



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Sampling and Sterility Testing of Nitrogen Gas and Compressed Air	Effective Date:
Supersedes: Nil	Review Date:
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1.0 OBJECTIVE:

To lay down a procedure for Sampling and Sterility Testing of Nitrogen Gas and Compressed Air.

2.0 SCOPE:

This SOP is applicable for Sampling and Sterility Testing of Nitrogen Gas and Compressed Air of Aseptic Area.

3.0 RESPONSIBILITY:

Operating Person - Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

FTM	Fluid Thioglycollate Medium
LAF	Laminar Air Flow
Ltd.	Limited
No.	Number
QA	Quality Assurance
QC	Quality Control
SCM	Soyabean Casein Digest Medium
SOP	Standard Operating Procedure

6.0 PROCEDURE:

6.1 Procedure for Sampling of Nitrogen Gas & Compressed Air:

- 6.1.1 Take a 38 X 200 mm test tube containing 100 ml of 0.9 % saline (normal saline), flow meter and polyurethane pipe wrapped with barrier paper.
- 6.1.2 Sterilize the test tube containing 100 ml of 0.9 % saline (normal saline), flow meter and Polyurethane pipe in Steam Sterilizer.
- 6.1.3 After sterilization of test tube containing 100 ml of 0.9 % Saline (normal saline), flow meter and Polyurethane pipe; transfer to respective area for sampling.
- 6.1.4 At a time of sample; remove the barrier paper from the Polyurethane Pipe and flow meter and connect to the sampling point and adjust the air flow on the flow meter to achieve 100 LPM (liters per minute).
- 6.1.5 Open the inlet valve and allow the purging of Nitrogen Gas/Compressed Air for 10 min in 0.9 % Saline Solution (normal saline).
- 6.1.6 Collect the 1000 liters total volume of Nitrogen Gas/Compressed Air.
- 6.1.7 Close the valve after completion of 1000 liters of Nitrogen Gas/Compressed Air.
- 6.1.8 After collection; disconnect the pipe from the sampling point.



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6.1.9 Repeat the section 6.1.4 to 6.1.7 for next sampling point.

6.1.10 Transfer the samples to microbiology laboratory for analysis.

6.1.11 Samples should be taken by appropriate precautions to avoid contamination.

6.2 Procedure for Sterility Testing of Nitrogen Gas / Compressed Air:

6.2.1 Enter into Sterility Area as per SOP, title: "Entry, Exit and gowning procedure for sterility area".

6.2.2 Check the temperature & Relative Humidity of Sterility Room as per SOP.

6.2.3 Clean the working bench of LAF with lint free duster moistened in sporocidal disinfectant.

6.2.4 After completion of sterilization cycle; unload the sterilized articles from autoclave to mobile LAF and transfer from cooling zone to LAF of Sterility test room.

6.2.5 Transfer the pre incubated FTM & SCM tubes, rinsing fluids and sample to buffer zone through dynamic pass box and after that to LAF of Sterility Room with mobile LAF.

6.2.6 Before starting sterility test, expose the SCA plates at specified location through testing as per SOP. Personnel monitoring shall be carried out after the completion of sterility test by contact plate method as per SOP. After personnel monitoring sanitize hand gloves and contact site of boiler suit with 0.22 μ filtered 70% IPA.

6.2.7 Assemble the filtration unit and connect the manifold assembly with reservoir properly and reservoir to vacuum pump with silicon tubes. Aseptically put the membrane filter with sterile SS forcep (pour size 0.45 μ m and diameter 47mm) in filtration cup.

6.2.8 Prior the test; pre-wet the membrane filter with approximately 20 ml of fluid A (0.1% peptone water) and filter the sample. After filtration; wash the membrane filter with 100 ml of 0.1% peptone water.

6.2.9 After completion of filtration, switch OFF the vacuum pump and disassemble the filtration cup carefully. Aseptically cut the membrane filter into two halves with sterile SS forcep and scissor and transfer one half to SCM tube and other one half to FTM tube with sterile forcep. Label the SCM / FTM tubes.

6.2.10 Frequently sanitize the hand and LAF working station with 0.22 μ filtered 70% IPA during sterility test.

6.2.11 Similarly perform negative control by using another filtration assembly and pre-wet the membrane filter with approximately 20 ml of fluid A (0.1% sterile peptone water) and filter 100 ml of fluid A (0.1% sterile peptone water). After filtration aseptically cut the membrane filter into two halves with sterile SS forcep and scissor and transfer one half to SCM tube and other one half to FTM tube with sterile forcep. These tubes will serve as negative control so that label the tubes as "negative control" along with date of test and date of release.



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6.2.12 After completion of work; transfer all inoculated media, Environmental Monitored plates and other materials to dynamic pass box of incubation room and exit from sterility area as per SOP, title: "Entry/exit and Gowning Procedure for Sterility area".

6.2.13 Collect the FTM, SCM tubes and environmental monitored plates from dynamic pass box of incubation Room and Label the SCM/FTM tubes/container with following information: Sample Name, Sampling Point, Media Reference No., Date of Testing, Date of Release and Tested By as per format.

6.2.14 Incubate FTM tubes at $32.5^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ and SCM tubes at $22.5^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ for 14 days. Incubate the SCA and DNA contact plates of environmental and personal monitoring at $22.5^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ for NLT 72 hrs. and further $32.5^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ for NLT 48 hrs.

6.2.15 Daily visual observation shall be performed of all tested sample tubes including negative control of FTM & SCM for 14 days.

6.2.16 Record the nitrogen gas samples receiving details in format.

6.2.17 Record the results of nitrogen gas in format.

6.2.18 Record the compressed air samples receiving details in format.

6.2.19 Record the results of compressed air in format.

6.2.20 Frequency for compressed air sampling: during media fill study.

6.3 Acceptance Criteria:

6.3.1 During the incubation period observe the medium for growth by means of turbidity. If no evidence of growth is found, sample being examined passes the test for sterility.

6.3.2 If evidence of microbial growth is found investigate the cause of failure as follows:

6.3.2.1 Check the membrane filter which is connected to filtration line and sampling procedure for collecting the sample.

6.3.2.2 Observe the negative control tube for growth by means of turbidity.

6.3.2.3 If the above parameters are found to comply then sample being examined doesn't pass the test for sterility.

7.0 ANNEXURES:

NA

ENCLOSURES: SOP Training Record.



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

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8.0 DISTRIBUTION:

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

9.0 REFERENCES:

Not Applicable

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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