VALIDATION PROTOCOL

FOR

EVALUATION OF SAMPLE SIZE

FOR MICROBIOLOGICAL ANALYSIS OF

PURIFIED WATER

VALIDATION PROTOCOL FOR EVALUATION OF SAMPLE SIZE FOR MICROBIOLOGICAL ANALYSIS OF PURIFIED WATER

CONTENTS

VALIDATION PROTOCOL FOR EVALUATION OF SAMPLE SIZE FOR MICROBIOLOGICAL ANALYSIS OF PURIFIED WATER

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				

VALIDATION PROTOCOL FOR EVALUATION OF SAMPLE SIZE FOR MICROBIOLOGICAL ANALYSIS OF PURIFIED WATER

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 standard testing procedure.

2.0 Scope

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

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

SOP’s 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 to solidify for 30 – 45 minutes.

9.5 After completion of solidification of agar medium, incubate these Petri plates at 32 $\pm$ 2°C for 48 hours and further incubate these plates at 22 $\pm$ 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 $\pm$ 2°C for 48 hours and further incubate these plates at 22 $\pm$ 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 $\pm$ 2°C for 48 hours and further incubate these plates at 22 $\pm$ 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 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 – 2.

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.