



Title: Microbiological Analysis of Water for Injection/Pure Steam

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1.0 OBJECTIVE:

To lay down a procedure for Microbiological Analysis of Water for Injection/Pure Steam.

2.0 SCOPE:

This SOP is applicable for Microbiological Analysis of Water for Injection/Pure Steam (**TAMC & BET**) in Microbiological Lab. of Quality Control at

3.0 RESPONSIBILITY:

3.1 Officer/Executive–QC (Microbiologist)

4.0 ACCOUNTABILITY:

4.1 Head – QC

5.0 DEFINITION:

5.1 Water for injection by definition is water that is intended for use in the manufacture of parenteral (i.e. injectable) drugs whose solvent is water.

5.2 Both pure steam and water for injection (WFI) are used in many areas of the pharmaceutical industry. Pure steam is mainly used for sterilizing tanks, filters and piping systems, as well as products in sterilizers. Moreover, it is used for air-moistening in clean room systems. WFI is used for the production of medicaments as well as for the final cleaning of equipment.

6.0 PROCEDURE:

6.1 Total Aerobic Microbial Count by Membrane Filtration Method:

6.1.1 Sample the Water for Injection/Pure Steam as per SOP.

6.1.2 Pre incubated R2A media plates shall be use for analysis.

6.1.3 R2A media shall be prepared as per SOP.

6.1.4 Assemble the sterile filter set to the filtration unit in the laminar air flow station.

6.1.5 Aseptically place the sterilized or pre sterilized membrane filter using forceps in the base of filtration cup.



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- 6.1.6** After fixing the sterilized or Pre sterilized Membrane filter, mount the top portion with filtration cup.
- 6.1.7** Transfer 200 ml of Water Sample through 0.45 μ Membrane Filter.
- 6.1.8** Apply the vacuum and filter the sample and ensure that the sample is completely filtered.
- 6.1.9** Transfer the filtered membrane to the surface of Pre incubated R2A plate by slowly placing the Membrane Filter from the edge of membrane filter to the complete portion in the center of the agar surface.
- 6.1.10** Ensure that no air bubble is trapped inside the Membrane filter and entire surface of Membrane filter should be in contact of Agar surface.
- 6.1.11** Label the plates with Media Name, Sampling Point, and Date of Testing.
- 6.1.12** Incubate the Plate in incubator at 30°C-35°C for Not Less than 5 (Five) Days in inverted position.
- 6.1.13** For negative control, Filter 200 ml Sterile Peptone, Bacteriological solution (0.1%) through Membrane filter and place the membrane filter to the surface of Pre incubated R2A media plates
- 6.1.14** Label the plates with Media Name, -ve control and Date of Testing.
- 6.1.15** Incubate the Plate in incubator at 30°C-35°C for Not Less than 5 (Five) Days in inverted position.
- 6.1.16** For positive control, spread 0.1 ml culture suspension (*Staphylococcus aureus/Pseudomonas aeruginosa/Bacillus subtilis*) of 10-100 cfu/ml into the surface of Pre incubated R2A media plate with the help of sterile L-Spreader.
- 6.1.17** Label the plates with Media Name , +ve control, Culture Name and Date of Testing.
- 6.1.18** Incubate the Plate in incubator at 30°C-35°C for Not Less than 5 (Five) Days in inverted position.
- 6.1.19** **Examine the Plate for the Growth:** Count the Number of Colonies after incubation with the help of Colony Counter (if required). Express the result for the plate in term of the number of the cfu/100 ml by dividing observed cfu with 2 for Water for Injection / Pure Steam and positive control.

(Note: Sample and Result observation on holiday shall be done next working day).

6.1.20 Acceptance Criteria: Water for Injection / Pure Steam

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| Acceptance Criteria |
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| NMT: 10 CFU/100ml |
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6.2 Bacterial Endotoxin Test:

6.2.1 Test Procedure: Refer the SOP.

6.2.2 Acceptance Criteria: Not more than 0.25 EU/ml

7.0 ABBREVIATIONS:

| | |
|--------|-------------------------------|
| BET | Bacterial Endotoxin Test |
| Cfu | Colony Forming Unit |
| Hrs | Hours |
| MI | Milliliter |
| NMT | Not More Than |
| No. | Number |
| QA | Quality Assurance |
| SOP | Standard Operating Procedure |
| TAMC | Total Aerobic Microbial Count |
| WFI | Water for Injection |
| °C | Degree Celsius |
| QM | Microbiology |
| QC | Quality Control |
| S. No. | Serial Number |
| LAF | Laminar Air Flow |
| μ | Micron |
| TAMC | Total Aerobic Microbial Count |

8.0 ANNEXURES:

| ANNEXURE No. | TITLE OF ANNEXURE | FORMAT No. |
|--------------|---|------------|
| Annexure-I | Microbiological Analysis Report of Water For Injection / Pure Steam | |



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ANNEXURE-I

MICROBIOLOGICAL ANALYSIS REPORT OF WATER FOR INJECTION/ PURE STEAM

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|-------------------------|--|-------------------|--|---------------------|--|
| Date of Sampling | | Sampled By | | Sampled Qty. | |
|-------------------------|--|-------------------|--|---------------------|--|

Test Method:

Membrane Filtration Method: 200 ml Water for injection / Pure Steam filtered through 0.45 micron membrane filter paper. Membrane filter transferred to Pre-incubated R2A Agar (B. No.: _____) and incubated at 30-35°C for Five days (Incubator ID: _____).

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|-------------------------|--|----------------------------|--|
| Date of Analysis | | Date of Incubation | |
| Analyzed By | | Date of Observation | |

| S. No. | Sampling Point No. | Observation | | Remark |
|--------|--------------------|---|---|--------|
| | | Total Aerobic Microbial Count (CFU/200) ml (A) | Total Aerobic Microbial Count (CFU/100) ml (A/2) | |
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|-------------|--|-------------|--|
| +ve Control | | -ve Control | |
|-------------|--|-------------|--|

Sample Name: Water for Injection / Pure Steam

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|---------------------------|
| Limit |
| NMT: 10 CFU/100 ml |

Remarks: The Total Aerobic Microbial Count result for above samples complies/does not comply as per Acceptance Criteria.

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| ---- | Observed By (Microbiologist) | Checked By |
| Signature | | |
| Date | | |