



## STANDARD OPERATING PROCEDURE

<b>Department:</b> Microbiology	<b>SOP No.:</b>
<b>Title:</b> Microbial Limit Test of Water, Non-Sterile Product & Material	<b>Effective Date:</b>
<b>Supersedes:</b> Nil	<b>Review Date:</b>
<b>Issue Date:</b>	<b>Page No.:</b>

### 1.0 OBJECTIVE:

To lay down the Harmonised procedures for enumeration of Total aerobic Microbial count, total combined yeast and mould count and microbiological examination of specified microorganisms for Water, Raw material, In Process and finished product & stability sample.

### 2.0 SCOPE:

This procedure applies to carry out total aerobic microbial counts, total combined yeast and mould counts and microbiological examination of specified microorganisms for Water, Raw material, intermediates and finished product & stability sample.

### 3.0 RESPONSIBILITY:

Q.C- Microbiologist.  
Head -Quality Control.

### 4.0 PROCEDURE:

4.1 **For Water:** Refers SOP for Microbiological Enumeration method.

**For Non Sterile Product / Material:** Refer GTP Test for Total Aerobic Microbial Count As per Harmonized and IP method.

4.2 Record the result for water in Annexure-II and For Non sterile Product / Material in Annexure-III.

4.3 **For Microbiological Examination of Specified Microorganisms:**

4.4 Refer GTP Test for Specified Microorganisms As per Harmonized and IP method.

Examined Sample should be free from following specified microorganisms as below:

- 1) Escherichia coli.
- 2) Salmonella spp.
- 3) Staphylococcus aureus.
- 4) Pseudomonas aeruginosa.
- 5) Shigella boydii
- 6) Bile tolerant Gram negative bacteria



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7)Candida albicans

8)Clostridium Sporogenes

9) Or As per individual monographs

4.5 For investigation of any out of specification test result follow the SOP.

### 5.0 ANNEXURE (S):

Annexure –I: Sample Inward register for Microbiological Examination of Non-sterile product / material

Annexure-II: Analytical Test Report for Microbiological examination of Water

Annexure- III: Analytical Test Report for Microbiological examination of Non-Sterile Products / Material.

### 6.0 REFERENCE (S):

GTP: Test for Total Aerobic Microbial Count As Per Harmonized and IP Method.

GTP: Test for Specified Microorganisms As Per Harmonized and IP Method.

Pharmacopoeia: As per current version of IP, BP, USP and European Pharmacopoeia.

SOP: Investigation of out of specification of test results in microbiology.

SOP: Sampling, Testing, Release & Rejection of Water.

SOP: Preparation, Approval, Distribution control, revision and Destruction of Standard operating Procedure (SOP).

### 7.0 ABBREVIATION (S) /DEFINITION (S):

GTP: General test procedure.

SOP: Standard Operating Procedures

Q.C.: Quality control.

QCM: Quality Control Micro.

IP: Indian Pharmacopoeia

BP :British Pharmacopoeia

USP: United States Pharmacopoeia



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EP: European Pharmacopoeia

TAMC : Total Viable Aerobic Microbial Count (TAMC)

TYMC : Total Yeast and Mould Count (TYMC)

**REVISION CARD**

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
1	00	---	---	New SOP	---





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**Annexure 2**  
**ANALYTICAL TEST REPORT FOR MICROBIOLOGICAL EXAMINATION OF WATER**

<b>MICROBIAL LIMIT TEST REPORT</b>	
<b>Types of Water :</b>	<b>A.R. No.:</b>
<b>Sample Point No:</b>	<b>Sampled by:</b>
<b>Date of Testing:</b>	<b>Date of Report :</b>
<b>Specification No.:</b>	<b>GTP No. :</b>

<b>A)TOTAL AEROBIC MICROBIAL COUNT (TAMC) :</b>				
<b>Method used:</b>	Membrane Filtration method			
	Pour plate method			
<b>Media used:</b>	<b>B.No/ Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at Temperature</b>
<b>Observation:</b>	<b>Positive control:</b>		<b>Negative Control:</b>	
PLATE-1:		PLATE-2:		Average Count : 
RESULT:	Average count x Dilution factor = <span style="float: right;">CFU/ml</span>			
Limit:	CFU/ml		Observed by :	

<b>B)TOTAL YEASTS AND MOULDS COUNT (TYMC):</b>				
<b>Method used:</b>				
<b>Media used:</b>	<b>Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at:</b>
<b>OBSERVATION:</b>	<b>Positive control:</b>		<b>Negative Control:</b>	
PLATE-1:		PLATE-2:		Average Count : 
RESULT:	Average count x Dilution factor = <span style="float: right;">CFU/ml</span>			
Limit:	CFU/ml		Observed by :	



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**C)TEST FOR SPECIFIED MICRO-ORGANISMS:**

**Enrichment for Pathogens**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator I.D	Incubated at:
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**i)TEST FOR *Escherichia coli***

**Primary Test:**

Media used:	B.No./Lot. No.:	Autoclave Load No	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**Secondary Test:**

Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**Confirmative Test:**

Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**ii)TEST FOR *Salmonella*:**

<b>Primary test</b>	
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Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

<b>Secondary Test:</b>				
Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

<b>Confirmative Test:</b>				
Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

<b>iii)TEST FOR <i>Staphylococcus aureus</i>:</b>				
<b>Primary test</b>				
Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		



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**Confirmative Test : Coagulase Test**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	Growth observed / not observed			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**iv)TEST FOR *Pseudomonas aeruginosa*:**

Primary test				
Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	Growth observed / not observed			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Secondary Test :**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	Growth observed / not observed			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Confirmative Test : Oxidase Test**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	Growth observed / not observed			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Remarks:** The Above water sample **complies/does not comply** as per Specification.





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**Annexure 3**  
**ANALYTICAL TEST REPORT FOR MICROBIOLOGICAL EXAMINATION OF NON-STERILE PRODUCT / MATERIAL**

MICROBIAL LIMIT TEST REPORT	
<b>Product / Material Name :</b>	<b>A.R. No.:</b>
<b>Batch No.:</b>	<b>Sampled Date:</b>
<b>Date of Testing:</b>	<b>Date of Report :</b>
<b>Specification No.:</b>	<b>GTP No. :</b>

**Sample Preparation:** Weigh and transfer \_\_\_g or \_\_\_ml sample to \_\_\_ml Soybean Casein Digest Medium (SCDM)(MEDIA Ref No. (T)\_\_\_\_\_)Give heat treatment at 40°C - 45°C for NMT 30 Min. if necessary.

For Pour Plate Take 1ml of Pretreatment SCDM (T) in duplicate plate OR

For membrane filtration take 10ml of pretreated SCDM (T) followed by washing with \_\_\_ml of Buffered Sodium Chloride Peptone Solution (BSCP) for each test

A)TOTAL AEROBIC MICROBIAL COUNT (TAMC) :				
<b>Method used:</b>	Membrane Filtration method			
	Pour plate method			
<b>Media used:</b>	<b>B.No/ Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at Temperature</b>
<b>OBSERVATION:</b>	<b>Positive control:</b>		<b>Negative Control:</b>	
PLATE-1:		PLATE-2:		Average Count : <input type="text"/>
RESULT:	Average count x Dilution factor = <input type="text"/> CFU/ml			
Limit:	CFU/ml <input type="text"/>		Observed by : <input type="text"/>	
B)TOTAL YEASTS AND MOULDS COUNT (TYMC):				
<b>Method used:</b>				
<b>Media used:</b>	<b>Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at:</b>
<b>OBSERVATION:</b>	<b>Positive control:</b>		<b>Negative Control:</b>	
PLATE-1:		PLATE-2:		Average Count : <input type="text"/>
RESULT:	Average count x Dilution factor = <input type="text"/> CFU/ml			
Limit:	CFU/ml <input type="text"/>		Observed by : <input type="text"/>	



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**TEST FOR SPECIFIED MICRO-ORGANISMS:**

**Sample Preparation:** Take \_\_\_\_\_ gm Sample for Solid & \_\_\_\_\_ ml Sample for liquid.  
Inoculate the sample in \_\_\_\_\_ ml diluents or medium.

**C)TEST FOR SPECIFIED MICRO-ORGANISMS:**

**Enrichment for Pathogens**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator I.D	Incubated at:
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**i)TEST FOR *Escherichia coli***

**Primary Test:**

Media used:	B.No./Lot. No.:	Autoclave Load No	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**Secondary Test:**

Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**Confirmative Test:**

Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>		<b>Observed by:</b>	

**ii)TEST FOR Salmonella:**

<b>Primary test</b>	
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Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Secondary Test:**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Confirmative Test:**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**iii) TEST FOR Staphylococcus aureus:**

**Primary test**

Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		



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**Confirmative Test : Coagulase Test**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**iv)TEST FOR Pseudomonas aeruginosa:**

Primary test				
Media used:	B.No./Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Secondary Test :**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Confirmative Test : Oxidase Test**

Media used:	B.No. / Lot. No.:	Autoclave Load No.	Incubator ID.	Incubated at
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		



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**V)TEST FOR Enterobacteria (bile gram negative bacteria)**

<b>Primary test</b>				
<b>Media used:</b>	<b>B.No./Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at</b>
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Secondary Test :**

<b>Media used:</b>	<b>B.No. / Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at</b>
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Confirmative Test :**

<b>Media used:</b>	<b>B.No. / Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at</b>
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**VI)TEST FOR Shigella boydi**

<b>Primary test</b>				
<b>Media used:</b>	<b>B.No./Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at</b>



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<b>Observation:</b>	<b>Growth observed / not observed</b>	
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>

<b>Secondary Test :</b>				
<b>Media used:</b>	<b>B.No. / Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at</b>
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

<b>Confirmative Test :</b>				
<b>Media used:</b>	<b>B.No. / Lot. No.:</b>	<b>Autoclave Load No.</b>	<b>Incubator ID.</b>	<b>Incubated at</b>
<b>Observation:</b>	<b>Growth observed / not observed</b>			
<b>Positive control:</b>	<b>Negative Control:</b>	<b>Observed by:</b>		

**Remarks:** The Product / Material **complies /does not comply** as per above mentioned Specification.