STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling and Testing of Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 PURPOSE:

To lay down the procedure for Sampling and Testing of Purified Water.

2.0 SCOPE:

This Standard Operating Procedure is applicable at Microbiology Department.

3.0 REFERENCE:

3.1 Aseptic Technique for Microbiological Testing.

3.2 Quality Monitoring of Water for Pharmaceutical Use.

3.3 SOP: Analysis of Water Samples.

3.4 IP/BP/USP

4.0 RESPONSIBILITY:

4.1 Officer or Executive of Microbiology department shall be responsible for preparation of new or revision of existing SOP.

4.2 Head of the Department / Designee of respective areas & QA shall be responsible for reviewing the SOP's.

4.3 Site Quality Head and Head QA shall be responsible for approval of SOP.

5.0 ABBREVIATIONS:

5.1 CCR : Change Control Record

5.2 CFU : Colony Forming Unit

5.3 COA : Certificate of Analysis

5.4 °C : Degree Celsius

5.5 HCL : Hydrochloric Acid

5.6 IPA : Isopropyl Alcohol

5.7 LIMS : Laboratory Information Management System

5.8 ML : Millilitre

5.9 NA : Not Applicable

5.10 PPB : Part per Billion

5.11 QA : Quality Assurance

5.12 QC : Quality Control

5.13 SOP : Standard Operating Procedure

5.14 TOC : Total Organic Carbon

6.0 DEFINITION:

6.1 **SOP:** A written authorized procedure, which gives instructions for performing operations

6.2 **Alert Limit:** Microbial levels, specified in the standard operating procedure or specifications, when exceeded should result in an investigation to ensure that the process is still within control. Alert levels are specific for a given facility and are established on the basis of a baseline developed under an activity monitoring program. These Alert levels can be modified depending on the trend analysis done in the monitoring program. Alert levels are always lower than Action levels.

7.0 PROCEDURE:

7.1 Preparation of Containers for sampling:

7.1.1 **For Chemical Analysis:** Clean 1000 ml Clear glass bottles with cap. Rinse with purified water.

7.1.2 **For TOC analysis:** Wash 125 ml Amber glass or suitable bottles with stopper with 2.0 M HCL or 2.0% nitric acid and rinse with purified water and dry the bottles at 105 ºC for at least one hour.

7.1.3 **For Microbiological Analysis:** Clean 250 ml Clear glass bottles with cap for membrane filtration method or pour plate method and rinse with purified water. Close the bottle with cap and wrap the neck with aluminium foil and Sterilize the bottle at 121 ºC (15 lbs.) for validated time.

7.2 **Sampling Schedule:**

7.2.1 Refer Annexure-2 for Sampling Schedule shall be made in such manner that generation point (For Chemical & Microbiology Analysis) & return loop (For Microbiology Analysis only) covered on daily basis and other user points (For Microbiology Analysis only) to be covered in rotation within a month.

7.2.2 Sampling schedule (Tentative) shall be prepared first working day of every New Year.

7.2.3 Write the sampling details or affix the label on the bottle as per Annexure-3.

7.2.4 Carry the sampling containers to the sampling point as per sampling schedule. Sanitize the hands with 70 % v/v IPA. Wear gloves and nose mask before sampling. Open the valve of the sampling point and allow the water to drain for about 20-30 seconds.

7.3 **Collection of purified water for Chemical and TOC analysis:**

7.3.1 Rinse the container at least one to two times with purified water from the sampling point.

7.3.2 Fill the containers with 1000 ml and 125 ml or upto the neck of purified water for chemical and TOC analysis respectively, and close the lid immediately.

7.3.3 Analyze the TOC sample as quickly as possible on arrival at the laboratory. If immediate analysis is not possible acidify the sample with 2-3 drop of 2.0 M HCL and store the sample under refrigeration at 2 to 8ºC. These stored samples should be analyzed within 6 hours.

7.4 **Collection of purified water for Microbiological analysis:**

7.4.1 Hold the sterile container near the sampling point. Unwrap the aluminium foil and open the bottle taking care not to touch the inner surface and neck of the bottle.

7.4.2 Collect the purified water sample (refer table 1) without rinsing and close it with stopper immediately. Do not fill the bottle up to the neck.

7.4.3 Wrap the neck with aluminium foil to avoid any contamination during transportation.

7.4.4 Close the valve and bring the samples to the laboratory and make entry in the purified water inward register. Perform the analysis as per current version of purified water specification. Analyze the sample as quickly as possible on arrival at the laboratory. If immediate analysis is not possible, store the sample under refrigeration at 2 to 8ºC. These stored samples should be analyzed within 6 hours.

Table 1

Test parameters	Sample quantity	Sample bottle
Chemical analysis	1000 ml	Non sterile
TOC analysis	125 ml	Non sterile
Microbiological analysis	250 ml	Sterile

7.5 **Issuance of water analysis template:**

7.5.1 Before analysis, sample shall be registered and template shall be generated from LIMS as per SOP current version of SOP.

7.5.2 A designated person shall issue the template.

7.6 Analyse the sample of purified water for Total Viable Count using filtration technique as per the current specification of Purified Water.

7.7 **Pathogen testing:** Analyse the sample of purified water for pathogens using filtration technique/Direct as per the current specification of Purified water.

7.8 Carry out the chemical analysis of samples as per current version of purified water specification.

7.9 **Sampling Precautions**:

7.9.1 Utility system sampling requires additional care because samples are collected in controlled and uncontrolled areas of the facility.

7.9.2 Sampling personnel shall wear clean smocks or gowns and sterile gloves prior to collecting any sample (As per area requirements).

7.9.3 Sampling apparatus and containers shall be clean, sterile and/or endotoxin-free as per test requirement.

7.9.4 Wipe any visible dust from the exterior of the sample port or valve using a lint free wipe/cloth moistened with an approved sanitizer – do not spray or wipe the interior of the port or valve. Ensure that the required contact time for the sanitizer has been achieved.

7.9.5 If a hose is already connected to the port or valve, collect the sample directly from that hose. If the hose is not connected, but is provided, attach the hose using procedures applicable for that system. Replace the used hose onto the provided clamp after sampling so that it fully drains.

7.9.6 If a hose is not present, a sterilized adapter and hose may be used to sample directly from the valve/port.

7.9.7 Flush the sample port/valve for the qualified time or volume and immediately open the labelled sample container and collect the sample. Close the container immediately and record the sampling time.

7.10 **Frequency:**

Sampling Locations	Type of Analysis	Frequency
Generation Point	• Chemical • TOC /Oxidisable Substance • Total Microbial Counts • Presence of Pathogens	Daily
Return Loop	• Total microbial counts • Presence of Pathogens	Daily
User Points	• Total microbial counts • Presence of Pathogens	Monthly

7.11 **Limit:** Total microbial count should not be more than 100 CFU/ml and pathogens should be absent. Total organic carbon should not more than 500 ppb.

7.12 **Alert and action limit:**

Name of Test	Alert limit	Action limit	Specification Limit
pH	6.5	6.8	5-7
Conductivity (µ/S)	3.5	4.4	5.1
ROE (%)	0.0008	0.0009	0.001
TOC (ppb)	400	450	500
TMC (cfu/ml)	65	83	100

7.13 **Recording of Results:**

7.13.1 After completion of test, record the results in LIMS generated data sheet and also enter the results in LIMS.

7.13.2 After completion of analytical report and LIMS entry, checked and approved by designated person and after that generate the COA (if required).

7.13.3 If any chemical parameter exceeds the limit, immediately inform the same QA, Production and Engineering Department.

7.13.4 If the Total bacterial count exceeds the Alert limit/Action limit, immediately inform the same to QA and Engineering personnel and investigation shall be done as per the current version of SOP “Handling of microbial Excursion in Water and Environment”.

7.13.5 A two phase approach is suggested where:

- Phase 1 focuses on the method and the analyst; the purpose of phase 1 is to verify that the data are valid. Analyst error should be rare. Immediate resampling of the specific location where the OOT/OOS occurred must be performed, to rapidly assess the condition of the system.
- Phase 2 follows the determination of data validity and investigates the water system and all potentially affected lots of product.
- Phase 2 requires a root cause analysis and a corrective action.

7.14 **Trending:**

7.14.1 Purified water quality parameter trending shall be done on Monthly basis, trend charts shall

be reviewed and conclusion shall be made (if required).

7.14.2 Final Summary report shall be prepared the end of Year.

7.15 **Identification of Microorganisms (Water Isolates):**

7.15.1 It is important to routinely identify organisms recovered from water systems when:

- Waterborne organisms may be detrimental to finished product or processes in which the water is used.
- There is a need to identify the source of contamination.
- Water system sanitization methods require assessment.

7.15.2 It is important to note that you don't have to exceed specification in order to submit a result for identification, it's a best practice to document and select and identify representative flora from the system.

8.0 DISTRIBUTION:

8.1 Quality Assurance

8.2 Quality Control

9.0 ANNEXURES:

9.1 Annexure-1: List of Sampling Points of Purified Water.

9.2 Annexure-2: Sampling Schedule of Purified Water.

9.3 Annexure-3: Sampling Details of Purified Water.

10.0 REVISION HISTORY:

Version Number	Revision Details	Effective Date	Ref. CCR Number
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ANNEXURE I

LIST OF SAMPLING POINTS OF PURIFIED WATER

S.No.	Name of Location	Sample ID No.

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE II

SAMPLING SCHEDULE OF PURIFIED WATER

Year: P= Purpose, A= Actual

S. No.	Name of Sampling Point	ID	Jan.		Feb.		Mar.		Apr.		May.		Jun.		Jul.		Aug.		Sep.		Oct.		Nov.		Dec.	
			P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A

Note: Water Sampling shall be done next day for which, the day falls on Holiday.

Remarks:

Prepared By: Checked By:

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ANNEXURE III

SAMPLING DETAIL OF PURIFIED WATER

MICROBIOLOGY DEPARTMENT	
Sampling Details of Purified Water	
Name of Sampling Point	
Sampling Point ID	
Date/Time of Sampling	
Sampled By	