PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down a procedure for sampling and testing of different types of water from different sampling points of water.

2.0 SCOPE:

This SOP is applicable for sampling and testing of raw water, Potable Water, Soft water, RO water and purified water from designated sampling points of water for chemical & microbiological analysis.

3.0 RESPONSIBILITY:

Microbiologist / Analyst - Quality Control

Head – Quality Control

4.0 PROCEDURE:

Sampling procedure

4.1 Preparation of Sampling Conical Flasks/ Glass bottles:

4.1.1 For chemical analysis cleaned and dried glassware shall be used.

4.1.2 For microbiological analysis, conical flasks shall be plugged with cotton and wrapped with aluminium foil on mouth of conical flask or alternatively glass

Bottle with lid can be used and then sterilized these bottles or conical flask in autoclave as per reference SOP. These sterile flasks / Bottles shall be used.

4.1.3 All conical flasks/ bottles used for sampling shall be properly stopper & kept in S.S. sampling kit before taking it to the sampling points.

4.2 Requirements:

4.2.1 Dry and clean stoppered glass bottles of 1000 ml capacity, for chemical testing.

4.2.2 Sterilize conical flask / bottle of 250 ml capacity for microbiology testing.

4.2.3 Filtered 70 %v/v IPA for sanitization of sampling point.

4.2.4 Sterile gloves and masks to prevent external contamination due to handling.

4.3 Sample collection:

4.3.1 Take the glassware (sterile for microbiology), mask, gloves & Filtered 70 % v/v IPA required for sampling to the respective sampling point in closed condition without exposing to the atmosphere, preferably in SS sampling kit.

4.3.2 Wear gloves, masks and wipe hands with Filtered 70 % v/v IPA.

4.3.3 Spray the exterior of sampling point with Filtered 70 % IPA v/v and leave it for a contact period of 1minute.

4.3.4 Open the sampling point valve fully and flush the water at least two to three liters.

4.3.5 Collect the water samples for Chemical and Microbiological analysis separately.

4.3.6 For microbiological sample collection, bring the Sterile conical flasks or Sterile Bottle close to the sampling point, open the plug/ Cap carefully to avoid any contamination from atmosphere, collect the sample without rinsing and close
the conical flasks/ bottle immediately.

4.3.7 Cover the conical flask/ bottle immediately with cotton plug to protect the samples from contamination. In case of bottle replace the cap immediately to avoid external contamination.

4.3.8 Rinse the glass bottles twice with water and then collect the sample for chemical analysis.

4.3.9 Label the conical flask/ bottle with details of type of water, Sampling point No., date of sampling, time of sampling and Sampled By as per Annexure-I.

4.4 Precautions during sampling:

4.4.1 Wear gloves and nose masks before collecting sample.

4.4.2 Collect the sample without connecting the hose pipe or silicone tube.

4.4.3 During sampling, the flow from the sampling point shall be restricted to permit filling of the conical flasks/ bottle without splashing.

4.4.4 During sampling remove the stopper / lid by taking all care to prevent any external contamination.

4.4.5 **NOTE:** As far as possible the sample shall be tested with in about 2 hours of collection and the sample shall be held at refrigerated temperature (2-8 °c) for maximum of about 24 hours.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

4.5 Chemical testing of water :

4.5.1 For chemical testing of Raw water, Potable water, Soft water and RO water refer Specification and refer Standard testing procedure.

4.5.2 For chemical testing of Purified water refers Specification and Standard testing procedure.

4.6 Microbiological testing of water

4.6.1 **Test for total viable aerobic microbial count by pour plate method**
(For Raw water, Chlorinated water, Soft water and RO water)

4.6.2 If necessary dilute the sample 10 times i.e. 1: 10 in buffered sodium chloride – peptone solution pH 7.0 / saline solution and take 1 ml diluted sample for analysis.

4.6.3 Transfer 1 ml of the prepared dilution in duplicate plate for detection of Bacteria and Fungi in duplicates.

4.6.4 Pour approximately 15 to 20 ml of previously cooled (NMT45°C), R2A Agar / Soya bean casein Digest Agar (SCDA) and Sabouraud Dextrose Agar (SDA)/ Sabouraud Chloramphenicol Agar (SCA) for bacteria and fungi respectively.

4.6.5 Cover the Petri dishes, mix the sample by rotating the dish clockwise & anticlockwise, and then allow the contents to solidify at room temperature.

4.6.6 Invert the Petridishes & incubate the R2A Agar/ SCDA plates at 30°C - 35°C for 5 days and SDA/SCA plates at 20°C - 25°C for 5 days.

4.6.7 Count the number of colonies developed & expresses the counts as cfu/ml of the sample examined. (If dilutions were made multiply number of cfu/ml with the dilution factor.)

4.6.8 Record the observations in the respective analytical test data sheet.

4.6.9 **Test for Total Aerobic Microbial Count (TAMC) and Total Yeast And Mould Count (TYMC) by membrane filtration method for Purified Water**

4.6.10 Use cellulose nitrate or cellulose acetate filters 47 mm in diameter and having a nominal pore size not greater than 0.45 µm.

4.6.11 Assemble sterile filtration assembly on the sterile filtration flask. Separate top portion of the filtration assembly by loosening the clamp and place sterile membrane filter with the help of sterile forceps in the apparatus.

4.6.12 Based on the microbial load data obtained, dilute the sample in sterile buffered sodium chloride -peptone solution pH 7.0 to get about 30-70 colonies and filter through 0.45 µm membrane filter.

4.6.13 Wash the membrane filter by filtering through it, three or more successive quantities each of about 100 ml of sterile purified water or buffered sodium chloride-peptone solution pH 7.0. After washing, transfer the membrane filter on to the surface of pre- incubated R2A agar plates in duplicate for bacterial count and SDA plate for Yeast and Mould count with the help of sterile forceps.

4.6.14 Invert the plates and incubate R2A Agar plate at 30°C to 35 °C for 3-5 days and SDA plates at 20°C to 25°C for 3 to 5 days. Observe the plates for growth of bacteria and fungus.

4.6.15 **Preparation of negative control.**

4.6.16 Transfer the flask containing 100 ml of sterile purified water or buffered sodium chloride-peptone solution pH 7.0 (diluting fluid used) in to the filtration Funnel and filter it by vacuum.

4.6.17 Aseptically transfer the filter paper with the aid of sterilized forceps on the surface of pre-incubated R2A agar plate and incubate at 30°C to 35 °C for 3 to 5 days and SDA plate at 20°C to 25°C for 3 to 5 days.
Observe the plate for growth of bacteria and fungus.

4.6.18 If no growth found in the plate it assures that the purified water used for dilution is sterilized properly and all analysis is valid.

4.6.19 If growth observed in negative control, all the analysis carried out by this purified water is invalid and investigation shall be carried out to detect the causes.

4.7 Precaution:

4.7.1 Do not use the pre incubated plates of R2A agar if any growth is found.

4.7.2 Take the utmost care not to keep the vacuum “ON” after completion of filtration of the sample to avoid desiccation and damage of microbial cells.

4.8 Test for pathogens of water:

(For Raw water, Chlorinated water, Soft water ,RO and Purified water)

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

4.8.1 **Enrichment Method**

Add 100 ml of sample to 100 ml of sterile Soyabean Casein Digest Medium (SCDM) Shake well and incubate at 30°C - 35°C for 18 to 24 hours (Enrichment sample-I).

4.8.2 **Test for Escherichia coli (E. coli)**

4.8.3 **Primary Test:** Add 1 ml of above enrichment sample-I to 20 ml of MacConkey broth (Durham's tube should be fully immersed in medium). Incubate at 43°C - 45°C for 24 to 48 hours. If acid and gas formation is observed (indicated by change in colour from purple to yellow with gas bubble observed in inverted Durham's tube), carry out secondary test. If acid and gas formation is not observed, conclude as 'E coli absent'.

4.8.4 **Secondary Test (Indole test):** Add 0.1 ml of the content of the above incubated tube to 5 ml of MacConkey broth and to 5 ml of 1 % peptone water .Incubate both the tubes at 30°C - 35°C for18 to 24 hours. After incubation observe the MacConkey broth for acid and gas formation. To the 1% peptone water, add 0.5ml of Kovac's reagent. Shake well; allow to stand for one minute. If cherry red colour is produced in the reagent layer, Indole is present. The presence of acid and gas formation along with Indole formation indicates the presence of E. coli.

4.8.5 **Alternative Test No. 1**

Add 1 ml of enrichment sample -I to 100 ml of MacConkey broth and incubate at 43-45 °C for 18-24 hours. Subculture on MacConkey agar plate and incubate at 35-37°C for 18-72 hours. If there is a growth of red, non-mucoid colonies, perform Gram staining.

4.8.6 If gram staining shows presence of gram negative rods, carry out indole test as shown above. The presence of red, non-mucoid colonies, showing gram negative rods along with indole formation indicates the presence of E. coli.

4.8.7 **Alternative Test No. 2**

4.8.8 Streak a portion from the enrichment sample-I on the surface of MacConkey agar plate and incubate at 30°C to 35°C for 18-72 hours. If there is presence of brick-red colonies, having a surrounding zone of precipitated bile, transfer the suspected colony on Eosin-Methylene Blue agar Levin and incubate. The presence of characteristic metallic sheen on the colonies under reflected light and a blue-black appearance under transmitted light indicates the presence of E. coli.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

4.9 Test for Salmonella sp.

4.9.1 **Primary Test**: Take 1 ml from enrichment sample -I to each 10 ml of Rappaport Vassiliadis Salmonella Enrichment Broth (RVS) and incubate at 30°C - 35°C for 18-24 hours. From each of these cultures, sub culture any one of following media and incubate at 30°C to 35°C for 24 to 48 hours. Observe for characteristic growth on each medium.

Xylose-lysine-deoxycholate agar

Brilliant green agar

Deoxycholate citrate agar

Bismuth Sulphite Agar.

4.9.2 **Medium: Description of Colony**

Xylose-lysine-deoxycholate agar : Red with/without black center

Brilliant green agar : Small transparent colorless pink/white (frequently surrounded by a pink or red zone)

Deoxycholate citrate agar : Colorless and opaque with/without black center

Bismuth sulphite agar : Black or green

4.9.4 If any colonies conforming to the above description are observed, proceed for secondary test.

4.9.5 **Secondary Test :** Subculture any colony which is showing above description on Triple Sugar Iron Agar by first inoculating the surface, then stab the culture

Or inoculate in Urea broth tube. Incubate at 30°C - 35°C for 18 to 24 hours. The formation of acid and gas in the stab culture (with or without concomitant blackening), absence of acidity from the surface growth in the Triple sugar iron agar or absence of red colour in the Urea broth, indicates the presence of Salmonella.

4.10 Test for Pseudomonas aeruginosa :

4.10.1 Take a loopful from enrichment sample -I and streak on the surface of Cetrimide agar medium and incubate at 30°C-35°C for 48 to 72 hours. If, upon examination, no growth is observed having the characteristics listed in Table given below, Pseudomonas aeruginosa is absent.

Medium	Characteristic Colonial Morphology	Fluorescence in UV light	Oxidase Test	Gram Stain
Cetrimide agar	Generally greenish	Greenish	Positive	Negative rods

4.10.2 Secondary Test: If above described colonies are found to be present, streak representative suspect colonies from the agar surface of Cetrimide agar on the surface of pseudomonas agar medium for detection of pyocyanin and pseudomonas agar medium for detection of fluorescein. Cover and invert the inoculated media plate and incubate at 35°C - 37°C for not less than 3 days

4.10.3 Examine the streaked surfaces under ultra - violet light. Examine the plates to Determine colonies as described in below table. If the colonies do not show below characteristics, sample is free from Pseudomonas aeruginosa. If growth of suspect colonies occurs carry out oxidase test and Gram staining.

Pseudomonas agar medium for detection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for detection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

4.10.4 **Oxidase Test :** Place 2 or 3 drops of a freshly prepared 1% w/v solution of N,N,N1,N1 - tetramethyl-4-phenylenediamine dihydrochloride on suspected colony or use the readymade Oxidase discs at suspected colony, if there is no development of a colour changing to purple, the sample meets the requirements of the test for absence of Pseudomonas aeruginosa.

4.10.5 Gram Staining: If no gram negative rods are observed the sample meets the requirements of the test for absence of Pseudomonas aeruginosa.

4.11 Test for Staphylococcus aureus:

4.11.1 Take a loopful from an Enrichment sample-I and steak on the surface of Mannitol-salt agar medium or Vogel- Johnson agar and incubate at 30°C-35°C for 48 to 72 hours.

If, upon examination, no growth is observed having the characteristics listed in Table given below, Staphylococcus aureus is Absent.

Selective medium	Characteristic colonial morphology	Gram stain
Mannitol-salt agar	Yellow colonies with yellow zones	Positive cocci (in clusters)
Vogel-Johnson agar	Black surrounded by yellow zones	Positive cocci (in clusters)
Baird-Parker agar	Black,shiny, surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)

4.12 Membrane Filtration method:

4.12.1 **Enrichment**

4.12.2 Filter aseptically 100 ml of water sample through a suitable membrane filter (0.45 micron) mounted on a sterile filtration assembly and transfers the membrane filter to 100 ml of pre-sterilized Soyabean Casein Digest Medium. Shake well and incubate at 30°C-35°C for 18 to 24 hours (Enrichment sample-II).

4.12.3 **Test for E.coli:** From the above Enrichment sample-II, proceed further as mentioned in point No. From 4.8.2 to 4.8.8.

4.13 Test for Salmonella :

4.13.1 From the above Enrichment sample-II, streak the enrichment sample on any one of media mention as point No. from 4.9.1 to 4.9.5.

4.14 Test for Pseudomonas aeruginosa:

4.14.1 From the above Enrichment sample-II, proceed further as mentioned in point No. from 4.10.1 to 4.10.4.

4.15 Test for Staphylococcus aureus:

4.15.1 From the above Enrichment sample-II, proceed further as mentioned in point No. 4.11.1

4.16 Frequency of water testing for different sampling point :

4.16.1 For Phase-I & Phase II validation shell be performed in procedure mentioned in Performance Qualification Protocol of water system. and in Phase-I & Phase II validation water sample shell be collected for chemical as well as microbial analysis on daily basis.

4.16.2 After successful completion of Phase I & Phase II validation study

Water sampling and testing frequency of individual sampling point shall be reduced as per phase III Performance Qualification Protocol of water system.

4.16.3 **Microbial Limit for water:**

Type of water	**Limit**			
	Alert Limit	**Action Limit**	**Specification**	
			Limit	**Reference No.**
Raw Water	NA	NA	500 cfu/ml	
Potable Water	NA	NA	250cfu/ml	
Soft Water	NA	NA	250cfu/ml	
Outlet of RO1	NA	NA	200 cfu/ml	
Outlet of RO2	NA	NA	100 cfu/ml	
Purified water	40 cfu/ml	45 cfu/ml	50 cfu/ml	

* Current version of respective specification.

4.17 Trend preparation

4.17.1 Prepare monthly trends for the sample which is analysed, after three months Trends should be reviewed by Head- Quality Control.

4.17.2 Based on the trend data corrective and preventive action should be taken.

On the basis of trend data alert and action limit can be established.

4.18 Release / Rejection of water :

4.18.1 After testing of water, if it is complies as per Specification, analyst shall make the COA as per respective format and shall approve.

4.18.2 Assign the AR Number of water as per respective SOP.

4.18.3 If water is out of specification, analyst shall immediately inform to Head-QC.

4.18.4 QC Head shall inform the all concerned departments (Engineering, QA & Production).

4.18.5 Handle the OOS (Out of specification) result as per respective SOP.

5.0 ANNEXURE (S):

Annexure – I : Specimen label for water sample

Annexure – II : Sample receipt log book for testing of water

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Annexure – III : Sampling point location, Sampling ID, Testing Parameter

Annexure – IV : Sampling schedule for purified water system

6.0 REFERENCE (S):

SOP: Sterilization of glass wares/ apparatus/ accessories and garments used in microbiology laboratory.

SOP: Investigation of out of specification of test result.

SOP: Allocation of Analytical Reference Number

SOP: Preparation, Approval, Distribution control, revision and Destruction of Standard operating Procedure (SOP).

7.0 ABBREVIATION (S) /DEFINITION (S) :

IPA : Isopropyl Alcohol

RO : Reverse Osmosis

SDA :Sabaraoud Dextrose Agar

RVSEB : Rappaport Vassiliadis Salmonella Enrichment Broth

SCDM : Soyabean Casein Digest Medium

REVISION CARD

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
01	00	---	---	New SOP	---

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Annexure 1

SPECIMEN LABEL FOR WATER SAMPLE

WATER SAMPLE

Type of water:

Sampling point No.:

Date of sampling:

Time of sampling:

Sampled by:

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Annexure 2

SAMPLE RECEIPT LOG BOOK FOR TESTING OF WATER

S.No.	Date of receipt	Name of Sample	Sampling point No.	Analysis Started on	Analysis Completed on	A.R. No.	Remark

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Annexure 3

SAMPLING POINT LOCATION, SAMPLING ID. , TESTING PARAMETER

S.No.	Sampling Point ID.	Location	Method of analysis	Test to be performed
1		Before NaOCl dosing system	Pour plate	As per Raw water Specification
2		After NaOCl dosing system	Pour plate	Description , pH , Free Chlorine Total Hardness & Microbiological Analysis
3		Inlet MGF 101	Pour plate	Description, Free Chlorine, Total Hardness, Total suspended Solid & Microbiological Analysis.
4		Inlet MGF 102	Pour plate	Description, Free Chlorine, Total Hardness, Total Suspended Solid & Microbiological Analysis.
5		Outlet MGF 101	Pour plate	Description, Total Hardness, Total Suspended Solid & Microbiological Analysis.
6		Outlet MGF 102	Pour plate	Description, Total Hardness, Total Suspended Solid & Microbiological Analysis.
7		Outlet SF 101	Pour plate	Description, Total Hardness & Microbiological Analysis.

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

S.No.	Sampling	Location	Method of	Test to be performed
8		Outlet SF 102	Pour plate	Description, Total Hardness & Microbiological Analysis.
9		Outlet of cartridge filter and inlet of pre- UF system (150 micron)	Pour plate	Description, pH, Total Suspended Solids, Total Dissolved Solids & Microbiological Analysis.
10		Permeate line of UF System	Pour plate	Description, pH, TOC/Oxidisable Substances & Microbiological Analysis.
11		Before SMBS Dosing System	Pour plate	Description, pH, Total Hardness, Free Chlorine & Microbiological Analysis.
12		After SMBS Dosing System	Pour plate	Description, Free Chlorine, pH, Total Dissolved Solids & Microbiological Analysis.
13		After ADS Dosing System	Pour plate	Description, pH, Total Dissolved Solids & Microbiological Analysis.
14		Outlet of 5 micron cartridge filter (CF201)	Pour plate	Description, pH, Total Dissolved Solids, Total Suspended Solid & Microbiological Analysis.
15		Permeate line of ROH-201 (Pass-1 RO)	Pour plate	Description, pH, Conductivity, Total Dissolved Solid & Microbiological Analysis.

STANDARD OPERATING PROCEDURE

Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

S.No.	Sampling	Location	Method of	Test to be performed
16		Permeate line of ROH-202 (Pass-1 RO)	Pour plate	Description, pH, Conductivity, Total Dissolved Solid & Microbiological Analysis.
17		Permeate Header of RO Pass-1	Pour plate	Description, pH, Conductivity, Total Dissolved Solid & Microbiological Analysis.
18		Permeate Line of ROH-203 (Pass-2 RO)	Pour plate	Description, pH, Conductivity, Total Dissolved Solid & Microbiological Analysis.
19		Permeate line of ROH-204 (Pass-2 RO)	Pour plate	Description, pH, Conductivity, Total Dissolved Solid & Microbiological Analysis.
20		Permeate Header of RO Pass-2	Pour plate	Description, pH, Conductivity, Total Dissolved Solid & Microbiological Analysis.
21		Permeate line of EDI Unit (EDI-201)	Pour plate	Description, pH, Conductivity, TOC/Oxidisable Substances, Total Dissolved Solid & Microbiological Analysis.
22		Before PW distribution loop pump (PW storage tank)	Filtration	Complete Testing as per Purified water Specification.
23		Before UV System	Filtration	Description, pH, TOC/Oxidisable Substances, Conductivity & Microbiological Analysis.

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

S.No.	Sampling	Location	Method of	Test to be performed
24		After UV System	Filtration	Description, pH, TOC/Oxidisable Substances, Conductivity & Microbiological Analysis.

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling, Testing, Release and Rejection of Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Annexure 4

SAMPLING SCHEDULE FOR PURIFIED WATER SYSTEM

MONDAY	TUESDAY	WEDNSDAY	THURSDAY	FRIDAY	SATURDAY
Daily Sampling Points					
Alternate Days Sampling Points					
Once in a week Sampling Points					
		------	------	-----	------

Note:

1. Sampling shall be on daily basis except weekly off / Holiday.
2. If holiday occur in working day then sampling of that day shall on next Working day.