PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sampling and Bio-burden Testing of Compressed Air and Nitrogen Gas	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE:

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

2.0 SCOPE:

This SOP is applicable for Sampling and Bioburden Testing of Compressed Air and Nitrogen Gas being used in Manufacturing of product by Microbiology Section of Quality Control Area.

3.0 RESPONSIBILITY:

Operating Person – Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

Cfu	Colony Forming Unit
LVP	Large Volume Parenteral
IPA	Isopropyl Alcohol
NLT	Not Less Than
No.	Number
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
SS	Stainless Steel

6.0 PROCEDURE:

6.1 Take a conical head of air sampler, flow meter and Polyurethane pipe wrapped with barrier paper.

6.2 Sterilize the conical head, Flow meter and Polyurethane pipe in Steam Sterilizer.

6.3 Put all the required material for intended purpose and pre–incubated SCA media plates into the SS container previously sanitized outer and inner surface with 0.22µ filtered 70 % IPA and transfer the container to respective area.

6.4 All the required material for sampling of Compressed air/Nitrogen Gas shall be shifted at the sampling points and assemble the compressed air/Nitrogen Gas component.

6.5 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)

6.6 Connect the Polyurethane Pipe and flow meter to the desired point of sampling. The inlet valve should be in a closed position.

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6.7 Label the soyabean casein digest agar media plate as follow: sampling point, grade, location, date of sampling, sampled by after that Insert soyabean casein digest agar media plate in the head space and screw it.

6.8 Open the line of compressed air/Nitrogen Gas and adjust the air flow on the flow meter at 100 LPM (liters per minute) and pressure of 2 Bar or 2 kg/cm².

6.9 Close the inlet.

6.10 Connect the regulated supply to sampler head through connector.

6.11 Open the inlet valve and start countdown of sampling for 10 minute.

6.12 Close the valve after completion of 1000 liters of air is collected.

6.13 Unscrew the head, remove the Petri dish, close the lid of the Petri dish and transfer it to the microbiology laboratory for incubation.

6.14 Soyabean casein digest agar media plate should have impaction of holes.

6.15 Repeat the above procedure for every sampling point.

6.16 Incubate the soyabean casein digest agar media plate at 20-25 ^{0}C for NLT 72 hours then transfer at 30-35 ^{0}C for NLT 48 hours.

6.17 After completion of incubation, count the microbial colonies of each plate and report the results as cfu/1000 liters of Compressed air/Nitrogen Gas.

6.18 Acceptance Criteria:

Class	Alert Limit Cfu/ 1000 liters	Action Limit Cfu/1000 liters	Specification Limit cfu/1000 liters
100	‹ 1	‹ 1	‹ 1
1,000	‹06	‹08	‹10
10,000	‹60	‹80	‹100
100,000	‹120	‹160	‹200

6.19 Frequency of Compressed Air: Six monthly in all Block ±5 days.

6.20 Frequency of Nitrogen Gas: Monthly ±5 days

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Compressed Air Sampling Log Book	
Annexure – II	Sampling Schedule of Compressed Air	
Annexure – III	Bioburden Report of Compressed Air	

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Annexure – IV	Nitrogen Gas Sampling Log Book	
Annexure –V	Bioburden Report of Nitrogen Gas	
Annexure – VI	Sampling Schedule of Nitrogen gas	

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:

Not Applicable.

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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

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ANNEXURE-I
COMPRESSED AIR SAMPLING LOG BOOK

S. No.	Date of Sampling	Area	Sampling Point No	Details of Sampling Point	Sampled By (Sign & Date)	Released By (Sign & Date)	Checked By (Sign & Date)

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ANNEXURE-II

SAMPLING SCHEDULE OF COMPRESSED AIR

Location	Sampling Point Details	Sampling Point	Grade	Frequency											
				Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
Granulation-01	RMG		D	√						√					
Coating-01	Auto coater		D		√						√				
Coating-02	Coating-02		D		√						√				
Liquid section	Filling		D			√						√			
Ointment section	Filling 01		D			√						√			

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Location	Sampling Point Details	Sampling Point	Grade	Frequency (Date)					
				5	10	15	20	25	30
Dry Powder	Vial Filling Area		A	√					
Dry Powder	Vial Washing & Sterilization Room		C	√					
Dry Powder	Autoclave cum bung processor		C	√					
Dry Powder	Equipment washing room		C	√					
Dry Powder	Disinfectant Preparation room		C	√					
Three Piece	CIP/SIP		D		√				
Three Piece	Manufacturing -1		C		√				
Three Piece	Manufacturing -2		C		√				
Three Piece	Filtration -01		A		√				
Three Piece	Filtration -02		A		√				
Three Piece	Filling room		A		√				
Three Piece	Equipment wash		C		√				
Ampoule line	Ampoule line (CIP & SIP)		D			√			
Ampoule line	Ampoule line (Manufacturing)		C			√			
Ampoule line	Ampoule line (Filtration)		A			√			
Ampoule line	Ampoule line (Ampoule filling room)		A			√			
Ampoule line	Ampoule line (Equipment wash)		C			√			
Ampoule line	Ampoule line (Ampoule washing & Sterilization Area)		C			√			
FFS Line	CIP & SIP		D				√		
FFS Line	FFS filling		B				√		

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Location	Sampling Point Details	Sampling Point	Grade	Frequency (Date)					
				5	10	15	20	25	30
LVP	Equipment washing		D					√	
LVP	Filling Room 01		C					√	
LVP	Filtration 01		C					√	
LVP	Manufacturing 01		C					√	
LVP	Disinfectant preparation room		D					√	

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ANNEXURE-III
BIOBURDEN REPORT OF COMPRESSED AIR

Date of Sampling		**Media Used**	Soybean casein digest agar
Sampled By		**Autoclave Media Ref. No.**	
Date of Incubation		**Time of Incubation**	
Incubated By		**Air Sampler ID**	
1st Incubation Temp & Time.	20 – 25° for NLT 72 hrs.	**2nd Incubation Temp & Time.**	30 – 35°C for NLT 48 hrs.
Incubator ID No.		**Incubator ID No.**	

S.No.	Area	Sampling Point No.	Grade	Observation (TMC)	Observed By (Sign & Date)	Checked By (Sign & Date)

LIMITS: Total Aerobic Microbial Count `

Grade	Alert Limits (cfu/1000 Liters)	Action Limits (cfu/1000 Liters)	Limits (cfu/1000 Liters)
Grade A	**<1**	**<1**	**<1**
Grade B	**<06**	**<08**	**<10**
Grade C	**<60**	**<80**	**<100**
Grade D	**<120**	**<160**	**<200**

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Done By
Date

Checked By
Date

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ANNEXURE-IV
NITROGEN GAS SAMPLING LOG BOOK

S. No.	Date of Sampling	Area	Sampling Point No	Details of Sampling Point	Sampled By (Sign & Date)	Released By (Sign & Date)	Checked By (Sign & Date)

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ANNEXURE-V
BIOBURDEN REPORT OF NITROGEN GAS

Date of Sampling		**Media Used**	Soyabean Casein Digest Agar
Sampled By		**Autoclave Media Ref. No.**	
Date of Incubation		**Time of Incubation**	
Incubated By		**Air Sampler ID**	
1st Incubation Temp & Time.	20 – 25°for NLT 72 hrs.	**2nd Incubation Temp & Time.**	30 – 35°C for NLT 48 hrs.
Incubator ID No.		**Incubator ID No.**	

S.No.	Area	Sampling Point No.	Grade	Observation (TMC)	Observed By (Sign & Date)	Checked By (Sign & Date)

LIMITS: Total Aerobic Microbial Count `

Grade	Alert Limits (cfu/1000 Liters)	Action Limits (cfu/1000 Liters)	Limits (cfu/1000 Liters)
Grade A	**<1**	**<1**	**<1**
Grade B	**<06**	**<08**	**<10**
Grade C	**<60**	**<80**	**<100**
Grade D	**<120**	**<160**	**<200**

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Done By
Date

Checked By
Date

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ANNEXURE-VI

SAMPLING SCHEDULE OF NITROGEN GAS

Location	Sampling Point Details	Sampling Point	Grade	Frequency (Date)					
				5	10	15	20	25	30
Dry powder	Filling room		A	√					
RM	Dispensing area Non sterile		C	√					
RM	Sampling area Non sterile		C	√					
RM	Sterile Sampling/ Dispensing Area		A		√				
Three Piece	Manufacturing -1		C		√				
Three Piece	Manufacturing -2		C		√				
Three Piece	Filtration-1		A		√				
Three Piece	Filtration-2		A		√				
Three Piece	Filling room		A		√				
Three Piece	Disinfectant preparation room		C		√				
Ampoule line	Manufacturing Room		C			√			
Ampoule line	Filtration room		A			√			
Ampoule line	Filling room		A			√			
FFS	Manufacturing Room		C				√		
FFS	Hold area		B				√		
LVP	Manufacturing 01		C					√	
LVP	Filtration 01		C					√	
LVP	Filling Room 01		C					√	