PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

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

2.0 SCOPE:

This SOP is applicable for Microbiological analysis of Raw Water & Purified Water in Microbiological Laboratory of Quality Control Department.

3.0 RESPONSIBILITY:

Officer / Executive – Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

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
USP	United State Pharmacopoeia
UV	Ultra Violet
cfu	Colony Forming Unit
TVC	Total Viable Count

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

6.0 PROCEDURE:

Prerequisite for microbiological analysis of Raw water & Purified water:

S.No.	Requirements
1	Water Sample for microbiological analysis
2	Calibrated Micropipette 100-1000 Micro litre
3	Sterilized Micropipette Tips 100-1000 Micro litre
4	Sterile SS filtration Assembly
5	Sterile Vacuum filtration flask
6	Sterile forceps
7	Sterile 0.45 µ Membrane Filter
8	Sterile SS Manifold filtration assembly
9	Sterile silicone tube and cork
10	Vacuum pump
12	SS Bucket
13	Sterile water
14	Preincubated R2A Media Plates
15	Preincubated Soyabean casein digest medium tube, Enterobacteria Enrichment broth, MacConkey broth, Rappaport Vassiliadis Salmonella Enrichment broth, Cetrimide agar, Mannitol salt agar, MacConkey agar, Xylose lysine deoxycholate agar, Violet red bile glucose agar plates
16	Sterile Glass test tube
17	Sterile petri plate

6.1 TOTAL VIABLE COUNT:

6.1.1 Sample the Raw water & Purified water as per SOP, Titled **"**Procedure for sampling of Water for Microbiological and Chemical Analysis**"**. Prepare required media as per SOP, Titled **"Receipt, Approval and Preparation of Culture Media"**

6.1.2 **For Raw Water Analysis: -** Take the 1 ml sample in 9 ml sterile water for Total Viable count and mix properly. After mixing take 1 ml of diluted raw water into two pre-sterilized Petri plates, and take 1 ml of sterile water into one pre-sterilized Petri plates for negative control and pour 20-25 ml sterilized R2A media ((cool up to 45°C, checks with IR gun)) and

rotate the plate gently in clockwise and anticlockwise direction for proper mixing of sample. Label the plates/tubes with Negative control/Sampling Point, Date of Testing and Media Reference Number.

6.1.3 Incubate the R2A media plates at 30 to 35^{0}C for 5 (five) days for Total viable count.

6.1.4 **For Purified Water Analysis**: Pre-wet the filter using approximately 10 ml of sterile water and Filter 1 ml of Purified water through 0.45µ sterile membrane filter and place the membrane filter onto pre-incubated R2A plate for Total Viable Count and For negative control; Pre-wet the filter using approximately 10 ml of sterile water and Filter 1 ml of sterile water through 0.45µ sterile membrane filter and place the membrane filter onto pre-incubated R2A plate. Label the plates with Sampling Point / Negative control, Date of Testing and Media Reference Number.

6.1.5 Incubate the R2A plates at 30 to 35^{0}C for 5 (five) days for Total viable count.

6.2 OBSERVATIONS AND RESULTS:

6.2.1 **Raw Water and Purified Water:** Examine the plates 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 and purified water.

6.3 TEST FOR SPECIFIED MICROORGANISMS:

6.3.1 **Pretreatment of Sample for Raw Water:**
Pre-wet the filter using approximately 10 ml of sterile water and Filter 100 ml of raw water sample through 0.45 µ sterile membrane filter. Transfer the membrane filter into 100 ml Soybean Casein Digest Medium.

6.3.2 Incubate the SCM tube at 20-25^{0}C for 2-5 hrs. after 2-5 hrs. incubation; perform the test of Bile-Tolerant Gram-Negative Bacteria After that incubate the SCM at 30-35^{0}C for 18-24 hrs for further analysis.

Test for Bile-Tolerant Gram-Negative Bacteria (*Enterobacteria*) for Raw Water: Transfer 1 ml of sample into 100 ml Enterobacter*ia Enrichment* Broth Mossel and Incubate at 30 to 35^{0}C for 24 to 48 hrs.

6.3.3 After completion of Incubation period, Streak on the plate of Violet Red Bile Glucose Agar and incubate at 30 to 35^{0}C for 18 to 24 hrs.

6.3.4 **Process Negative Control:** Pre-wet the filter using approximately 10 ml of sterile water and Filter 100 ml of sterile water through 0.45 µ sterile membrane filter. Transfer the membrane filter into 100 ml Soybean Casein Digest Medium.
Incubate the SCM tube at 20-25^{0}C for 2-5 hrs.
After 2-5 hrs. incubation; perform the test of Bile-Tolerant Gram-Negative Bacteria Transfer 1 ml SCM of negative control into 100 ml Enterobacteria Enrichment Broth Mossel and Incubate at 30 to 35^{0}C for 24 to 48 hrs.

After that incubate the SCM at 30-35^{0}C for 18-24 hrs. for further analysis.
After incubation completion of Enterobacteria Enrichment Broth Mossel streak a loop full on the Preincubated plate of Violet Red Bile Glucose Agar and incubate at 30 to 35^{0}C for 18 to 24 hrs.

6.3.5 Negative control should not show any growth.

6.3.6 After completion of Incubation period; examine the plates. upon examination, if none of the colonies confirm to the description given in **Table-1**, the sample meets the requirements for the absence of **Bile-Tolerant Gram-Negative Bacteria** (*Enterobacteria)*

6.3.7 If colonies show characteristic growth as per **Table-1**, carry out the identification by Vitek-2 compact identification system or outside Laboratory.

6.3.8 Pretreatment of Sample for Purified Water:

Pre-wet the filter using approximately 10 ml of sterile water and Filter 100 ml of Purified water sample through 0.45 µ sterile membrane filter Transfer the membrane filter into 100 ml Soyabean Casein Digest Medium.

6.3.9 Incubate the Soyabean Casein Digest Medium at 30-35^{0}C for 18-24 hrs for further analysis**.**

6.3.10 Test for *Escherichia coli* for Raw water and Purified water:

6.3.10.1 Shake the enriched SCM tube and transfer 1 ml of pretreated sample (SCM) into 100 ml of Mac Conkey Broth and incubate at 42 to 44^{0}C for 24 to 48 hrs.

6.3.10.2 After incubation completion; Streak a one loop full portion from MacConkey broth on the surface of MacConkey Agar Plate and incubate at 30 to 35^{0}C for 18 to 72 hrs.

6.3.10.3 After incubation completion; examine the plates. 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.*

6.3.10.4 Process Negative Control: Pre-wet the filter using approximately 10 ml of sterile water and Filter 100 ml of sterile water sample through 0.45 µ sterile membrane filter.
Transfer the membrane filter into 100 ml Soyabean Casein Digest Medium and Incubate the medium at 30-35^{0}C for 18-24 hrs. for further analysis**.**
After incubation completion; transfer 1 ml sample to sterile MacConkey broth and incubate at 42-44°C for 24-48 hrs. After incubation completion; subculture a loop full on pre-incubated plates of MacConkey agar (MCA) and incubate at 30-35°C for 18-72 hours in inverted position.

6.3.10.5 Negative control should not show any growth.

6.3.10.6 If colonies show characteristic growth as per **Table-1**, carry out the identification by Vitek-2 compact identification system or outside Laboratory.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

6.3.11 Test for *Salmonella spp*. for raw water and purified water:

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

6.3.11.2 After incubation completion: Streak a one loop full portion from the Rappaport Vassiliadis Salmonella Enrichment Broth on surface of Xylose Lysine Deoxycholate Agar Plate and incubate at 30 to 35^{0}C for 18 to 48 hrs.

6.3.11.3 After incubation completion; examine the plates. 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*.

6.3.11.4 If colonies show characteristic growth as per Table-1, carry out the identification by Vitek-2 compact identification system or outside Laboratory.

6.3.11.5 Process Negative Control: After incubation completion of negative control Soyabean casein digest transfer 0.1 ml SCM into 10 ml of sterile RVS Broth and incubate at 30- 35°C for 18-24 hrs. After incubation completion; subculture a loop full on Preincubated plates of Xylose lysine Deoxycholate Agar (XLD) and incubate at 30-35°C for 18-48 hours in inverted position.

6.3.11.6 Negative control should not show any growth.

6.3.12 Test for *Pseudomonas aeruginosa* for raw water and purified water:

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

6.3.12.2 After incubation completion; examine the plates. 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*.

6.3.12.3 If colonies show characteristic growth as per Table-1, carry out the identification by Vitek-2 compact identification system or outside Laboratory.

6.3.12.4 Process Negative Control: After incubation completion of negative control Soyabean casein digest medium subculture a loop full on pre-incubated plates of Cetrimide Agar (CTA) and incubate at 30 to 35°C for 18 to 72 hours in inverted position.

6.3.12.5 Negative control should not show any growth.

6.3.13 Test for *Staphylococcus aureus* for raw water and purified water:

6.3.13.1 Shake the enriched 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.

6.3.13.2 After incubation completion; examine the plates. 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*.

6.3.13.3 If colonies show characteristic growth as per Table-1, carry out the identification by Vitek-2 compact identification system or outside Laboratory.

6.3.13.4 **Process Negative Control:** After incubation completion of negative control Soyabean casein digest medium i subculture a loop full on pre-incubated plates of Mannitol salt agar plate (MSA) and incubate at 30 to 35°C for 18 to 72 hours in inverted position.

6.3.13.5 Negative control should not show any growth.

TABLE-1

Specified Microorganism	Media Name	Positive Growth Characteristics	Gram Staining Characteristics
E. coli	MacConkey Broth	Medium colour turns to yellow.	Gram Negative Rod
	MacConkey Agar	Pink/red coloured non-mucoid colonies.	
Salmonella	Rappaport Vassiliadis Salmonella Enrichment Broth	Medium colour turns to light green	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 Negative Enterobacteria	Enterobacteria Enrichment Broth, Mossel	Medium color turns to yellow.	Gram Negative
	Violet Red Bile glucose Agar	Pink/red colonies	

(Note: If there is a holiday on the day of release/transfer of media plates, take the observation/ transfer of media plates on next working day.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

7.0 ACCEPTANCE CRITERIA:

Purified Water:

Total viable count	Alert Limit	60 cfu/ml
	Action Limit	80 cfu/ml
	Specified Limit	100 cfu/ml

Specified Microorganisms	*Escherichia coli*	Should be absent
	Salmonella spp.	Should be absent
	Pseudomonas aeruginosa	Should be absent
	Staphylococcus aureus	Should be absent

Raw Water:

Total Viable Count	Alert Limit	300 cfu/ml
	Action Limit	400 cfu/ml
	Specified Limit	500 cfu/ml

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

8.0 ANNEXURES:

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

ENCLOSURES: SOP Training Record.

9.0 DISTRIBUTION:

- Controlled Copy No. 01 — Quality Assurance
- Controlled Copy No. 02 — Microbiology
- Master Copy — Quality Assurance

10.0 REFERENCES:

- United State Pharmacopoeia 39
- Indian Pharmacopoeia 2014
- British Pharmacopoeia 2012

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

11.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Change Control No.	Details of Changes	Reason for Change	Effective Date	Updated by

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE-I
MICROBIOLOGICAL ANALYSIS RECORD OF PURIFIED WATER

Date of Testing:				**Method:** Membrane Filtration							Date of report:
Tested By:				**Incubator ID.:**							
Test Name	**Media Reference**	**Incubation condition**	**A. R. No→**								**Observed by /date**
			Sampling point→								
TVC	**R2A/**	**30-35^{0}C for 5 days**	**Observation**								
Enrichment for Test for Specified Microorganisms: Filter 100 ml water sample by 0.45µ membrane and transfer the membrane into SCM Media **Test performed by/date:**											
SCM/		**30-35^{0}C for 18-24hrs.**	**Observation**								
PRIMARY TEST FOR SPECIFIED MICROORGANISM: **Test performed by/date:**											
Salmonella	**RVS/**	**30-35^{0}C for 18-24hrs.**	**Observation**								
E.coli	**MCB/**	**42-44^{0}C for 24-48hrs.**	**Observation**								
P.aeruginosa	**CTA/**	**30-35^{0}C for 18-72hrs.**	**Observation**								
S. aureus	**MSA/**	**30-35^{0}C for 18-72hrs.**	**Observation**								
SECONDARY TEST FOR SPECIFIED MICROORGANISM:											
For *E.coli* Tested by/date:											
E.coli	**MCA/**	**30-35^{0}C for 18-72 hrs.**	**Observation**								
Confirmatory identification test:			**Observation**								
For *Salmonella* Tested by/date:											
Salmonella	**XLD/**	**30-35^{0}C for 18-48hrs.**	**Observation**								
Confirmatory identification test:			**Observation**								
For *Pseudomonas aeruginosa* Tested by/date:											
Confirmatory identification test:			**Observation**								
For *Staphylococcus aureus* Tested by/date:											
Confirmatory identification test:			**Observation**								
P→ Characteristic growth observed, N→ No Characteristic growth observed,											
CONCLUSIONS											
Test Organism	*E. coli*										**NA**

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

	Salmonella spp.								
	P. aeruginosa								
	S. aureus								

A→ Absent, P→ Present

Acceptance criteria of Total Viable count and Specified Microorganisms :

Total Viable count	Alert Limit	60 cfu/ml
	Action Limit	80 cfu/ml
	Specified Limit	100 cfu/ml

Specified Microorganisms	*Escherichia coli*	Should be absent
	Salmonella spp.,	Should be absent
	Pseudomonas aeruginosa	Should be absent
	Staphylococcus aureus	Should be absent

Remarks: The above samples comply/do not comply as per IH specification.

Microbiologist:
Date:

Reviewed By:
Date:

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE – II
MICROBIOLOGICAL ANALYSIS RECORD OF RAW WATER

Date of Testing:	**Method:** Membrane Filtration &Pour plate	Date of report:
Tested By:	**Incubator ID.:**	

Test Name	Media Reference	Incubation condition										Observed by /date
			A. R. No→									
			Sampling point→									
TVC	R2A/	30-35^{0}C for 5 days	Observation	P1								
				P2								
			Average									
			Count /ml									
Enrichment for Test for Specified Microorganisms: Filter 100 ml water sample by 0.45µ membrane and transfer the membrane into SCM Media **Test performed by/date:**												
SCM/		30-35^{0}C for 18-24hrs	Observation									
PRIMARY TEST FOR SPECIFIED MICROORGANISM:												
After 2-5 hrs. at 20-25^{0}C of enrich sample for Bile Tolerant Gram Negative Bacteria **Test performed by/date:**												
EEM/		30-35^{0}C for 24-48hrs	Observation									
After 18-24 hrs. at 30-35^{0}C of enrich sample for *Salmonella, E. coli, P. aeruginosa, S. aureus* **Test performed by/date:**												
Salmonella	RVS/	30-35^{0}C for 18-24hrs	Observation									
E. coli	MCB/	42-44^{0}C for 24-48hrs	Observation									
P.aeruginosa	CTA/	30-35^{0}C for 18-72hrs	Observation									
S. aureus	MSA/	30-35^{0}C for 18-72hrs	Observation									
SECONDARY TEST FOR SPECIFIED MICROORGANISM:												
For Bile Tolerant Gram Negative Bacteria **Test performed by/date:**												
VBA/		30-35^{0}C for 18-24hrs	Observation									
For *E.coli* Tested by/date:												
E.coli	MCA/	30-35^{0}C for 18-72 hrs.	Observation									
Confirmatory identification test:			Observation									
For *Salmonella* Tested by/date:												
Salmonella	XLD/	30-35^{0}C for 18-48hrs	Observation									

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Analysis of Raw Water & Purified Water	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Confirmatory identification test:	**Observation**								
For *Pseudomonas aeruginosa* Tested by/date:									
Confirmatory identification test:	**Observation**								
For *Staphylococcus aureus* Tested by/date:									
Confirmatory identification test:	**Observation**								

P→ Characteristic growth observed, N→ No Characteristic growth observed

CONCLUSION

Test Organism	**Sampling point →**								NA
	Bile Tolerant Gram Negative Bacteria								
	E. coli								
	Salmonella spp.								
	P. aeruginosa								
	S. aureus								

A→ Absent, P→ Present, P1→Plate-1, P2→Plate-2,

Acceptance criteria of Total Viable count and Specified Microorganisms :

Total Viable count	Alert Limit	300 cfu/ml
	Action Limit	400 cfu/ml
	Specified Limit	500 cfu/ml

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

Remarks: The above samples comply/do not comply as per IH specification.

Microbiologist:
Date:

Reviewed By:
Date: