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 suitable media for microbiological limit test of water, with respect to use three different types of basic media to ensure that the capability to support the microbial recovery from water samples, when operated as per the pre approved analysis method.

2.0 Scope

The protocol shall be applicable at formulation Plant of ……. The validation study of the comparative study of media for microbiological limit test of water shall be carried out for one time to check the efficiency of media and analysis method is suitable for recovery of microbial load.

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
SCS	: Standardized Cell Suspension
LAF	: Laminar Air Flow

CFU : Colony Forming Unit

TSA : Tryptone Soy Agar

PCA : Plate Count Agar

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*, *Salmonella*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* (SCS)

7.1.2 Tryptone Soy Agar

7.1.3 R2A Agar

7.1.4 Plate Count Agar

7.1.5 Glass Bottles (100 ml & 500 ml capacity)

7.1.6 Measuring cylinders (10 & 100 ml capacity)

7.1.7 Normal saline

7.1.8 70% Iso Propyl Alcohol

7.1.9 Petri plates (90 mm)

7.1.10 Colony counter

7.1.11 Calibrated Incubators

7.1.12 Calibrated Compound Microscope

7.1.13 Validated Autoclave

7.1.14 Validated LAF unit

7.1.15 Membrane Filtration Apparatus

7.1.16 0.45µ membrane filters

7.1.17 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 All the three media 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 the samples as per routine sampling plan and follow the procedure as per SOP on 'sampling procedure for water samples'.

9.2 Transfer the samples to the microbiology laboratory for analysis in a closed condition.

9.3 Analyze the samples in duplicate mode followed by Membrane filtration technique with a sample quantity of 10.0 ml, in triplicate as follows;

9.3.1 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.

9.3.2 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).

9.3.3 Aseptically remove the membrane with the help of a sterile forceps and put on a sterile pre incubated Tryptone Soy Agar plate.

9.4 Analyze the same samples followed by point no. 9.3 to 9.3.2, aseptically remove the membrance with the help of a sterile forceps and put on a sterile pre incubated R2A Agar plate & follow the same procedure for PCA.

9.5 Incubate these Petri plates at 32.5 $\pm$ 2.5°C for 72 hours and observe for every 24 hours.

9.6 Pipette out 1.0 ml of each SCS (10 – 100 cfu) and add into 10 ml of sterile normal saline & filter this solution through sterilized 0.45µ membrane filter using sterilized filtration assembly with vacuum pump.

9.7 Rinse the membrane with 100 ml of sterile saline solution.

9.8 Aseptically remove the membrane with the help of a sterile forceps and put on a sterile pre incubated Tryptone Soy Agar plate for Positive Control on TSA & incubate along with samples & observe for every 24 hours.

9.9 Follow the same procedure from point no. 9.6 to 9.8 for R2A Agar and PCA.

9.10 Keep one no. of each TSA, R2A Agar and PCA plates as negative control and incubate along with samples & observe for every 24 hours.

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

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

9.13 After completion of this study, report shall be prepared on the basis of results obtained at 72 hours incubation.

9.14 Based on the test results the optimum media usage 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 Cover page of the Report (as per Annexure-1).

12.1.2 Validation Report Approval Sheet (as per Annexure-2).

12.1.3 Data Sheets (as per Annexure-3).

12.1.4 Validation Report summary (as per Annexure-4).

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.

ANNEXURE I

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

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

ANNEXURE II

DATA SHEET

Sampling Point Name: **Sampling Point No.:**

Sampled on & Time: **Sampled By:**

Analyzed on & Time: **Analyzed By:**

Method of Analysis: Membrane Filtration Technique **Analyzed Sample Quantity: 10 ml**

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

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

Date	Incubation Time	Observation (Sample)															Observed By	Remarks
		R2A Agar					Tryptone Soy Agar					Plate Count Agar						
		Plate-1	Plate-2	Avg.	+ ve	- ve	Plate-1	Plate-2	Avg.	+ ve	- ve	Plate-1	Plate-2	Avg.	+ ve	- ve		
	After 24 hours																	
	After 48 hours																	
	After 72 hours																	

Checked By:

Date:

ANNEXURE III

Summary & Conclusion:

The comparative study of media for Microbial Analysis of Purified Water was carried out for consecutive 30 days as per the routine sampling schedule, using R2A agar, Plate Count Agar and Soyabean Casein Digest Agar by Membrane Filtration Technique.

During the study it has been observed that the microbial counts are within the minimum and maximum range as given below.

Name of Medium	No. of Counts Observed (cfu)	
	Minimum	Maximum
Soyabean Casein Digest Agar (TSA)	25	54
R2A Agar	22	49
Plate Count Agar	22	55

As per the above summary, the obtained results in all three mediums are within the same series. Hence it is concluded that Soyabean Casein digest Agar is the suitable medium for analysis of water samples. So, Soyabean Casein Digest Agar shall be continued as earlier it being used.

Prepared By
Date
(Microbiologist)

Checked By
Date
(Quality Assurance)