CONTENTS

PROTOCOL APPROVAL SHEET

Prepared by

Functional Area	Name	Designation	Signature	Date
Microbiology				

Checked by

Functional Area	Name	Designation	Signature	Date
In charge, Microbiology				
Quality Assurance				

Approved by

Functional Area	Name	Designation	Signature	Date
Head, Quality Control				
Head, Quality Assurance				

1.0 Objective

The objective of this study is to determine the sample quantity of purified water for microbiological analysis, with respect to increase in sample quantity and the analysis method to ensure that the capability to support the microbial recovery from purified water samples, when operated as per the set SOPs.

2.0 Scope

The protocol shall be applicable at …….... The validation study of the Purified water testing with respect to the sample size for Microbiological evaluations shall be carried out for one time to check the sample quantity and analysis method is suitable for recovery of microbial load, even in small quantity from purified water samples.

3.0 Responsibility

Microbiology	:	Preparation and review of validation protocol; conducting the experiment as per the approved protocol and compilation of data; preparation of validation report.
Quality Assurance	:	Review of validation protocol, results and report.
Head, QC	:	To check the protocol with respect to its intended purpose and to make evaluations on compiled data from the test; Final approval of protocol and report.
Head, QA	:	Review of protocol for the correctness and adequacy of the text and the experiment, regulatory compliance; final approval of protocol. Review of report for the correctness of the data and compliance of the protocol and approval of the report.

4.0 Validation Team members

Validation team shall comprise of the representatives from following functions:

- Microbiology
- Quality Assurance

5.0 Abbreviations

SOP : Standard Operating Procedure

SCDA : Soya bean Casein Digest Agar

CFU : Colony Forming Unit

SCS : Standardized Cell Suspension

LAF : Laminar Air Flow

6.0 Safety Considerations

6.1 All the procedures should be carried out aseptically.

6.2 All the materials that are to be used should be sterile.

6.3 All the materials used for handling the microorganisms should be decontaminated.

7.0 Pre-requisites for Validation

7.1 The following pre-requisites are to be used for purified water testing with respect to the sample size for Microbiological evaluations.

7.1.1 Standardized cell suspension of *Escherichia coli* or *Salmonella* or *Pseudomonas aeruginosa* or *Staphylococcus aureus* or *Bacillus subtilis* (SCS)

7.1.2 SCDA medium

7.1.3 Glass Bottles (100 ml & 500 ml capacity)

7.1.4 Measuring cylinders (10 & 100 ml capacity)

7.1.5 Normal saline

7.1.6 70 % Iso Propyl Alcohol

7.1.7 Petri plates (90 mm)

7.1.8 Colony counter

7.1.9 Calibrated Incubators

7.1.10 Calibrated Compound Microscope

7.1.11 Validated Autoclave

7.1.12 Validated LAF unit

7.1.13 Membrane Filtration Apparatus

7.1.14 0.45µ membrane filters

7.1.15 Forceps

7.2 Documents

SOPs of the relevant instruments and testing procedures.

8.0 Acceptance Criteria

8.1 The estimated number of cells in positive control shall be less than 100 for the Microbial Limit Test.

8.2 The recovery shall be not less than 70% for the CFU obtained from positive control.

8.3 SCDA medium should pass 'Growth Promotion Test for Microbiological Culture Meida'.

8.4 The negative control should not show any growth**.**

9.0 Procedure

9.1 Collect 350 ml of purified water sample in a 500 ml sterilized bottle from purified water storage tank, same from Purified water return from distribution and Coating as per SOP on 'sampling procedure for water samples.

9.2 Take the samples to the microbiology laboratory for analysis.

9.3 Analyze the sample by Pour plate method with a sample quantity of 1.0 ml, in triplicate as follows

9.4 Pipette out 1.0ml of sample into each sterile petridishes and pour 15-20 ml of sterile SCDA medium in both petridishes and allow solidifying for 30 – 45 minutes.

9.5 After completion of solidification of agar medium, incubate these Petri plates at 32±2°C for 48 hours and further incubate these plates at 22±2°C for 72 hours & observe for every 24 hours.

9.6 Analyze the same sample followed by Membrane filtration technique with a sample quantity of 10.0 ml, in triplicate as follows;

9.6.1 Filter the sample (Aseptically collected in sterile measuring cylinder from sample bottle), through sterilized 0.45µ membrane filter using sterilized filtration assembly with vacuum pump.

9.6.2 Rinse the membrane with 100 ml of sterile saline solution (Sterile normal saline shall be prepared by dissolving sodium chloride in purified water in the ratio of 9.0 g to 1000 ml respectively).

9.6.3 Aseptically remove the membrane and put on a sterile pre incubated SCDA plate.

9.7 Incubate these Petri plates at 32±2°C for 48 hours and further incubate these plates at 22±2°C for 72 hours & observe for every 24 hours.

9.8 Analyze the same sample followed by Membrane filtration technique with a sample quantity of 50.0 ml, in triplicate as per the procedure given point no. 9.6.1 to 9.6.3.

9.9 Incubate these Petri plates at 32±2°C for 48 hours and further incubate these plates at 22±2°C for 72 hours & observe for every 24 hours.

9.10 Analyze the same sample followed by Membrane filtration technique with a sample quantity of 100.0 ml, in triplicate as per the procedure given point no. 9.6.1 to 9.6.3.

9.11 Incubate these Petri plates at 32±2°C for 48 hours and further incubate these plates at 22±2°C for 72 hours & observe for every 24 hours.

9.12 Follow the procedure from point no. 9.3 to 9.11 for purified water samples collected from Purified water return from distribution and Coating.

9.13 Pipette out 1.0 ml of SCS into two sterile petridishes and pour 15-20 ml of sterile SCDA medium in both petridishes and allow to solidify for 30 – 45 minutes. After completion of solidification of agar medium, incubate these Petri plates at 32±2°C for 72 hours and observe for every 24 hours (Positive Control).

9.14 Keep one SCDA plate as negative control and incubate along with samples.

9.15 Record the details in given Data Sheet as per Annexure – 3.

9.16 Follow the same procedure for consecutive 30 days and monitor the trend.

9.17 After completion of this study, report shall be prepared on the basis of results obtained at 120 hours incubation.

9.18 Based on the test results the optimum sample size shall be confirmed and shall be recommended for the routine practice.

10.0 Re-validation

10.1 In the view of following listed, the test shall be re-conducted if there is:

10.1.1 Any major change in the specification of purified water.

10.1.2 Any major change in the testing procedure of purified water.

11.0 Deviations and Investigations

11.1 Any deviation to this protocol and thereupon investigation shall be recorded as per SOP.

12.0 Validation Report

12.1 Based on the outcome from this validation study, a report shall be prepared by Microbiology and Quality Assurance. The validation report shall be reviewed and then approved by all functional heads of all the concerned departments. Validation Report shall include following:

12.1.1 Validation Report Approval Sheet (as per Annexure-1).

12.1.2 Data Sheets (as per Annexure-2).

12.1.3 Validation Report summary (as per Annexure-3).

13.0 List of Annexures/Formats attached

1.	Validation Report Approval Sheet	Annexure-1
2.	Data Sheet	Annexure-2
3.	Validation Report Summary & Conclusion	Annexure-3

14.0 References

14.1 In – House Method.

DATA SHEET

Sampling Point Name: Sampling Point No.:

Sampled on & Time: Sampled By:

Analyzed on & Time: Analyzed By:

Method of Analysis: Pour Plate Method / Membrane Filtration Technique Analyzed Sample Quantity:

Autoclave I.D. No.: LAF I.D. No.:

Media Lot No.: Incubator I.D. No.:

Date	**Incubation Time**	**Observation (Sample)**				**Positive Control**	**Negative Control**	**Observed By**	**Remarks**
		Plate – 1	Plate – 2	Plate – 3	Average (Cfu / ___ml)				
	After 24 hours								
	After 48 hours								
	After 72 hours								
	After 96 hours								
	After 120 hours								

Checked By:

Date:

Summary & Conclusion:

The study for Evaluation of Sample Size for Microbial Analysis of Purified Water carried out for consecutive one month (30 days) on different sampling points viz. S-10 (Purified Water Storage Tank Outlet), S-12 (Purified Water Return from Distribution) and S-17 (User Point in Coating-2), using 1 ml sample by pore plate method and 10, 50 and 100 ml sample using Membrane Filtration Technique.

During the study it has been concluded that 10 ml is the suitable sample size to enumerate the microbial counts in Purified Water by using membrane filtration technique because in 10 ml sample the recovery is more accurate as compare to 1 ml by pore plate method. While in 50 ml and 100 ml sample the accurate counting is not possible due to dens growth on the membrane filter.

Hence it is concluded that Membrane Filtration Technique, using 10 ml sample is suitable method for Microbiological Analysis of Purified Water.

Prepared By
Date
(Microbiologist)

Checked By
Date
(Quality Assurance)

REPORT APPROVAL SHEET

Prepared by

Functional Area	Name	Designation	Signature	Date
Microbiology				

Checked by

Functional Area	Name	Designation	Signature	Date
In charge, Microbiology				
Quality Assurance				

Approved by

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Head, Quality Control				
Head, Quality Assurance				