

Title: Microbiological Analysis of Raw Water, Process Potable Water & Purified Water				
SOP No.:	R	evision No.:	00	
Effective Date:	Su	upersedes No.	Nil	
Review Date:	Pa	age 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.



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.	2 of 14		

- 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° 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^oC 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



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.	3 of 14		

(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.	Туре	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^oC 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^oC 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^oC for 18 to 72 hrs.



PHARMA DEVILS

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.	4 of 14	
Review Date:		Page No.	4 of 14	

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^oC 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^oC 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^oC 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^oC 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.



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.	5 of 14	

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.

S.No.	Specified Microorganism	Media NamePositive Growth Characteristics		Gram Staining Characteristics
1. E. coli		MacConkey Broth	Medium colour turns to yellow.	Gram Negative
1. <i>L. con</i>	MacConkey Agar	Pink/red coloured non- mucoid colonies.	Rod	
2.	Salmonella	Rappaport Vassiliadis Salmonella Enrichment Broth Xylose lysine Deoxycholate Agar	Medium colour turns to reddish pink. Red colonies with or without black centers.	Gram Negative Rod
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

TABLE-1



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.	6 of 14

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



Title: Microbiological Analysis of Raw Water, Process Potable Water & Purified Water			
SOP No.:	Revision N	No.: 00	
Effective Date:	Supersede	es No. Nil	
Review Date:	Page No.	7 of 14	

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		



Title: Microbiological Analysis of Raw Water, Process Potable Water & Purified Water Revision No.: 00 Supersedes No. Nil Revision No.: 00 Supersedes No. Nil Revision No.: Output Date of Sampling Sampled By Sampled Qty. Test Method: (a) Membrane Filtration Method: Iml purified water diluted in 100 ml Bacteriological Peptone (0.1% (B. No.:									
SOP No.: Revision No.: 00 Strective Date: Supersedes No. Nil Review Date: Page No. 8 of 14 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 (Incubator ID:). (b) Pour Plate Method: 1 ml purified water sample add into two petri plates and pour R2A Agar / SCD (B. No.:) in each petri plates and rotate clockwise and anticlockwise. After solidif of the media plates, incubated at 30-35°C for Five days (Incubator ID:). Date of Analysis Date of Analysis Agar / B. No.:) Cobservation Sampling Point No. Observation Sample Rame: Purified Water Limit NMT: 100 CFU/ml Result for above samples complies/does not comply as rescribe Acceptance Criteria. <td< td=""><th>Fitle: M</th><td>licrobiologica</td><td>al Analysis of Ra</td><td>aw Water, P</td><td>rocess Potab</td><td>le Water & F</td><td>Purified Water</td><td></td><td></td></td<>	Fitle: M	licrobiologica	al Analysis of Ra	aw Water, P	rocess Potab	le Water & F	Purified Water		
Stepersedes No. Nil Review Date: Page No. 8 of 14 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: Iml purified water diluted in 100 ml Bacteriological Peptone (0.1% (B. No.:) and filtered through 0.45 micron membrane filter paper. Membrane filte transferred to Pre-incubated R2A Agar (B. No.:) and incubated at 30-35°C for Five (Incubator ID:). (b) Pour Plate Method: 1 ml purified water sample add into two petri plates and pour R2A Agar / SCD (B. No.:) in each petri plates and rotate clockwise and anticlockwise. After solidif of the media plates, incubated at 30-35°C for Five days (Incubator ID:). Date of Analysis Analyzed By Sampling Point No. Observation Sample Water Limit NMT: 100 CFU/ml Vec Control Sample Mame: Purified Water Limit NMT: 100 CFU/ml Checked By Analysed By Checked By Analyse By Checked By Analysed By	OP No).:	-				Revision No.:	00	
teview Date: Page No. 8 of 14 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 filte transferred to Pre-incubated R2A Agar (B. No.:) and incubated at 30-35°C for Five (Incubator ID:). (b) Pour Plate Method: 1 ml purified water sample add into two petri plates and pour R2A Agar / SCD (B. No.:) in each petri plates and rotate clockwise and anticlockwise. After solidif of the media plates, incubated at 30-35°C for Five days (Incubator ID:). Date of Analysis Analyzed By Observation S.No. Sampling Point No. Observation Sample Name: Purified Water Limit Wet Control Imit in the Total Aerobic Microbial Count result for above samples complex/does not comply as escribe Acceptance Criteria. Wet Control Imit in the Total Aerobic Microbial Count result for above samples complex/does not comply as escribe Acceptance Criteria. <td< td=""><th>ffectiv</th><td>e Date:</td><td></td><td></td><td></td><td></td><td>Supersedes No.</td><td>, Nil</td><td></td></td<>	ffectiv	e Date:					Supersedes No.	, Nil	
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: Iml purified water diluted in 100 ml Bacteriological Peptone (0.1% (B, No.:) and filtered through 0.45 micron membrane filter paper. Membrane filte transferred to Pre-incubated R2A Agar (B. No.:) and incubated at 30-35°C for Five (Incubator ID:). (b) Pour Plate Method: 1 ml purified water sample add into two petri plates and pour R2A Agar / SCD (B. No.:) in each petri plates and rotate clockwise and anticlockwise. After solidif of the media plates, incubated at 30-35°C for Five days (Incubator ID:). Date of Analysis Date of Observation SNo. Sampling Point No. Sampling Point No. Sample Name: Purified Water Limit NMT: 100 CFU/ml emarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as escribe Acceptance Criteria. <u> Analysed By Checked By Approved By Signature</u>	Review	Date:					Page No.	8 of 1	4
Date of Sampling Sampled By Sampled Qty. Test Method:		N	IICROBIOLO MIC	GICAL AN ROBIAL C	ANNEX IALYSIS RE COUNT OF I	URE–I CPORT OF ' PURIFIED V	TOTAL AEROBI WATER	С	
Test Method: (a) Membrane Filtration Method: Iml purified water diluted in 100 ml Bacteriological Peptone (0.1% (B. No.:) and filtered through 0.45 micron membrane filter paper. Membrane filte transferred to Pre-incubated R2A Agar (B. No.:) and incubated at 30-35°C for Five (Incubator ID:). (Incubator ID:) and incubated at 30-35°C for Five (Incubator ID:). (B. No.:) in each petri plates and rotate clockwise and anticlockwise. After solidif of the media plates, incubated at 30-35°C for Five days (Incubator ID:). Date of Analysis Analyzed By Observation S.No. Sampling Point No. Observation Imit Imit Imit <t< td=""><th>Date o</th><td>f Sampling</td><td></td><td>Sam</td><td>pled By</td><td></td><td>Sampled Qt</td><td>ty.</td><td></td></t<>	Date o	f Sampling		Sam	pled By		Sampled Qt	t y.	
(B. No.:) in each petri plates and rotate clockwise and anticlockwise. After solidif of the media plates, incubated at 30-35°C for Five days (Incubator ID:). Date of Analysis	Test M (a) M (E tra (I (b) Pe	Iethod: Iembrane Fi 3. No.: ansferred to H ncubator ID: our Plate Me	Itration Method) an Pre-incubated R2 ethod: 1 ml puri	d: 1ml purif d filtered the 2A Agar (B.). ified water s	ied water dilu rough 0.45 m No.: ample add in	uted in 100 n icron membr) an to two petri j	nl Bacteriological F rane filter paper. M nd incubated at 30- plates and pour R2/	eptone (embrane 35°C for A Agar /	0.1%) filter Five days SCD Aga
Date of Analysis Date of Incubation Analyzed By Date of Observation S.No. Sampling Point No. Observation Plate 1 Plate 2 Average Dilution Factor Result Result Plate 1 Plate 1 Plate 2 Average Dilution Factor Result Result Result	(E of	3. No.: f the media pl) in ates, incubated a	each petri p at 30-35°C f	lates and rota for Five days	te clockwise (Incubator I	and anticlockwise	After so	olidificatio
Date of Observation S.No. Sampling Point No. Observation Plate 1 Plate 2 Average Dilution Factor Result Result S.No. Dilution Factor Result Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4">Colspan="4"Colspan="4">Colspan="4"Colspan="4"Colspan="4"Colspan="4">Colspan="4"Colspan="4"Colspan	Date of	f Analysis			Γ	Date of Incul	bation		
S.No. Sampling Point No. Observation Total Aerobic Microbial Count (CFU/ml) Plate 1 Plate 2 Average Dilution Factor Result Result Plate 1 Plate 2 Average Dilution Factor Result	Analyz	ed By			Γ	Date of Obse	ervation		
+ve Control -ve Control ample Name: Purified Water -ve Control Limit NMT: 100 CFU/ml emarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as escribe Acceptance Criteria. Analysed By Checked By Approved By Signature Jate Jate Jate Jate Jate Jate	S.No.	Samplir	ıg Point No.	Plate 1	Total A Plate 2	Obse erobic Micr Average	ervation obial Count (CFU Dilution Factor	/ml) Result	Remar
+ve Control -ve Co									
Limit Limit NMT: 100 CFU/ml emarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as escribe Acceptance Criteria. Analysed By Checked By Approved By Signature Date Image: Colspan="2">Image: Colspan="2">Checked By	+ve Co	ntrol			-	ve Control			
Limit NMT: 100 CFU/ml emarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as escribe Acceptance Criteria. Analysed By Checked By Approved By Signature Date	ample	Name: Purif	ied Water						
NMT: 100 CFU/ml emarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as escribe Acceptance Criteria. Analysed By Checked By Approved By Signature Image: Complex of the second seco					Limit				
emarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as escribe Acceptance CriteriaAnalysed ByChecked ByApproved BySignatureImage: Complex of the same section				N	MT: 100 CF	U/ml			
Analysed By Checked By Approved By Signature	emark escribe	s: The Total Acceptance	Aerobic Microb Criteria.	oial Count re	esult for abov	ve samples co	omplies/does not co	omply as	
Signature Date			Analys	sed By		Checked By	y A	Approve	d By
Date	Signati	ure							
	Date								



	icrobiologics	al Analysis of Ra	aw Water, P	rocess Potal	ole Water & P	urified Wate	r		
Title: M	leiobloiogie	,	,			unned wate	/1		
SOP No	.:					Revision	No.:	00	
Effective	e Date:					Supersed	les No.	Nil	
Review	Date:					Page No.		9 of 14	1
	M	IICROBIOLO ICROBIAL CO	GICAL AN DUNT OF I	ANN ALYSIS R RAW WAT	EXURE–II EPORT OF ' ER/PROCES	FOTAL AE SS POTABL	ROBIC LE WAT	C FER	
Date of	f Sampling		Sam	pled By		Samp	oled Qty	″∙	
Test M (a) M (B tra (In (b) Po	Iethod: Iembrane Fill Iembr	Itration Method) and Pre-incubated R2 ethod: Make dil	d: 1ml purified filtered the 2A Agar (B.). ution if nece	ied water dil rough 0.45 r No.: essary by usi	luted in 100 m nicron membr) au ing Peptone, H	nl Bacteriolog rane filter pag nd incubated Bacteriologic	gical Pe per. Me at 30-3	ptone (0 mbrane 5°C for 1	0.1%) filter Five days
(B As	8. No.: gar (B. No.: _). 1	ml purified	water sampl betri plates a	e add into two	o petri plates kwise and an	and por	ur R2A A	Agar / SCD ter
(B Aş so	B. No.: gar (B. No.: _ lidification o). 1	ml purified _) in each p es, incubate	water sampl betri plates a d at 30-35°C	e add into two nd rotate cloc C for Five day	o petri plates kwise and an s (Incubator	and porticlocky	ur R2A . wise. Af	Agar / SCD ter).
(B Ag so Date of A	B. No.: gar (B. No.: _ lidification o). 1	ml purified _) in each p es, incubate	water sampl petri plates a d at 30-35°C	e add into two nd rotate cloc c for Five day Date of Incubat	o petri plates kwise and an s (Incubator	and por nticlocky ID:	ur R2A A	Agar / SCD ter).
(B Ag SO Date of A Analyzed	B. No.: gar (B. No.: _ lidification o Analysis d By	f the media plate	ml purified _) in each p es, incubate	water sampl petri plates a d at 30-35°C	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa	o petri plates kwise and an s (Incubator ion ation	and por nticlocky ID:	ur R2A . wise. Af	Agar / SCD ter).
(B Ag SO Date of A Analyzed S.No.	B. No.: gar (B. No.: _ lidification o Analysis d By Sampli). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa Observa Observa Average	o petri plates kwise and an s (Incubator ion ation ervation cobial Count (Cl Dilution Facto	FU/ml)	ur R2A A	Agar / SCD ter).
(B As SO Date of A Analyzed S.No.	B. No.: gar (B. No.: _ lidification o Analysis d By Sampli). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa Observa Observa Average	o petri plates kwise and an s (Incubator ion ation ervation robial Count (Cl Dilution Facto	FU/ml)	ur R2A A	Agar / SCD ter). Remark
(B As SO Date of A Analyzed S.No.	B. No.: gar (B. No.: _ lidification o Analysis d By Sampli ntrol). 1	ml purified _) in each p es, incubate Plate 1	water sampl betri plates a d at 30-35°C	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa- Observ	o petri plates kwise and an s (Incubator ion ation ervation cobial Count (Cl Dilution Facto	FU/ml)	ur R2A A	Agar / SCD ter).
(B Ag SO Date of A Analyzed S.No. +ve Con Sample N	B. No.: gar (B. No.: _ lidification o Analysis d By Sampli ntrol Iame: Process F). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C Plate 2 Limit NMT: 100 CF	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa O	o petri plates kwise and an s (Incubator ion ation ervation robial Count (Cl Dilution Facto	FU/ml)	ur R2A /	Agar / SCD ter). Remark
(B Ag SO Date of A Analyzed S.No. +ve Con Sample N Sample N	8. No.: gar (B. No.: _ lidification o Analysis d By Sampli Sampli htrol Name: Process F). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C Plate 2 Difference Limit NMT: 100 CF	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa O	o petri plates kwise and an s (Incubator ion ation ervation cobial Count (Cl Dilution Facto	FU/ml)	ur R2A A	Agar / SCD ter).
(B As SO Date of A Analyzed S.No. +ve Con Sample N Sample N	B. No.: gar (B. No.: _ lidification o Analysis d By Sampli Sampli ntrol Iame: Process F). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C Plate 2 Limit NMT: 100 CF	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa O	o petri plates kwise and an s (Incubator ion ation ervation obial Count (Cl Dilution Facto	FU/ml)	ur R2A A	Agar / SCD ter). Remark
(B As so Date of A Analyzed S.No. +ve Con Sample N Sample N	B. No.: gar (B. No.: lidification o Analysis d By Sampli Sampli Introl Hame: Process F). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates and d at 30-35°C Plate 2 Limit NMT: 100 CF Limit NMT: 500 CF	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa Observa Observa Observa Observa U/ml U/ml U/ml U/ml	o petri plates kwise and an s (Incubator ion ation ervation robial Count (Cl Dilution Facto	and por nticlocky ID: FU/ml) or	ur R2A A	Agar / SCD ter).
(B As SO Date of A Analyzed S.No. +ve Con Sample N Sample N Sample N Remarks:	8. No.: gar (B. No.: _ lidification o Analysis d By Sampli Sampli Introl Iame: Process F). 1	ml purified) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C Plate 2 Limit NMT: 100 CF Limit NMT: 500 CF ove samples co	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa Observa Observa Observa Observa Observa U/ml U/ml U/ml U/ml Omplies/does not	o petri plates kwise and an s (Incubator ion ation cobial Count (Cl Dilution Facto	and pounticlocky ID: FU/ml) or r prescribe	ur R2A A	Agar / SCD ter). Remark
(B As SO Date of A Analyzed S.No. +ve Con Sample N Sample N Sample N	B. No.: gar (B. No.: lidification of Analysis d By Sampli Sampli Introl Hame: Process F Name: Raw Wa The Total Aero). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C Plate 2 Difference Limit NMT: 100 CF Limit NMT: 500 CF ove samples co	e add into two nd rotate cloc C for Five day Date of Incubat Date of Observa O	o petri plates kwise and an s (Incubator ion ation ervation obial Count (Cl Dilution Facto	and pounticlocky ID: FU/ml) pr prescribe	ur R2A A wise. Af Result	Agar / SCD ter). Remark
(B As SO Date of A Analyzed S.No. +ve Con Sample N Sample N Sample N Sample N Sample N	8. No.: gar (B. No.: lidification o Analysis d By Sampli sampli Introl lame: Process F Name: Raw Wa The Total Aero). 1	ml purified _) in each p es, incubated Plate 1	water sampl betri plates a d at 30-35°C Plate 2 Difference Limit NMT: 100 CF UNMT: 500 CF ove samples co	e add into two nd rotate cloci C for Five day Date of Incubat Date of Observa Obse otal Aerobic Mice Average U/ml U/ml U/ml Dutyml Duty	o petri plates kwise and an s (Incubator ion ation cobial Count (Cl Dilution Facto	and pounticlocky ID: FU/ml) or prescribe	ur R2A A wise. Af Result	Agar / SCD ter). Remark



Title: Microbiological Analysis of Raw Water, Process Potable Water & Purified WaterSOP No.:Revision No.:00Effective Date:Supersedes No.NilReview Date:Page No.10 of 14

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►		Escherio	chia coli	Salmonella spp.		P. aerug- inosa	S. aureus	Rem- ark
		Pretreatment of sample	lm Im ([CB dia	lm l	1 gar	reated edia	reated edia	
	Procedure	Filter 100 ml water sample & transfer to 100 ml SCM tube. / Add 100 ml water sample to double strength SCM.	Shake and Transfer 1 Pretreated sample to 100 MCB Medium tube.	Streak loop full from M broth to MCA Agar me plate.	Shake and Transfer 0.1 Pretreated sample to 10 RVSE broth tube.	Streak loop full from RVSE broth to XLD A, media plate.	Streak loop full from Pretu sample to CTA Agar me plate.	Streak loop full from Pret sample to MSA Agar m 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								
	Nedia 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



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.	11 of 14				

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			



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.	12 of 14			

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		Escherie	chia coli	Salmonella spp.		P. aerug- inosa	S. aureus	Rem- ark
		D							
		sample	o ml	MCB ledia	1 ml 0 ml	m Agar	streated nedia	treated nedia	
	Procedure	Filter 100 ml water sample & transfer to 100 ml SCM tube. / Add 100 ml water sample to double strength SCM.	Shake and Transfer 1 Pretreated sample to 10 MCB Medium tub	Streak loop full from l broth to MCA Agar m plate.	Shake and Transfer 0. Pretreated sample to 1 RVSE broth tube.	Streak loop full fro RVSE broth to XLD / media plate.	Streak loop full from Pre sample to CTA Agar n plate.	Streak loop full from Pre sample to MSA Agar r 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								
	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



Title: Microbiological Analysis of Raw Water, Process Potable Water & Purified Water									
SOP No.:						Revis	ion No.:	00	
Effective Date:						Super	rsedes No.	Nil	
Review Date:						Page	No.	13 of 14	
									Ok
Positive Control									
observation									
Negative Control									
observation									ĺ

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			



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.	14 of 14		

ANNEXURE–V CHARACTERISTIC GROWTH IDENTIFICATION OF MICROORGANISM

Date of Sampling	Sampled By	
Analyzed By	Date of Observation	

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