



**STANDARD OPERATING PROCEDURE**

<b>Department:</b> Microbiology	<b>SOP No.:</b>
<b>Title:</b> Procedure for Microbiological Analysis of Water for Injections/Pure Steam Condensate	<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 a procedure for Microbiological Analysis of Water for Injections and Pure Steam Condensate.

**2.0 SCOPE:**

This SOP is applicable for Microbiological Analysis of Water for Injections and Pure Steam Condensate in Microbiological Lab of Quality Control Area.

**3.0 RESPONSIBILITY:**

Officer / Executive – Microbiologist

**4.0 ACCOUNTABILITY:**

Head – QC

**5.0 ABBREVIATION:**

Cfu	Colony Forming Unit
IP	Indian Pharmacopeia
Hrs	Hours
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 Pharmacopeia
UV	Ultra Violet

**6.0 PROCEDURE:**



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#### Prerequisite for microbiological analysis of Water for Injections / Pure Steam Condensate:

S.No.	Requirements
1.	Water Sample for microbiological analysis
2.	Sterile SS filtration Assembly
3.	Sterile Vacuum filtration flask
4.	Sterile forceps
5.	Sterile 0.45 $\mu$ Membrane Filter
6.	Sterile SS Manifold filtration assembly
7.	Sterile silicone tube and cork
8.	Vacuum pump
9.	Glass bid sterilizer
10.	Extension Board
11.	SS Bucket
12.	Sterile water/sterile 0.1% peptone water
13.	Preincubated R2A Media Plate
14.	Glass test tube

#### 6.1 TOTAL AEROBIC MICROBIAL COUNT:

**6.1.1** Water for Injections/Pure Steam condensate sampling perform as per respective SOP. Preparation of R2A media as per respective SOP.

**6.1.1.1** Filter approx. 10 ml of sterile water /0.1% Sterile peptone water for prewetting through 0.45 $\mu$  sterile membrane filter, then filter 100 ml of Water for Injections / Pure Steam condensate sample through sterile 0.45 $\mu$  membrane filter, place the membrane filter to pre-incubated R2A plate for Total Aerobic Microbial Count. Label the plates with sampling point, Date of Testing and Media Reference No.

**6.1.2** Incubate the R2A media plates at 30°C - 35°C for 5 days for Total Aerobic Microbial Count.

**6.1.3** After Incubation period, or on the next working day, if there is a holiday on the day of release count the number of Colonies. Express the result for the plate in term of the number of the Microorganisms per 100 ml of Water for Injections / Pure Steam condensate.

#### 6.2 ACCEPTANCE CRITERIA:

**6.2.1 Water For Injections/Pure Steam condensate:**

**6.2.1.1 Total Aerobic Microbial Count**

**Alert Limit:** 6 cfu/100 ml

**Action Limit:** 8 cfu/100 ml

**Limit:** 10 cfu/100 ml



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**7.0 ANNEXURES:**

<b>ANNEXURE No.</b>	<b>TITLE OF ANNEXURE</b>	<b>FORMAT No.</b>
Annexure – I	Microbiological Analysis of Water for Injections/ Pure Steam condensate	

**ENCLOSURES:** SOP Training Record

**8.0 DISTRIBUTION:**

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

**9.0 REFERENCES:**

United State Pharmacopoeia -39  
Indian Pharmacopoeia  
British Pharmacopoeia

**10.0 REVISION HISTORY:**

**CHANGE HISTORY LOG**

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



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**ANNEXURE – I**

**MICROBIOLOGICAL ANALYSIS RECORD OF WATER FOR INJECTIONS/ PURE STEAM CONDENSATE**

**Test** : Total aerobic Microbial Count  
**Test Method** : Membrane Filtration  
**Name of media** : R2A **Media Reference No.:**R2A/ .....  
**Volume of Sample** : 100 ml  
**Incubation Temp.** : 30-35<sup>o</sup>C for 5 days  
**Date of Incubation** :  
**Tested by:**  
**Date:**

<b>A. R. No</b> →												
<b>Sampling points</b> →												
<b>Observation</b>												
<b>Negative control</b>												

**Remarks**.....

**Observed By**  
**Date**

**Checked By:**  
**Date**

**LIMIT:** Total Aerobic Microbial Count

<b>Alert Limit:</b> 6 CFU/100 ml	<b>Action Limit:</b> 8 CFU/100 ml	<b>Limit:</b> 10 CFU/100 ml
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