and Materials

Equipment

- Portable chlorine meter/colorimeter or comparator
- Clean sample cell/cuvette
- Stopwatch or timer
- Sample collection bottles

Reagents

- DPD Free Chlorine Reagent (powder pillow, tablet, or liquid)
- Distilled or deionized water (for cleaning)

7. Sample Collection and Preservation

1. Collect water samples in clean containers.
2. Analyze samples immediately after collection.
3. Avoid exposure of samples to direct sunlight.
4. Minimize agitation and delay between sampling and analysis.
5. Testing should preferably be completed within 15 minutes of sample collection.

8. Procedure

8.1 Sample Preparation

1. Rinse the sample cell three times with the water sample.
2. Fill the sample cell to the marked level (typically 10 mL or as specified by the instrument manufacturer).
3. Ensure that the sample is free from air bubbles.

8.2 Analysis

1. Add one DPD Free Chlorine reagent tablet or powder pillow to the sample.
2. Cap the sample cell and mix gently until the reagent dissolves completely.
3. Allow color development according to the manufacturer's instructions (typically 30 seconds to 1 minute).
4. Insert the sample cell into the chlorine meter/colorimeter.
5. Read and record the free residual chlorine concentration in mg/L (ppm).

8.3 Visual Comparator Method (if applicable)

1. Add DPD reagent to the sample.
2. Mix thoroughly until the pink color develops.
3. Compare the developed color against the comparator color standards.
4. Record the corresponding chlorine concentration.

Schematic flow of the free residual chlorine testing is given as below in Figure 25.

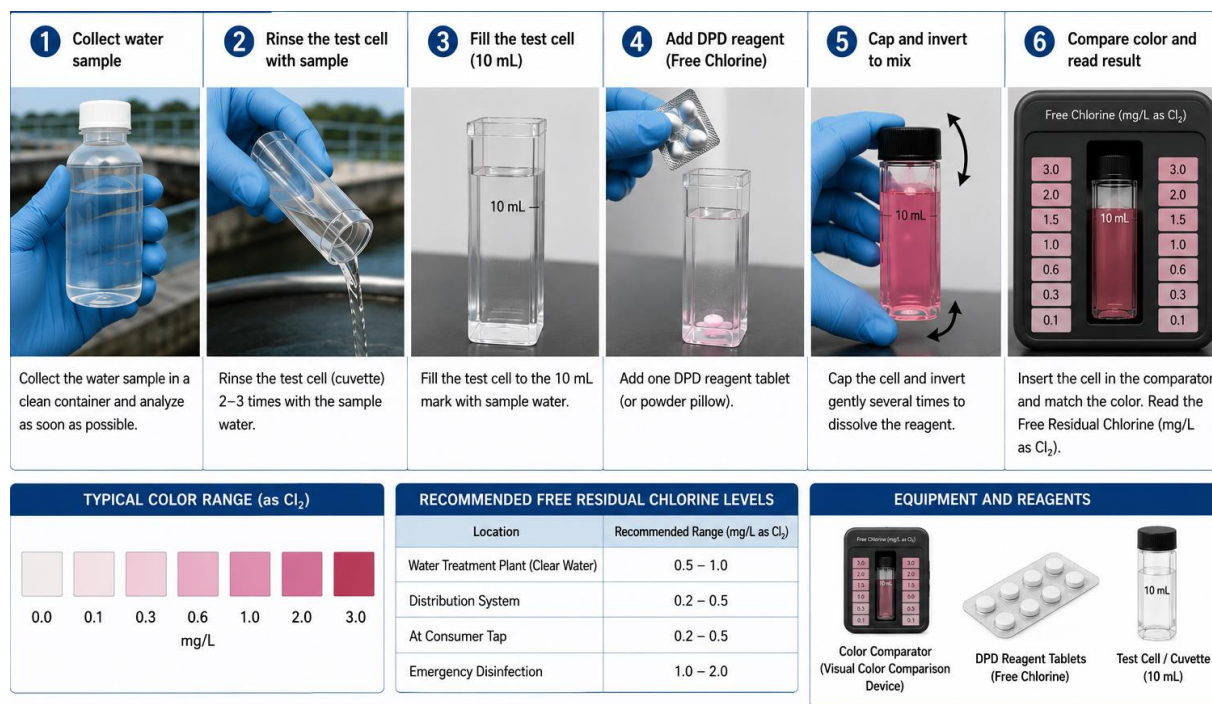


Figure 25: Determination of Free Residual Chlorine Using the DPD Color Comparator Method

9. Quality Control

- Analyze a reagent blank daily.
- Verify instrument calibration according to manufacturer recommendations.
- Analyze duplicate samples at a minimum frequency of 10%.
- Use certified chlorine standards when available.
- Record all quality control results.

10. Calculation

The instrument directly displays the concentration of free residual chlorine.

Result (mg/L as Cl_2) = Meter Reading

No additional calculations are required unless dilution is performed.

11. Interpretation of Results

Free Residual Chlorine (mg/L)	Interpretation
< 0.2	Insufficient disinfection
0.2 – 0.5	Acceptable residual chlorine in distribution systems
0.5 – 1.0	Effective disinfection
> 1.0	Excess chlorine; may affect taste and odor

The recommended free residual chlorine concentration at consumer points is generally 0.2–0.5 mg/L, while higher levels may be maintained at treatment plants or during emergency chlorination programs.

12. Recording and Reporting

Record the following information:

- Sample ID
- Sampling location
- Date and time of collection
- Date and time of analysis
- Free residual chlorine concentration (mg/L)
- Analyst name
- Remarks

Results shall be reported as:

Free Residual Chlorine = ____ mg/L (as Cl₂)

13. References

1. APHA, AWWA, WEF. (2023). *Standard Methods for the Examination of Water and Wastewater*, 24th Edition.
2. World Health Organization (WHO). *Guidelines for Drinking-water Quality*, Latest Edition.
3. Pakistan National Standards for Drinking Water Quality (NSDWQ).
4. USEPA Method 330.5 – Chlorine (Total Residual).

پاکستان تحقیقاتی کونسل برائے آبی وسائل
وزارت سائنس و ٹیکنالوجی
گورنمنٹ آف پاکستان



گھریلو پانی کو جراثیم سے پاک کرنے کا طریقہ

اگر آپ کے گھر کا پانی بیکٹیریا یا جراثیم کی وجہ سے غیر محفوظ پایا جاتا ہے تو اس کو روکنے کے لیے اور اسے محفوظ پانی کے طور پر استعمال کرنے کے لیے اقدامات کرنا انتہائی ضروری ہے۔ گھریلو سطح پر مندرجہ ذیل اقدامات ممکن ہیں۔

1- پانی ذخیرہ کرنے والے ٹینک کی صفائی: ہر تین ماہ بعد زیر زمین اور اوور ہیڈ پانی کے ٹینکوں کو صاف کرنا ضروری ہے۔ اگر پانی واٹر پلائی لائن سے فراہم کیا جاتا ہے تو مین لائن اور ڈسٹری بیوشن لائن کے کنکشن میں کوئی لیکج نہیں ہونا چاہیے اور وہ مناسب طریقے سے بند ہوں۔ لیکج جراثیم کش آلودگی کا سبب بن سکتا ہے۔ آلودگی سے بچنے کے لیے اوور ہیڈ اور زیر زمین پانی کے اندر ٹینکوں کی صفائی کرنا بھی بہت ضروری ہے۔

2- ابالنے کا طریقہ: حیاتیاتی جراثیم کی آلودگی کا علاج 10 منٹ کے لیے 105 ڈگری تک پانی کو ابال کر کیا جاسکتا ہے۔

3- کلورینیشن: پینے کے پانی کے لئے سب سے قابل اعتماد جراثیم کش کلورین کا استعمال ہے۔ پینے کے پانی میں کلورین کی مقدار 0.2 سے 0.5 ملی گرام فی لٹر ہے جو پاکستان کے پینے کے پانی کے قومی معیار کے مطابق ہے۔ کلورین بطور کیمیشم ہائپوکلورائٹ (Calcium Hypochlorite) کے دانے دار ذرات ٹینک کی پانی ذخیرہ کرنے کی کل گنجائش کا حساب کتاب کر کے زیر زمین اور اوور ہیڈ پانی ذخیرہ کرنے والے ٹینک میں شامل کی جاسکتی ہے۔ کلورین کی گولیاں مارکیٹ میں بھی دستیاب ہیں۔ ایک کلورین کی گولی سے 20 سے 30 لٹر پانی کو جراثیم سے پاک کیا جاسکتا ہے۔

4- گھر کے استعمال کے لئے فلٹر: مارکیٹ میں دستیاب ہاؤس ہولڈ (گھر میں استعمال ہونے والے) فلٹرز (کارٹرٹج فلٹر، جی اے سی فلٹر کارٹرٹج اور یو وی لیپ پر مشتمل ہے) کو واٹر فلٹریشن اور جراثیم کو مارنے کے لئے بھی استعمال کیا جاسکتا ہے۔ تاہم ان فلٹرز کارٹرٹج کی باقاعدگی سے تبدیلی ضروری ہے۔

5- الٹرا وائلٹ جراثیم کش: حیاتیاتی جراثیم سے آلودہ پانی کو الٹرا وائلٹ تابکاری لیپ کا استعمال کر کے علاج کیا جاسکتا ہے۔ اعلیٰ توانائی سے UV تابکاری کا استعمال کرتے ہوئے پانی کی صفائی اور جراثیم دور کرنے کے ماحول دوست طریقہ کار موجود ہیں۔ خصوصی UV لیپ حیاتیاتی جراثیم کو ختم کرتے ہیں جس میں بیکٹیریا، وائرس اور کسی حد تک پروٹوزون بھی شامل ہیں۔ یہ طریقہ پانی کی بو یا ذائقہ پر اثر انداز نہیں ہوتا۔

پاکستان تحقیقاتی کونسل برائے آبی وسائل

خیابان جوہر، H-8/1 اسلام آباد

رابطہ نمبر: 051-9101282-3

Annexure-A: National Drinking Water Quality Standards of Pakistan

A. Physical Requirements

S. #.	Characteristics	Unit	MAC*
1	pH	-	6.5-8.5
2	Turbidity	NTU	5
3	Colour	TCU	15
4	Taste & Odor	-	Unobjectionable

B. Chemical Requirements

1	Alkyl Benzyl Sulfates	mg/l	-
2	Calcium (Ca)	mg/l	-
3	Total Hardness (CaCO ₃)	mg/l	500
4	Chloride (Cl)	mg/l	250
5	Sulfate (SO ₄)	mg/l	250
6	Nitrate (NO ₃)	mg/l	50
7	Total Dissolved Solids	mg/l	1000
8	Iron (Fe)	mg/l	0.3
9	Fluoride (F)	mg/l	1.5
10	Nitrite (NO ₂)	mg/l	3
11	Total Ammonia	mg/l	-
12	Hydrogen Sulfide	mg/l	-
13	Zinc (Zn)	mg/l	5.0
14	Manganese (Mn)	mg/l	0.05
15	Copper (Cu)	mg/l	1.0
16	Magnesium (Mg)	mg/l	-

C. Limits of Toxic Substances

1	Arsenic (As)	mg/l	0.05
2	Cadmium (Cd)	mg/l	0.01
3	Chromium (Cr)	mg/l	0.05
4	Cyanide (Cn)	mg/l	0.05
5	Lead (Pb)	mg/l	0.05
6	Selenium (Se)	mg/l	0.01

D. Limits for Bacteriological contaminants

Source/Organisms	Guideline Value
All water intended for drinking (E. coli or Thermotolerant Coliform Bacteria)	Must not be detectable in any 100 ml sample
Treated water entering the distribution system (E. coli or Thermotolerant Coliform and Total Coliform Bacteria)	Must not be detectable in any 100 ml sample

Source/Organisms	Guideline Value
Treated water in the distribution system (<i>E. coli</i> or Thermotolerant Coliform and Total Coliform bacteria)	Must not be detectable in any 100 ml sample In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period.

* Maximum Acceptable Concentration

The figures indicated under the column “Acceptable” are the limits up to which the water is generally acceptable to the consumers.

Figures in excess of those mentioned under “acceptable” render water not acceptable, but still may be tolerated in absence of alternative and better source but up to the limits indicated under column “Marginal” above which the supply will have to be rejected.

E. Bacteriological Standards

- i) Water entering the distribution system coliform count in any sample of 100 ml should be zero.

- ii) Water in the distribution system shall satisfy all the three criteria indicated below;

E.coli count in 100 ml of any sample should be zero

Coliform organisms no more than 10 per 100 ml shall be present in any sample

Coliform organisms should not be detectable in 100 ml of any two consecutive samples or more than 50% of the samples collected for the year.

Individual or small community supplies.

E.coli count should be zero in any sample of 100 ml and coliform organisms should not be more than 3 per 100 ml.

F. Virological Aspects

A level of 0.5 mg/l of free chlorine residual for one hour is sufficient to inactivate virus, even in water that was originally polluted. This free chlorine residual is to be insisted in all disinfected supplies in areas suspected of endemicity of infectious hepatitis to take care of the safety of the supply from virus point of view, which incidentally takes care of the safety from the bacteriological point of view as well. For other areas 0.2 mg/l of free chlorine residual for half an hour should be insisted.

Annexure-B: WHO Guidelines for Drinking Water

A. Bacteriological Qualities

Source/Organisms	Guideline Value
All water intended for drinking (E. coli or Thermotolerant Coliform Bacteria)	Must not be detectable in any 100 ml sample
Treated water entering the distribution system (E. coli or Thermotolerant Coliform and Total Coliform Bacteria)	Must not be detectable in any 100 ml sample
Treated water in the distribution system (E. coli or Thermotolerant Coliform and Total Coliform Bacteria)	Must not be detectable in any 100 ml sample

B. Chemicals of Health Significance

Inorganic	mg/l	Inorganic	mg/l	Inorganic	mg/l
Antimony	0.02	Copper	2.000	Molybdenum	occurs in drinking-water at concentrations well below those of health concern
Arsenic	0.010	Fluoride	1.500		
Barium	1.3	Lead	0.010		
Boron	2.4	Manganese	0.08		
Cadmium	0.003	Mercury	0.006		
Chromium	0.050	Nickel	0.07		
		Nitrate(NO ₃)	50.00		
		Nitrite(NO ₂)	03.00	Cyanide	Occurs in drinking-water at concentrations well below those of health concern, except in emergency situations following a spill to a water source
		Selenium	0.04		
		Uranium	0.03		

C. Other Parameters

Parameter	mg/l	Parameter	mg/l	Parameter	mg/l
Color	15 TCU	1,2 dichlorobenzene	1	Hardness, Zinc,	-Not of health concern at levels found in drinking-water
Taste, Odor.	-	1,4-dichlorobenzene	0.3	SO ₄ , Na, Fe,	
Turbidity	5 NTU	Tetrachlorethene	0.1	Hydrogen Sulfide,	The health-based value exceeds practicable levels based on optimization of the coagulation process in drinking-water plants using aluminum-based coagulants: 0.1 mg/l or less in large water treatment facilities and 0.2 mg/l or less in small facilities
Toluene	0.70	Ethylbenzene	0.3	TDS, PH, Cl,	
Xylenes	0.5	Copper	2	Monochlorobenzene,	
Ethyl-benzene	0.3	DO	0.05	Ammonia	
Styrene	0.02			Al	

D. Pesticides

Pyridate	Not persistent and only rarely found in drinking-water 0.04	Chlorotoluron	0.03 occurs in drinking-water or drinking-water sources at concentrations well below those of health concern
1,2-dichloropropane		Bentazon	