Title: Microbiological Analysis of Raw Water, Process Potable Water & Purified Water			
SOP No.:		**Revision No.:**	00
Effective Date:		**Supersedes No.**	Nil
Review Date:		**Page No.**	1 of 14

1.0 OBJECTIVE:

To lay down a procedure for Microbiological Analysis of Raw Water, Process Potable Water & Purified Water.

2.0 SCOPE:

This SOP is applicable for Microbiological analysis of Raw Water, Process Potable Water & Purified Water in Microbiological Lab of Quality Control at ……..

3.0 RESPONSIBILITY:

3.1 Officer/Executive – QC (Microbiologist)

4.0 ACCOUNTABILITY:

4.1 Head – QC

5.0 DEFINITION:

5.1 Bacteriological water analysis is a method of analyzing water to estimate the number of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations.

6.0 PROCEDURE:

6.1 Total Aerobic Microbial Count by Membrane Filtration Method:

6.1.1 Collect the water sample as per SOP**.**

6.1.2 Prepare the R2A media as per SOP.

6.1.3 Pre incubated R2A media plates shall be use for analysis.

6.1.4 Assemble the sterile filter set to the filtration unit in the laminar air flow station.

6.1.5 Aseptically place the sterilized or pre sterilized Membrane using forcep in the base of filtration cup.

6.1.6 After fixing the sterilized/pre sterilized Membrane filter, mount the top portion with filtration cup.

6.1.7 For Total Aerobic Microbial Count for Purified water, Raw water and Process Potable Water dilute 1 ml of sample in 100 ml Bacteriological Peptone (0.1%) and filter the entire content of solution through 0.45µ Membrane Filter.

6.1.8 Place the Membrane Filter to pre-incubated R2A media plate for bacterial count. Label the plates with Sampling Point, Date of Testing and Media Name.

6.1.9 Ensure that no air bubble is trapped inside the Membrane filter and entire surface of Membrane filter should be in contact of Agar surface.

6.1.10 Incubate the R2A media plates at 30 to 35^{0}C for 5 (five) days for total aerobic microbial count.

6.1.11 For Negative control filter 100 ml Bacteriological Peptone (0.1%) and filter through 0.45µ Membrane filter.

6.1.12 Place the Membrane Filter to pre-incubated R2A media plate for bacterial count. Label the plates with Sampling Point, Date of Testing and Media Name.

6.2 Total Aerobic Microbial Count by Pour Plate Method:

6.2.1 Collect the water sample as per SOP**.**

6.2.2 Prepare the required media such as Soyabean Casein Digest Agar or R2A media as per SOP and sterilize the media at 121°C & 15 lbs or psi.

6.2.3 Take the water sample to the analysis room through dynamic pass box and perform the test under Laminar Air Flow.

6.2.4 Make dilutions if necessary by using diluents such as Peptone, Bacteriological (0.1%). Label the petri plate with Sampling Point, Date of Testing and Media Name.

6.2.5 Then pipette 1 ml water sample in each of the two petri plates and pour 20 ml SCDA / R2A media in each petri plates.

6.2.6 After that rotate the plates clockwise and anti clockwise. Keep the media plates for solidify.

6.2.7 After solidification of the media plates, Incubate the SCDA / R2A media plates in incubator at 30 to 35^{0}C for 5 (five) days in inverted position for total aerobic microbial count.

6.3 Total Aerobic Microbial Count:

6.3.1 Observations and Results:

6.3.1.1 Purified Water, Raw Water and Process Potable Water: Examine the plates for the growth at 5th Day of incubation and count the number of colonies manually or with the help of colony counter

(if required). Express the count in term of the number of microorganisms per ml of Purified water, Raw water and Potable water.

6.3.1.2 Analysis activity shall record as **Annexure –I and Annexure -II** Titled as **"Microbiological Analysis Report for Total Aerobic Microbial Count of Purified Water"** and **"Microbiological Analysis Report for Total Aerobic Microbial Count of Raw Water / Process Potable Water".**

6.3.1.3 Acceptance criteria for Total Aerobic Microbial Count of Purified water, Raw water and Process Potable water as below:

S.No.	Type	Acceptance Criteria
1.	Purified Water	NMT 100 CFU/ml
2.	Process Potable Water	NMT 100 CFU/ml
3.	Raw Water	NMT 500 CFU/ml

6.4 Test For Specified Microorganisms:

6.4.1 Pretreatment of Sample by Membrane Filtration Method:

6.4.1.1 Filter 100 ml of water sample through membrane filter of nominal pore size NMT 0.45µm and 47 mm diameter.

6.4.1.2 Transfer the filter to 100 ml Soya bean Casein Digest Medium.

6.4.2 Pretreatment of Sample by Pour Plate Method:

6.4.2.1 Add 100 ml water sample to 100 ml double strength Soybean Casein Digest Medium.

6.4.3 Incubate the pretreatment sample at 30-35^{0}C for 18-24 hrs**.**

6.4.4 Examine the medium for turbidity.

6.4.5 Test for specified Microorganism perform for point like Raw Water, Process Potable Water, Purified Water.

6.4.6 Run Positive and Negative Control with test.

6.4.7 Test for *Escherichia coli*:

6.4.7.1 Shake the tube and transfer 1 ml of pretreated sample (SCM) to 100 ml of MacConkey Broth and incubate 42 to 44^{0}C for 24 to 48 hrs.

6.4.7.2 Streak a portion from MacConkeybroth on the surface of MacConkey Agar media and incubate at 30 to 35^{0}C for 18 to 72 hrs.

6.4.7.3 Run Positive and Negative Control with test.

6.4.8 Test for *Salmonella spp*.

6.4.8.1 Shake the tube and transfer 0.1 ml of pretreated sample to 10 ml of Rappaport Vassiliadis Salmonella Enrichment Broth and incubate at 30 to 35^{0}C for 18 to 24 hrs.

6.4.8.2 Streak a portion from the Rappaport Vassiliadis Salmonella Enrichment Broth on surface of Xylose Lysine Deoxycholate Agar Medium and incubate 30 to 35^{0}C for 18 to 48 hrs.

6.4.8.3 Run Positive and Negative Control with test.

6.4.9 Test for *Pseudomonas aeruginosa*:

6.4.9.1 Shake the tube and streak one loop full pretreated sample (SCM) on to the plate of Cetrimide Agar Medium and incubate 30 to 35^{0}C for 18 to 72 hrs.

6.4.9.2 Run Positive and Negative Control with test.

6.4.10 Test for *Staphylococcus aureus*:

6.4.10.1 Shake the tube and streak one loop full pretreated sample (SCM) on to the plate of Mannitol Salt Agar Medium and incubate at 30 to 35^{0}C for 18 to 72 hrs.

6.4.10.2 Run Positive and Negative Control with test.

6.4.11 Evaluation of Results Purified Water Analysis:

6.4.11.1 If none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of the ***Escherichia coli, Salmonella spp., Pseudomonas aeruginosa & Staphylococcus aureus*.**

6.4.11.2 Purified water analysis activity shall record as **Annexure -III** Titled as **"Microbiological Analysis Report for Specified Microorganism of Purified Water".**

6.4.11.3 If colonies show characteristic growth, carry out gram staining as per SOP.

6.4.11.4 If colonies show characteristic growth as per **Table-1**, carry out the identification by outside lab up to species level.

6.4.11.5 Characteristic growth identification of microorganism shall be recorded as **Annexure-V** Titled as **"Characteristic Growth Identification of Microorganism"**.

6.4.11.6 Acceptance criteria for specified microorganisms of Purified Water : *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus* should be absent/100 ml.

6.4.12 Evaluation of Results of Raw Water/Process Potable Water Analysis:

6.4.12.1 **If** none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of the ***Escherichia coli, Salmonella spp., Pseudomonas aeruginosa & Staphylococcus aureus*.**

6.4.12.2 Raw/Potable water analysis activity shall record as **Annexure -IV** Titled as **"Microbiological Analysis Report for Specified Microorganism of Raw Water/Process Potable Water"**

6.4.12.3 If colonies show characteristic growth, carry out gram staining as per SOP.

6.4.12.4 If colonies show characteristic growth as per **Table-1**, carry out the identification by outside lab up to species level.

6.4.12.5 Characteristic growth identification of microorganism shall be recorded as **Annexure-V** title as **"Characteristic Growth Identification of Microorganism"**.

6.4.12.6 **Acceptance criteria for specified microorganisms of Raw Water / Process Potable Water** :

Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus should be absent/100 ml.

TABLE-1

S.No.	Specified Microorganism	Media Name	Positive Growth Characteristics	Gram Staining Characteristics
1.	*E. coli*	MacConkey Broth	Medium colour turns to yellow.	Gram Negative Rod
		MacConkey Agar	Pink/red coloured non-mucoid colonies.	
2.	*Salmonella*	Rappaport Vassiliadis Salmonella Enrichment Broth	Medium colour turns to reddish pink.	Gram Negative Rod
		Xylose lysine Deoxycholate Agar	Red colonies with or without black centers.	
3.	*Pseudomonas aeruginosa*	Cetrimide Agar	Greenish yellow colonies	Gram Negative Rod
4.	*Staphylococcus aureus*	Mannitol Salt Agar	Yellow colonies surrounded by yellow zones.	Gram Positive Cocci

7.0 ABBREVIATIONS:

SOP	Standard Operating Procedure
No.	Number
QA	Quality Assurance
QC	Quality Control
USP	United State Pharmacopoeia
IP	Indian Pharmacopoeia
BP	British Pharmacopoeia
Ml	Milliliter
cfu	Colony Forming Unit
SCM	Soya bean Casein Digest Medium
UV	Ultra Violet
Hrs.	Hours
spp.	Species
NMT	Not More Than
°C	Degree Celsius
QM	Microbiology
QC	Quality Control
S. No.	Serial Number
IPA	Iso Propyl Alcohol
LAF	Laminar Air Flow
NLT	Not More Than
hrs	Hours
mm	Milimeter
Qty	Quantity
psi	Pounds Per Square Inch
SCDA	Soya bean Casein Digest Agar

8.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure-I	Microbiological Analysis Report for Total Aerobic Microbial Count of Purified Water	
Annexure-II	Microbiological Analysis Report for Total Aerobic Microbial Count of Raw Water / Process Potable Water	
Annexure-III	Microbiological Analysis Report for Specified Microorganism of Purified Water	
Annexure-IV	Microbiological Analysis Report for Specified Microorganism of Raw Water/Process Potable Water	

9.0 DISTRIBUTION:

- Master Copy — Quality Assurance Department
- Controlled Copy No. 01 — Quality Assurance Department
- Controlled Copy No. 02 — Quality Control (Microbiology)

10.0 REFERENCES:

- United State Pharmacopeia (USP)
- Indian Pharmacopeia (IP)
- British Pharmacopeia (BP)
- SOP, Titled **"Sampling of Raw Water, Process Potable Water, Purified Water and Water for Injection / Pure Steam for Microbiological Analysis"**
- SOP, Titled **"Preparation of Culture Media"**
- SOP, Titled **"Gram Staining"**.

11.0 REVISION HISTORY:

Revision No.	Change Control No.	Details of Changes	Reason of Changes	Effective Date	Done By
00	Not Applicable	Not Applicable	New SOP		

ANNEXURE–I

MICROBIOLOGICAL ANALYSIS REPORT OF TOTAL AEROBIC MICROBIAL COUNT OF PURIFIED WATER

Date of Sampling		**Sampled By**		**Sampled Qty.**	

Test Method:

(a) **Membrane Filtration Method:** 1ml purified water diluted in 100 ml Bacteriological Peptone (0.1%) (B. No.: _____) and filtered through 0.45 micron membrane filter paper. Membrane filter transferred to Pre-incubated R2A Agar (B. No.: _____) and incubated at 30-35°C for Five days (Incubator ID: _____).

(b) **Pour Plate Method:** 1 ml purified water sample add into two petri plates and pour R2A Agar / SCD Agar (B. No.: _____) in each petri plates and rotate clockwise and anticlockwise. After solidification of the media plates, incubated at 30-35°C for Five days (Incubator ID: _____).

Date of Analysis		**Date of Incubation**	
Analyzed By		**Date of Observation**	

S.No.	**Sampling Point No.**	**Observation**					
		Total Aerobic Microbial Count (CFU/ml)					
		Plate 1	**Plate 2**	**Average**	**Dilution Factor**	**Result**	**Remark**

+ve Control		-ve Control	

Sample Name: Purified Water

Limit
NMT: 100 CFU/ml

Remarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as per prescribe Acceptance Criteria.

----	**Analysed By**	**Checked By**	**Approved By**
Signature			
Date			

ANNEXURE–II

MICROBIOLOGICAL ANALYSIS REPORT OF TOTAL AEROBIC MICROBIAL COUNT OF RAW WATER/PROCESS POTABLE WATER

Date of Sampling		**Sampled By**		**Sampled Qty.**	

Test Method:

(a) **Membrane Filtration Method:** 1ml purified water diluted in 100 ml Bacteriological Peptone (0.1%) (B. No.: _____) and filtered through 0.45 micron membrane filter paper. Membrane filter transferred to Pre-incubated R2A Agar (B. No.: _____) and incubated at 30-35°C for Five days (Incubator ID: _____).

(b) **Pour Plate Method:** Make dilution if necessary by using Peptone, Bacteriological (0.1%) (B. No.: _____). 1 ml purified water sample add into two petri plates and pour R2A Agar / SCD Agar (B. No.: _____) in each petri plates and rotate clockwise and anticlockwise. After solidification of the media plates, incubated at 30-35°C for Five days (Incubator ID: _____).

Date of Analysis		**Date of Incubation**	
Analyzed By		**Date of Observation**	

S.No.	**Sampling Point No.**	**Observation**					
		Total Aerobic Microbial Count (CFU/ml)					
		Plate 1	**Plate 2**	**Average**	**Dilution Factor**	**Result**	**Remark**

+ve Control		-ve Control	

Sample Name: Process Potable Water

Limit
NMT: 100 CFU/ml

Sample Name: Raw Water

Limit
NMT: 500 CFU/ml

Remarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as per prescribe Acceptance Criteria.

----	**Analysed By**	**Checked By**	**Approved By**
Signature			
Date			

ANNEXURE–III

MICROBIOLOGICAL ANALYSIS REPORT FOR SPECIFIED MICROORGANISM OF PURIFIED WATER

Date of Sampling		**Sampled By**	
Analyzed By		**Date of Observation**	

S. No.	Microorganism Name →		*Escherichia coli*		*Salmonella spp.*		*P. aerug-inosa*	*S. aureus*	Rem-ark
	Procedure →	Pretreatment of sample ↓ / Filter 100 ml water sample & transfer to 100 ml SCM tube. / Add 100 ml water sample to double strength SCM.	Shake and Transfer 1 ml Pretreated sample to 100 ml MCB Medium tube.	Streak loop full from MCB broth to MCA Agar media plate.	Shake and Transfer 0.1 ml Pretreated sample to 10 ml RVSE broth tube.	Streak loop full from RVSE broth to XLD Agar media plate.	Streak loop full from Pretreated sample to CTA Agar media plate.	Streak loop full from Pretreated sample to MSA Agar media plate.	--
	Incubation Temp.	30-35°C	42-44°C	30-35°C	30-35°C	30-35°C	30-35°C	30-35°C	--
	Incubation Time	18-24 hrs	24-48 hrs	18-72 hrs	18-24 hrs	18-48 hrs	18-72 hrs	18-72 hrs	--
	Incubation								--
	Incubated By								--
	Incubator ID								--
	Media Reference No.								--
	Incubation Completion Date								--
	Sample Point No.	Observation in form of Turbidity	Observation in form of turbidity	Pink/Red coloured non-mucoid colonies	Observation in form of turbidity	Red colonies with or without black center	Green colour colonies	Yellow or white colonies surrounded by yellow zone	--
		+Ve/-Ve	+Ve /-Ve	Yes/No	+Ve/-Ve	Yes /No	Yes/No	Yes/No	Ok / Not Ok

Positive Control observation								
Negative Control observation								

Acceptance Criteria: *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa & Staphylococcus aureus* should be absent/100 ml.

NOTE: Characteristic growth identification of microorganism shall be performed, if observed.

Remarks: The above samples complies / does not comply as per Acceptance Criteria.

----	**Analysed By**	**Checked By**	**Approved By**
Signature			
Date			

ANNEXURE–IV

MICROBIOLOGICAL ANALYSIS REPORT FOR SPECIFIED MICROORGANISM OF RAW WATER/PROCESS POTABLE WATER

Date of Sampling		**Sampled By**	
Analyzed By		**Date of Observation**	

S. No.	Microorganism Name →		*Escherichia coli*		*Salmonella spp.*		*P. aerug-inosa*	*S. aureus*	Rem-ark
	Procedure →	Pretreatment of sample ↓ Filter 100 ml water sample & transfer to 100 ml SCM tube. / Add 100 ml water sample to double strength SCM.	Shake and Transfer 1 ml Pretreated sample to 100 ml MCB Medium tube.	Streak loop full from MCB broth to MCA Agar media plate.	Shake and Transfer 0.1 ml Pretreated sample to 10 ml RVSE broth tube.	Streak loop full from RVSE broth to XLD Agar media plate.	Streak loop full from Pretreated sample to CTA Agar media plate.	Streak loop full from Pretreated sample to MSA Agar media plate.	--
	Incubation Temp.	30-35°C	42-44°C	30-35°C	30-35°C	30-35°C	30-35°C	30-35°C	--
	Incubation Time	18-24 hrs	24-48 hrs	18-72 hrs	18-24 hrs	18-48 hrs	18-72 hrs	18-72 hrs	--
	Incubation Started Date								--
	Incubated By								--
	Incubator ID								--
	Media Reference No.								--
	Incubation Completion Date								--
	Sample Point No.	Observation in form of Turbidity	Observation in form of turbidity	Pink/Red coloured non-mucoid colonies	Observation in form of turbidity	Red colonies with or without black center	Green colour colonies	Yellow or white colonies surrounded by yellow zone	--
		+Ve/-Ve	+Ve /-Ve	Yes/No	+Ve/-Ve	Yes /No	Yes/No	Yes/No	Ok / Not

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									Ok
Positive Control observation									
Negative Control observation									

Acceptance Criteria: *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa & Staphylococcus aureus* should be absent/100 ml.

NOTE: Characteristic growth identification of microorganism shall be performed, if observed.

Remarks: The above samples complies / does not comply as per Acceptance Criteria.

----	**Analysed By**	**Checked By**	**Approved By**
Signature			
Date			

ANNEXURE–V
CHARACTERISTIC GROWTH IDENTIFICATION OF MICROORGANISM

Date of Sampling		**Sampled By**	
Analyzed By		**Date of Observation**	

S. No.	**Microorganism Name** ⟶		***Escherichia coli***	***Salmonella spp.***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
	Characterstics growth Observation after gram staining ⟶		Gram Positive/ Gram Negative	Gram Positive/ Gram Negative	Gram Positive/ Gram Negative	Gram Positive/ Gram Negative
	Sample Point No.	Observation	Observation	Observation	Observation	Observation
			+ve/-ve	+ve/-ve	+ve/-ve	+ve/-ve
			+ve/-ve	+ve/-ve	+ve/-ve	+ve/-ve

NOTE: If colonies showing characteristics growth, carryout the identification by outside lab up to species level.

Remarks: The above samples complies/does not comply.

----	**Analysed By**	**Checked By**	**Approved By**
Signature			
Date			