

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE					
Department: Microbiology	SOP No.:				
Title: Microbiological analysis of Raw water, Potable water & Purified water	Effective Date:				
Supersedes: Nil	Review Date:				
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#### 1.0 OBJECTIVE:

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

#### 2.0 SCOPE:

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

#### 3.0 RESPONSIBILITY:

Officer / Executive – Microbiologist

#### 4.0 ACCOUNTABILITY:

Head – QC

#### **5.0 PROCEDURE:**

#### 5.1 TOTAL AEROBIC MICROBIAL COUNT:

- 5.1.1 Sample the Raw water, Potable water & Purified water as per SOP, Titled "Sampling of Water for Microbiological Analysis". Prepare the R-2Amedia as per SOP, Titled "Preparation of Culture Media" (Note: Sample and Result observation due on holiday shall be done next working day).
- 5.1.2 For Total Aerobic Microbial Count filter 1 ml of raw and potable water through 0.45µ Membrane Filter and wash the membrane with 100 ml sterile water and place the Membrane Filter to pre-incubated R-2A media plate for bacterial count. Label the plates with Sampling Point, Date of Sampling, Date of Testing, and Date of release and Media Reference No.
- **5.1.3** Incubate the R-2A media plates at 30 to 35°C for 5 (five) days for total microbial count.
- 5.1.4 For Total Aerobic Microbial Count filter 1 ml of Purified water and Process Potable water through 0.45µ Membrane Filter and wash the membrane with 100 ml sterile water and place the Membrane Filter to pre-incubated R-2A media plate for bacterial count. Label the plates with Sampling Point, Date of Sampling, Date of Testing, Date of release and Media Reference No.
- **5.1.5** Incubate the R-2A plates at 30 to 35°C for 5 (five) days for total microbial count.

#### **5.1.6** Observations and Results:

**5.1.6.1 Raw Water and Potable Water:** Examine the plates for the growth and count the number of colonies with the help of colony counter. Express the count in term of the number of microorganisms per ml of raw water or potable water.



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**5.1.6.2 Purified Water and Process Potable Water:** Examine the plates for the growth and count the number of colonies with the help of colony counter. Express the count in term of the number of microorganisms per ml of purified water.

#### **5.2 TEST FOR SPECIFIED MICROORGANISMS:**

#### **5.2.1** Pretreatment of Sample:

- **5.2.1.1** Filter 100 ml of water sample through membrane filter of nominal pore size NMT 0.45μm and 47 mm diameter.
- **5.2.1.2** Transfer the filter to 100 ml Soyabean Casein Digest Medium.
- **5.2.1.3** Incubate the medium at 30-35°C for 18-24 hrs.
- **5.2.1.4** Examine the medium for turbidity.

#### 5.2.2 Test for Escherichia coli:

- **5.2.2.1** Shake the tube and transfer 1 ml of pretreated sample (SCM) to 100 ml of Mac Conkey Broth and incubate 42 to 44°C for 24 to 48 hrs.
- **5.2.2.2** Streak a portion from MacConkey broth on the surface of MacConkey Agar media and incubate 30 to 35°C for 18 to 72 hrs.
- **5.2.2.3** Upon examination, if none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of the *E. coli*.
- **5.2.2.4** Run Positive and Negative Control with test.
- **5.2.2.5** If colonies show characteristic growth, carry out gram staining as per SOP, Titled "Techniques for Microbial Culture Staining".
- **5.2.2.6** If colonies show characteristic growth as per **Table-1**, carry out the identification by BBL Crystal ID System in plant-III.

#### 5.2.3 Test for Salmonella spp.

- **5.2.3.1** 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°C for 18 to 24hrs.
- **5.2.3.2** Streak a portion from the Rappaport Vassiliadis Salmonella Enrichment Broth on surface of Xylose Lysine Deoxycholate Agar Medium and incubate 30 to 35°C for 18 to 48 hrs.
- **5.2.3.3** Upon examination, if none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of the *Salmonella spp*.



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- **5.2.3.4** Run Positive and Negative Control with test.
- **5.2.3.5** If colonies show characteristic growth, carry out gram staining as per SOP, Titled "Techniques for Microbial Culture Staining".
- **5.2.3.6** If colonies show characteristic growth as per **Table-1**, carry out the identification by BBL Crystal ID System.

#### 5.2.4 Test for *Pseudomonas aeruginosa*:

- **5.2.4.1** Shake the tube and streak one loop full pretreated sample (SCM) on to the plate of Cetrimide Agar Medium and incubate 30 to 35°C for 18 to 72 hrs.
- **5.2.4.2** Upon examination, if none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of the *Pseudomonas aeruginosa*.
- **5.2.4.3 Oxidase Test:** If growth of suspected colonies occurs, place suspected colony on the oxidase disc or paper strips or discs that previously had been impregnated with N, N-dimethyl-p-phenylenediamine dihydrochloride. If there is no development of a pink color, changing to purple color, the sample meets the requirements of the test for absence of *Pseudomonasaeruginosa*.
- **5.2.4.4** Run Positive and Negative Control with test.
- **5.2.4.5** If colonies show characteristic growth, carry out gram staining as per SOP, Titled "Techniques for Microbial Culture Staining".
- **5.2.4.6** If colonies show characteristic growth as per **Table-1**, carry out the identification by BBL Crystal ID System.

#### 5.2.5 Test for Staphylococcus aureus:

- **5.2.5.1** 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°C for 18 to 72 hrs.
- **5.2.5.2** Upon examination, if none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of the *Staphylococcus aureus*.
- **5.2.5.3** Run Positive and Negative Control with test.
- **5.2.5.4** If colonies show characteristic growth, carry out gram staining as per SOP, Titled "Techniques for Microbial Culture Staining".



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- **5.2.5.5** If colonies show characteristic growth as per **Table-1**, carry out the identification by BBL Crystal ID System.
- 5.2.6 Test for Bile-Tolerant Gram-Negative Bacteria (*Enterobacteria*) for Raw Water Potable Water:
  - **5.2.6.1** Transfer 1 ml of sample to 100 ml Enterobacteria Enrichment Broth Mossel. Incubate the medium at 30 to 35°C for 24 to 48 hrs.
  - **5.2.6.2** After Incubation, Subculture on the plate of Violet Red Bile Glucose Agar and incubate at 30 to **350C for 18 to 24 hrs.**
  - **5.2.6.3** Upon examination, if none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of *Enterobacteria*.
  - **5.2.6.4** Run Positive and Negative Control with test.
  - **5.2.6.5** If colonies show characteristic growth, carry out gram staining as per SOP, Titled "Techniques for Microbial Culture Staining".
  - **5.2.6.6** If colonies show characteristic growth as per **Table-1**, carry out the identification by BBL Crystal ID System.

#### **TABLE-1**

Specified Microorganism	Media Name	Positive Growth Characteristics	Gram Staining Characteristics	
E. coli	MacConkey Broth	Medium colour turns to yellow.	Casa Na sativa Dad	
E. Cott	MacConkey Agar	Pink/red coloured non-mucoid colonies.	Gram Negative Rod	
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.		
Pseudomonas aeruginosa	Cetrimide Agar Greenish yellow colonies		Gram Negative Rod	
Staphylococcus aureus	Mannitol Salt Agar	Yellow colonies surrounded by yellow zones.	Gram Positive Cocci	
Bile Tolerant Gram	Enterobacteria Enrichment Broth, Mossel	Medium colour turns to yellow.	Gram Negative	
Negative Enterobacteria	Violet Red Bile glucose Agar	Pink/red colonies	-	



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#### **5.3 ACCEPTANCE CRITERIA:**

#### **5.3.1** Purified Water:

#### **5.3.1.1** Total Aerobic Microbial Count

Limit : 100 cfu/ml Action Limit : 80 cfu/ml Alert Limit : 60 cfu/ml

**5.3.1.2** Specified Microorganisms – Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus should be absent.

#### 5.3.1.3 Raw Water / Potable Water:

#### 5.3.1.4 Total Aerobic Microbial Count

Limit : 500 cfu/ml Action Limit : 400 cfu/ml Alert Limit : 300 cfu/ml

**5.3.1.5** Specified Microorganisms— Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus, Bile-Tolerant Gram-Negative Bacteria (Enterobacteria) should be absent

#### **6.0 REFERENCES:**

United State Pharmacopoeia 37 Indian Pharmacopoeia 2012 British Pharmacopoeia 2012

#### 7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Microbiological Analysis Record of Purified Water	
Annexure – II	Microbiological Analysis Record of Raw/Potable Water	

**ENCLOSURES:** SOP Training Record

#### **8.0 DISTRIBUTION:**

Controlled Copy No. 01
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#### 9.0 ABBREVIATIONS:

cfu Colony Forming Unit

Hrs. Hours

IP Indian Pharmacopoeia

ml Milliliter No. Number

NMT Not More Than
QA Quality Assurance
QC Quality Control

SOP Standard Operating Procedure SCM Soyabean Casein Digest Medium

spp. Species UV Ultra Violet

USP United State Pharmacopoeia

#### **10.0 REVISION HISTORY:**

#### **CHANGE HISTORY LOG**

Revision No.	Details of Changes	Reason for Change	<b>Effective Date</b>	Updated By



			STANDARD	OPERATING	G PROCED	URE			
Department:	Microbiolog	gy				SOP N	No.:		
Title: Microbio	STANDARD OPERATING PROCEDI Department: Microbiology Title: Microbiological analysis of Raw water, Potable water & Purified water impersedes: Nil SSURE-I SSURE-I MICROBIOLOGICAL ANALYSIS RECORD OF PURI Date of Sampling A. R. No Sampling points Volume sampled Date of Testing St : Total aerobic Microbial Count St Method : Membrane Filtration Sume of media : R-2A Media Reference No.: Summe of Sample :1 ml Cubation Temp. : 30-35°C for five days St of Incubation: Date of Observation: Servations:  Sampling Point No.  Pate  Average cfu. Results : Total Aerobic Microbial Count / ml of purified water  E Control  Pemarks:  MIT: Total Aerobic Microbial Count Alert Limit: 60 CFU/ml   Action Limit : 80 CFU/ml   Incubation Test:  Incubator Tip No.  Action Limit : 80 CFU/ml   Incubator ID No.  Date of Test:  Incubator ID No.  Date of Observation:  Sampling Point No.  Date of Observation:  Date of Observation:  Sampling Point No.  Date of Test:  Sampling Point No.  Date of Observation:  Sampling Po						ive Date:		
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				Inci	ıbator ID No				
Date of Testing	3								
Test	: Total aero	bic Microbia	1 Count						
Test Method									
Name of media			Reference No.:	,	· •				
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	-		•	Date of Obse	ervation:				
<b>Observations:</b>									
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-		oial Count	T						
Alert Limit: 60	O CFU/ml		Action Limit	: 80 CFU/ml		Limit : 100	CFU/ml		
Test	: Test for	Specified M	Iicroorganism	s Da	ate of Test:				
<b>Enrichment of</b>	Sample:								
<b>Test Method</b>	: Membra	ne Filtration							
Name of media	: Sc	yabean Case	ein Digest Med	ium					
Volume of Sam	_	00 ml		Med	ia Reference	No. :			
<b>Incubation Ten</b>	<b>ар. :</b> 30-35 <sup>0</sup> С	for 18 - 24 l	nrs						
Date of Incubat	tion :			Date	of Observati	ion :			
Sampling							Positive	Negative	
Points							control	control	
Observation									



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Remarks:  1.0 Test for Escherichia coli: (1) Date: Name of media : MacConkey Broth	Supe	rsedes: Nil							Review Date:			
1.0 Test for Escherichia coli:   (1) Date:	Issue	Date:							Page No	.:		
(I) Date :	Rema	nrks:										
Points   Control   Control   Control	( N I	I) Date: Name of media Incubation Ten	: MacCo	onkey Broth Cfor 24 -48	hrs							
Name of Media : MacConkey Agar   Media Reference No:		Points									_	
Observation   Gram Staining Test : Gram Positive / Negative / Cocci / Bacilli Confirmation Test : Remarks:  Remarks:  Name of Media : Rappaport Vassialiadis Enrichment Broth Incubation Temp. : 30-35°C for 18 - 24 hrs Date of Incubation : Date of Observation. :  Sampling Points   Positive Control Control Observation   Date of Incubation Temp. : 30-35°C for 18 - 48 hrs. Date of Incubation : Date of Observation.  Name of media: Xylose Lysine Deoxycholate Agar Media Reference No	I I	Incubation Tendate of Incubate Sampling	<b>ap.:</b> 30-35°	C for 18 - 72						Positive	Negative	
Confirmation Test: Remarks:	Ė	Observation	Test	: Gram Pos	itive / Nega	tive / Cocci /Ba	acilli			control	control	
2.0 Test for Salmonella spp:  (I) Date	(	Confirmation Test:										
Sampling Points  (II) Date Name of media: Xylose Lysine Deoxycholate Agar Media Reference No Incubation Temp.: 30-35°C for 18 - 48 hrs. Date of Incubation  Date of Observation.  Positive Control  Regative Control  Positive Negative control	( 1	Test for <i>Salmon</i> I) Date Name of Media	nella spp: 	 port Vassial	iadis Enrich							
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Points control control	ľ I	Name of media Incubation Ten	: Xylose Lys np. : 30-35%	C for 18 - 48	3 hrs.							
Observation											_	
		Observation										

**Gram Staining Test:** Gram Positive / Negative / Cocci /Bacilli

**Confirmation Test:** 



Salmonella spp.

## PHARMA DEVILS

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0	Test for Pseudo	monas aerugi	nosa		••••••	•••••			••••••		
	Date:       Reference No:         Name of Media: Cetrimide Agar       Reference No:         Incubation Temp.: 30-35°C for 18 - 72 hrs       Date of Observation:         Date of Observation:       Date of Observation:										
	Sampling							Positive	Negative		
	Points							control	control		
	Observation										
	Name of Test Reagent: N,N-dimethyl-p-phenyldiamine dihydrochloride (Strips/Discs)  Oxidase Test:  Date										
	Sampling							Positive	Negative		
	Points							control	control		
	Observation										
	Gram Staining Test : Gram Positive / Negative / Cocci /Bacilli Confirmation Test : Remarks:										
	Test for Staphylococcus aureus  Date  Name of media: Mannitol Salt Agar  Incubation Temp.: 30-35°C for 18 - 72 hrs.  Date of Incubation  Date of Observation.										
	Sampling							Positive	Negative		
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Sampling points									
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	Aerobic Mic	robial (	Count / ml	of purified water	r				
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Results : Total  ve Control  demarks:  IMIT: Total Aero Alert Limit: 300  Fest Enrichment of Sa Fest Method Name of media	: Test for Spemple: : Membrane F : Soyabe : 100 m	Count Ciltration ean Case 1 18 - 24 h	Action Lin	nit : 400 CFU/ml sms Dat edium Media	e of Test:	mit : 500 CFU/ml			



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Name of media: Mannitol Salt Agar Media Reference No										
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