



## STANDARD OPERATING PROCEDURE

<b>Department:</b> Microbiology	<b>SOP No.:</b>
<b>Title:</b> Microbiological analysis of Raw water, Potable water & Purified water	<b>Effective Date:</b>
<b>Supersedes:</b> Nil	<b>Review Date:</b>
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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-2A media 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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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<sup>0</sup>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 35°C 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**

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





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MICROBIOLOGY DEPARTMENT

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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	Effective Date	Updated By





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.....

### 1.0 Test for *Escherichia coli*:

(I) Date : .....

Name of media : MacConkey Broth

Media Reference No.: .....

Incubation Temp. : 42-44°C for 24 -48 hrs

Date of Incubation : .....

Date of Observation: .....

Sampling Points							Positive control	Negative control
Observation								

(II) Date: .....

Name of Media : MacConkey Agar

Media Reference No.: .....

Incubation Temp.: 30-35°C for 18 - 72 hrs

Date of Incubation: .....

Date of Observation. : .....

Sampling Points							Positive control	Negative control
Observation								

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

**Confirmation Test :**

**Remarks:**.....  
.....

### 2.0 Test for *Salmonella spp*:

(I) Date.....

Name of Media : Rappaport Vassialiadis Enrichment Broth

Media Reference No.....

Incubation Temp. : 30-35°C for 18 - 24 hrs

Date of Incubation : .....

Date of Observation. : .....

Sampling Points							Positive Control	Negative Control
Observation								

(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. ....

Sampling Points							Positive control	Negative control
Observation								

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

**Confirmation Test :**





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**Remarks:**.....  
 .....

### 3.0 Test for *Pseudomonas aeruginosa*

**Date :** .....

**Name of Media:** Cetrimide Agar

**Reference No:**.....

**Incubation Temp.:** 30-35°C for 18 - 72 hrs

**Date of Incubation:**.....

**Date of Observation:** .....

Sampling Points							Positive control	Negative control
Observation								

**Name of Test Reagent:** N,N-dimethyl-p-phenyldiamine dihydrochloride (Strips/Discs)

**Oxidase Test:**

**Date:**.....

Sampling Points							Positive control	Negative control
Observation								

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

**Confirmation Test :**

**Remarks:**.....  
 .....

### 4.0 Test for *Staphylococcus aureus*

**Date:**.....

**Name of media:** Mannitol Salt Agar

**Media Reference No:**.....

**Incubation Temp. :** 30-35°C for 18 - 72 hrs.

**Date of Incubation:**.....

**Date of Observation:** .....

Sampling Points							Positive Control	Negative Control
Observation								

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

**Confirmation Test:**

**Remarks:**.....  
 .....

### CONCLUSIONS:

Test Organism	Sampling Points						
<i>E. coli</i>							
<i>Salmonella spp.</i>							



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<i>P. aeruginosa</i>						
<i>S. aureus</i>						

**Comments:**.....

.....

+ve = Growth with specified colony characteristic or specified positive response which indicate presence of specified organism (s) or Positive Test

- ve = No Growth with specified colony characteristic or specified negative response which indicate absence of specified organism (s) or Negative Test

**Microbiologist:**

**Checked By:**

**Date**

**Date**



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**ANNEXURE – II**

**MICROBIOLOGICAL ANALYSIS RECORD OF RAW/POTABLE WATER**

<b>Date of Sampling</b>					
<b>A. R. No</b>					
<b>Sampling points</b>					
<b>Volume sampled</b>				<b>Incubator ID No.</b>	
<b>Date of Testing</b>					

**Test** : Total aerobic Microbial Count  
**Test Method** : Membrane Filtration  
**Name of media** : R-2A **Media Reference No.:** .....  
**Volume of Sample** : 1 ml  
**Incubation Temp.** : 30-35<sup>0</sup>C for five days  
**Date of Incubation:**..... **Date of Observation:**.....

**Observations:**

Date	Sampling Point No.				
<b>Average cfu.</b>					

<b>Results : Total Aerobic Microbial Count / ml of purified water</b>					

**-ve Control** \_\_\_\_\_

**Remarks:**.....  
 .....

**LIMIT:** Total Aerobic Microbial Count

<b>Alert Limit: 300 CFU/ml</b>	<b>Action Limit : 400 CFU/ml</b>	<b>Limit : 500 CFU/ml</b>
--------------------------------	----------------------------------	---------------------------

**Test** : Test for Specified Microorganisms **Date of Test:** \_\_\_\_\_

**Enrichment of Sample:**

**Test Method** : Membrane Filtration  
**Name of media** : Soyabean Casein Digest Medium  
**Volume of Sample** : 100 ml **Media Reference No.** :.....  
**Incubation Temp.** : 30-35<sup>0</sup>C for 18 - 24 hrs  
**Date of Incubation** :..... **Date of Observation** :.....



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Sampling Points							Positive control	Negative control
Observation								

**Remarks:**.....  
.....

**5.0 Test for *Escherichia coli*:**

**(I) Date :**.....

**Name of media :** MacConkey Broth

**Media Reference No.:** .....

**Incubation Temp. :** 42-44<sup>0</sup>C for 24 -48 hrs

**Date of Incubation :**.....

**Date of Observation:** .....

Sampling Points							Positive control	Negative control
Observation								

**(II) Date:** .....

**Name of Media :** MacConkey Agar

**Media Reference No:** .....

**Incubation Temp.:** 30-35<sup>0</sup>C for 18 - 72 hrs

**Date of Incubation:**.....

**Date of Observation. :** .....

Sampling Points							Positive control	Negative control
Observation								

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

**Confirmation Test :**

**Remarks:**.....  
.....

**6.0 Test for *Salmonella spp*:**

**(II) Date**.....

**Name of Media :** Rappaport Vassialiadis Enrichment Broth

**Media Reference No**.....

**Incubation Temp. :** 30-35<sup>0</sup>C for 18 - 24 hrs

**Date of Incubation :**.....

**Date of Observation. :** .....

Sampling Points							Positive Control	Negative Control
Observation								

**(II) Date**.....

**Name of media:** Xylose Lysine Deoxycholate Agar

**Media Reference No**.....

**Incubation Temp. :** 30-35<sup>0</sup>C for 18 - 48 hrs.

**Date of Incubation**..... **Date of Observation.** .....



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Sampling Points							Positive control	Negative control
Observation								

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

**Confirmation Test :**

**Remarks:**.....  
.....

### 7.0 Test for *Pseudomonas aeruginosa*

**Date :** .....

**Name of Media:** Cetrimide Agar

**Reference No:**.....

**Incubation Temp.:** 30-35°C for 18 - 72 hrs

**Date of Incubation:**.....

**Date of Observation:** .....

Sampling Points							Positive control	Negative control
Observation								

**Name of Test Reagent:** N,N-dimethyl-p-phenyldiamine dihydrochloride (Strips/Discs)

**Oxidase Test:**

**Date:**.....

Sampling Points							Positive control	Negative control
Observation								

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

**Confirmation Test :**

**Remarks:**.....  
.....

### 8.0 Test for *Staphylococcus aureus*

**Date:**.....

**Name of media:** Mannitol Salt Agar

**Media Reference No:**.....

**Incubation Temp. :** 30-35°C for 18 - 72 hrs.

**Date of Incubation:**.....

**Date of Observation:** .....

Sampling Points							Positive Control	Negative Control
Observation								

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

**Confirmation Test:**

**Remarks:**.....  
.....



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### 9.0 Test for Bile – Tolerant Gram Negative Bacteria (*Enterobacteria*)

(I) **Date**.....

**Name of media:** Enterobacteria Enrichment Broth Mossel **Media Reference No.**.....

**Incubation Temp. :** 30 – 35°C for 18 - 48 hrs.

**Date of Incubation**.....

**Date of Observation.** .....

Sampling Points							Positive control	Negative control
Observation								

(II) **Date**.....

**Name of media:** Violet Red Bile Glucose Agar

**Media Reference No.**.....

**Incubation Temp.:** 30-35°C for 18 - 24 hrs. **Date of Incubation**..... **Date of Observation.** .....

Sampling Points							Positive control	Negative control
Observation								

**Remarks:**.....  
.....

### CONCLUSIONS:

Test Organism	Sampling Points					
<i>E. coli</i>						
<i>Salmonella spp.</i>						
<i>P. aeruginosa</i>						
<i>S. aureus</i>						
<i>Bile – Tolerant Gram Negative Bacteria (Enterobacteria)</i>						

**Comments:**.....  
.....

+ve = Growth with specified colony characteristic or specified positive response which indicate presence of specified organism (s) or Positive Test

- ve = No Growth with specified colony characteristic or specified negative response which indicate absence of specified organism (s) or Negative Test

**Microbiologist:**

**Date**

**Checked By:**

**Date**