PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure for Microbiological Analysis of Purified Water Samples	**Effective Date:**
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1.0 Objective

To lay down a procedure for Microbiological Analysis of Purified Water.

2.0 Scope

This Standard Operating Procedure is applicable for formulation plant.

3.0 Responsibility

Executive/Officer - Microbiology : Shall be responsible to follow the procedure for Microbiological Analysis of Purified Water.

Head - QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

SOP	: Standard Operating Procedure
QC	: Quality Control
IPA	: Isopropyl Alcohol
CFU	: Colony Forming Unit
UV	: Ultra Violet
psi	: Per Square Inch
TBC	: Total Bacterial Count
TFC	: Total Fungal Count
µ	: Micron

5.0 Procedure

5.1 Tests for Total Bacterial Count

5.1.1 Properly disinfect the surface of sample bottles by IPA 70% and transfer to microbiology laboratory.

5.1.2 Sterilize, membrane filtration assembly by Autoclaving at 121°C and15 psi for 30 minutes.

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5.1.3 Aseptically place the sterilized membrane filter on the filtration assembly.

5.1.4 Transfer 10 ml sample of purified water or 1 ml sample of raw water and treated water to the membrane filtration assembly and filter it through 0.45 µ filter by applying vacuum and rinse the filter with 100 ml sterile water.

5.1.5 Aseptically remove the membrane filter from the assembly with the help of a sterilized forcep and transfer it to the surface of pre incubated plate of Soyabean Casein Digest Agar Medium in a rolling fashion to avoid entrapment of air below the filter membrane.

5.1.6 Invert the plate and incubate at 30 -35°C for 3 days.

5.1.7 After completion of incubation period count the number of colonies observed on plates.

5.1.8 Enumerate the counts as cfu/ml by using the formula given below

$$\text{TBC (cfu/ml)} = \frac{\text{Number of counts on membrane filter}}{\text{Quantity of sample filtered}}$$

5.1.9 Record the counts in format as per Annexure-1.

5.2 **Test for Total Fungal Counts**

5.2.1 Aseptically place another sterilized membrane filter on the filtration assembly.

5.2.2 Transfer 10 ml sample of purified water or 1 ml sample of raw water and treated water to the membrane filtration assembly and filter it through 0.45 µ filter by applying vacuum and rinse the filter with 100 ml sterile water.

5.2.3 Aseptically remove the membrane filter from the assembly with the help of a sterilized forcep and transfer it to the surface of pre incubated plate of Sabouraud Dextrose Agar Medium in a rolling fashion to avoid entrapment of air below the filter membrane.

5.2.4 Invert the plate and incubate at 20- 25°C for 5 days.

5.2.5 After completion of incubation count the number of colonies observed on plates.

5.2.6 Enumerate the fungal counts as cfu/ml by using the formula given below

$$\text{TFC (cfu/ml)} = \frac{\text{Number of counts on membrane filter}}{\text{Quantity of sample filtered}}$$

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5.2.7 Record the counts in format as per Annexure-1.

5.3 **Test for Specified microorganisms**

5.3.1 **Enrichment**

5.3.1.1 Place another sterilized membrane filter on the membrane filtration assembly.

5.3.1.2 Transfer 100 ml of sample to the assembly and filter it through 0.45µ membrane filter by applying vacuum.

5.3.1.3 Aseptically remove the filter from the assembly and inoculate the filter in a tube containing 100 ml of Soyabean casein digest medium, shake well and incubate the medium at 30-35°C for 18-24 hours.

5.3.2 **Test for *Escherichia coli***

5.3.2.1 Inoculate 1.0 ml of the enriched culture into the tube containing 100 ml of Macconkey broth.

5.3.2.2 Incubate the tube at 42-44°C for 24- 48 hours.

5.3.2.3 Subculture on a pre incubated plate of MacConkey agar and incubate the plates at 30-35°C for 18 - 72 hours.

5.3.2.4 After completion of incubation period observe the tubes for acid and gas formation and plates for the presence of Brick Red colonies. In case no characteristic results observed, the sample meets the requirement for the absence of *E. coli*

5.3.2.5 **Indole Test**

5.3.2.5.1 Transfer 0.1 ml of enrichment medium to a tube containing 5.0 ml of MacConkey broth and incubate at 43-45°C for 24 hours.

5.3.2.5.2 After completion of incubation period add 0.5 ml Kovac's reagent, shake well and allow to stand for one minute.

5.3.2.5.3 If red colour ring is observed in the upper layer of medium it confirms the presence of *Escherichia coli*.

5.3.3 **Test for *Salmonella* species**

5.3.3.1 Add 1.0 ml of enrichment culture to a tube containing 10.0 ml of Rappaport Vassiliadis *Salmonella* enrichment broth.

5.3.3.2 Incubate the tubes at 30-35°C for 18-48 hours and observe the tubes for colour change or turbidity.

5.3.3.3 After completion of incubation period from the tubes of Rappaport Vassiliadis *Salmonella* enrichment broth, streak on the surface of pre incubated plates of Xylose lysine deoxycholate agar medium.

5.3.3.4 Cover and invert the petriplates and incubate at 30-35°C for 18-48 hours.

5.3.3.5 After completion of incubation period if medium shows red colonies with or without black centers, it confirms the presence of *Salmonella* species.

5.3.4 **Tests for *Pseudomonas aeruginosa***

5.3.4.1 Streak one loop full of the enrichment culture on the surface of pre incubated plate of Cetrimide agar.

5.3.4.2 Incubate the plates in inverted position at 30-35°C for 18-72 hours.

5.3.4.3 Examine the presence of greenish colonies which gives florescence under UV light.

5.3.4.4 If no specific colonies observed then the sample passes the test for absence of *Pseudomonas aeruginosa*.

5.3.4.5 After completion of incubation period if medium shows red colonies with or without black centers, it confirms the presence of *Salmonella* species.

5.3.5 **Test for *Staphylococcus aureus***

5.3.5.1 Streak one loop full of the enriched culture on the surface of pre incubated plate of Mannitol-Salt agar.

5.3.5.2 Incubate the plates in inverted position at 30-35°C for 24-48 hours.

5.3.5.3 If upon examination no colony shows characteristic colonies of yellow/white surrounded by a yellow zone, the test meets the requirement for the absence of *Staphylococcus aureus*.

5.3.5.4 If any colony shows the characteristics as described above then perform coagulase test.

5.3.5.5 **Coagulase Test**

5.3.5.5.1 Transfer representative suspected colonies from the agar surface of Mannitol-Salt agar, to individual tubes containing 0.5 ml of mammalian preferably rabbit or horse plasma, with or without additives.

5.3.5.5.2 Incubate the tubes in water bath at 37°C. Examine the tubes after 3 hours and subsequently at suitable intervals up to 24 hours.

5.3.5.5.3 If there is no coagulation, the sample meets the requirements of the absence of *Staphylococcus aureus*.

5.3.6 **Test for Coli forms**

5.3.6.1 Filter 100 ml of test sample through membrane filter and transfer the filter to an absorbent pad impregnated in Lauryl tryptose broth and incubate at 30-35°C for 1 hour.

5.3.6.2 Aseptically transfer the membrane filter to a pre incubated plate of M-Endo agar and incubate at 30-35°C for 24 hrs.

5.3.6.3 Count the colonies which are golden green with a metallic surface sheen, the sheen may vary from pinpoint to complete coverage of colony.

5.3.6.4 Report the results as number of Coliforms colonies per 100 ml.

6.0 Forms and Records

6.1 Report for Microbiological Analysis of Purified Water: Annexure-1

7.0 Distribution

7.1	Master Copy	:	Documentation Cell (Quality Assurance)
7.2	Controlled Copies	:	Quality Control, Quality Assurance

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8.0 History

Date	Revision Number	Reason for Revision
	00	New SOP