STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbiological Monitoring of Drain Traps in Injection Block	**Effective Date:**
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1.0 OBJECTIVE:

To lay down a procedure for Microbiological Monitoring of Drain Traps.

2.0 SCOPE:

This SOP is applicable for Microbiological Monitoring of Drain Traps in Injection Block.

3.0 RESPONSIBILITY:

Officer / Executive – Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

µm	Micrometer
Cfu	Colony Forming Unit
CTA	Cetrimide Agar
Hrs.	Hours
IPA	Isopropyl Alcohol
LAF	Laminar Air Flow
LVP	Large Volume Parenteral
ml	Milliliter
No.	Number
QA	Quality Assurance
QC	Quality Control
SCA	Soyabean Casein Digest Agar
SOP	Standard Operating Procedure
XLA	Xylose lysine Deoxycholate Agar

6.0 PROCEDURE:

6.1 SAMPLING:

6.1.1 Take the required quantity of swab stick and aseptically add sterile 01 ml of 0.9% saline solution in each of individual swab tubes and wrap with sanitized aluminum foil and kept in SS container.

6.1.2 Transfer the SS container into respective area (Dry Powder Injection line/Three Piece line Block/Ampoule/FFS/ L Block etc.).

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6.1.3 Take swab stick and rub on the Drain Traps in unidirectional to cover the maximum surface of drain which is to be monitored. During swabbing, swab should not be dip in water.

6.1.4 Label the swab sample with drain point number, date, time and take the sample to Microbiology Laboratory for further analysis.

6.2 TEST FOR SPECIFIED MICROORGANISMS:

6.2.1 Pretreatment of Sample:

6.2.1.1 Vertex the swab tube and transfer the whole quantity of swab sample to 100 ml Soyabean Casein Digest Broth Medium.

6.2.1.2 Mix and incubate the SCM tube at 20-25°C for 2-5 hours.

6.2.1.3 After 2-5 hours, perform the analysis of Bile-Tolerant Gram-Negative Bacteria and incubate the medium at 30-35°C for 18-24 hours for further analysis (Pretreated Sample).

6.2.2 Test for Bile-Tolerant Gram-Negative Bacteria (Enterobacteria):

6.2.2.1 After 2-5 hours; transfer 10 ml of sample to 100 ml Enterobacteria Enrichment Broth Mossel and Incubate the medium at 30 to 35°C for 24 to 48 hrs.

6.2.2.2 After completion of Incubation, Subculture from Enterobacteria Enrichment Broth Mossel on Violet Red Bile Glucose Agar plate and incubate at 30 to 35°C for 18 to 24 hrs.

6.2.2.3 During observation, if none of the colonies confirm to the description given in Table-1, the sample meets the requirements for the absence of Enterobacteria.

TABLE-1

Specified Microorganism	Media Name	Positive Growth Characteristics	Gram Staining Characteristics
E. coli	MacConkey Agar	Pink/red coloured non-mucoid colonies.	Gram Negative Rod
Salmonella	Xylose lysine Deoxycholate Agar	Red colonies with or without black centers.	Gram Negative Rod
Pseudomonas aeruginosa	Cetrimide Agar	Greenish yellow colonies	Gram Negative Rod
Staphylococcus aureus	Mannitol Salt Agar	Yellow colonies surrounded by yellow zones.	Gram Positive Cocci
Bile Tolerant Gram Negative Enterobacteria	Violet Red Bile glucose Agar	Pink/red colonies	Gram Negative

6.2.2.4 If colonies show characteristic growth, carry out gram staining as per SOP, Titled **"Gram Staining" and perform identification through Vitek 2 Compact system**.

6.2.2.5 **Negative Control:** Incubate 100 ml SCM at 20-25°C for 2-5 hours. After incubation; transfer 10 ml SCM in to 100 ml of Enterobacteria Enrichment Broth-Mossel and rest quantity of SCM tube shall be Incubated at 30-35°C for 24-48 hours. After incubation; subculture from Enterobacteria Enrichment Broth-Mossel on Violet Red Bile Glucose Agar plate and Incubate at 30-35°C for 18 to 24 hrs.

6.2.2.6 Negative control should not show any growth.

6.2.3 Test for Escherichia coli:

6.2.3.1 Shake the Pretreated sample tube and transfer 1 ml of pretreated sample to 100 ml of MacConkey Broth and incubate at 42 to 44°C for 24 to 48 hrs.

6.2.3.2 Streak a portion from MacConkey broth on the surface of MacConkey Agar plate and incubate at 30 to 35°C for 18 to 72 hrs.

6.2.3.3 During Observation, if none of the colonies confirm to the description given in Table-1, the sample meets the requirements for the absence of the *E. coli*.

6.2.3.4 If colonies show characteristic growth, carry out gram staining as per SOP, Titled **"Gram Staining" and perform identification through Vitek-2 Compact system**.

6.2.3.5 **Negative Control:** Transfer 1 ml incubated SCM to 100 ml MacConkey broth and incubates at 42-44°C for 24-48 hours. After incubation; subculture on MacConkey agar plates and incubate at 30-35°C for 18-72 hours.

6.2.3.6 Negative control should not show any growth.

6.2.4 Test for Salmonella spp.:

6.2.4.1 Shake the pretreated sample tube and transfer 0.1 ml of pretreated sample to 10 ml of Rappaport Vassiliadis Salmonella Enrichment Broth and incubate at 30 to 35°C for 18 to 24 hours.

6.2.4.2 Streak a portion from the Rappaport Vassiliadis Salmonella Enrichment Broth on surface of Xylose Lysine Deoxycholate Agar plate and incubate 30 to 35°C for 18 to 48 hours.

6.2.4.3 During observation, if none of the colonies confirm to the description given in Table-1, the sample meets the requirements for the absence of the Salmonella spp.

6.2.4.4 If colonies show characteristic growth, carry out gram staining as per SOP, Titled **"Gram Staining" and perform identification through Vitek-2 Compact system**.

6.2.4.5 **Negative Control:** Transfer 0.1 ml incubated SCM to 10 ml RVS Broth and incubates at 30-35°C for 18-24 hrs. After incubation; subculture on XLD (Xylose lysine Deoxycholate Agar) plates and incubate at 30-35°C for 18-48 hrs.

6.2.4.6 Negative control should not show any growth.

6.2.5 Test for Pseudomonas aeruginosa:

6.2.5.1 Shake the tube and streak one loop full of pretreated sample on Cetrimide Agar plate and incubate 30 to 35°C for 18 to 72 hrs.

6.2.5.2 During observation, if none of the colonies confirm to the description given in Table-1, the sample meets the requirements for the absence of the Pseudomonas aeruginosa.

6.2.5.3 If colonies show characteristic growth, carry out gram staining as per SOP, Titled **"Gram Staining" and perform identification through Vitek-2 Compact system.**

6.2.5.4 Negative Control: Subculture from incubated SCM on Cetrimide Agar medium (CTA) plate and incubate at 30 to 35°C for 18 to 72 hrs.

6.2.5.5 Negative control should not show any growth.

6.2.6 Test for Staphylococcus aureus:

6.2.6.1 Shake the tube and streak one loop full of pretreated sample on Mannitol Salt Agar Medium plate and incubate at 30 to 35°C for 18 to 72 hrs.

6.2.6.2 During observation, if none of the colonies confirm to the description given in Table-1, the sample meets the requirements for the absence of the *Staphylococcus aureus*.

6.2.6.3 If colonies show characteristic growth, carry out gram staining as per SOP, Titled **"Gram Staining" and perform identification through Vitek-2 Compact system**.

6.2.6.4 Negative Control: Subculture from incubated SCM on Mannitol Salt Agar medium plate and incubate at 30 to 35ºC for 18 to 72 hrs.

6.2.6.5 Negative control should not show any growth.

6.2.7 Frequency of Monitoring: Monthly ±5 days.

6.2.8 Acceptance criteria:

Specified Microorganisms – *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus, Bile-Tolerant Gram-Negative Bacteria (Enterobacteria)* should be absent.

6.2.9 Drain point sampling shall be done as per Drain Point Sampling Schedule as shown in **Annexure-III**, Titled **"Drain Point Sampling Schedule"**.

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Sample Receipt and Analysis Record for Drain Swab Sample	

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Annexure – II	Drain Sample Analysis Report	
Annexure – III	Drain Point Sampling Schedule	

ENCLOSURES: SOP Training Record

8.0 DISTRIBUTION:

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

9.0 REFERENCES:

Drug and Cosmetic Act 1940, Schedule M

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
SAMPLE RECEIPT AND ANALYSIS RECORD FOR DRAIN SWAB SAMPLE

Date	Area	Location	Drain Point ID	Sampled By/Date	Analysis By/Date	Release By/Date	Checked By (Sign & Date)

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE – II
DRAIN SAMPLE ANALYSIS REPORT

Date of Testing:			**Area Name:**						
Tested By:			**Incubator ID.:**						
Test Name	**Media Reference**	**Incubation condition**	Drain Point ID →						**Observed by /date**
Enrichment for Test for Specified Microorganisms: Vertex the swab tube and transfer the whole quantity of swab sample to 100 ml SCM Media and incubate at 20-25°C for 2-5 hrs. **Test performed by/date:**									
PRIMARY TEST FOR SPECIFIED MICROORGANISM:									
After 2-5 hrs. at 20-25°C of enrich sample for Bile Tolerant Gram Negative Bacteria **Test performed by/date:**									
EEB/		**30-35°C for 24-48hrs.**	**Observation**						
After 18-24 hrs at 30-35°C of enrich sample for *Salmonella, E. coli, P. aeruginosa, S. aureus*									
SCM/		**30-35°C for 18-24hrs.**	**Observation**						
After 18-24 hrs at 30-35°C of enrich sample for *Salmonella, E. coli, P. aeruginosa, S. aureus* **Test performed by/date:**									
Salmonella	**RVS/**	**30-35°C for 18-24hrs.**	**Observation**						
E. coli	**MCB/**	**42-44°C for 24-48hrs.**	**Observation**						
P.aeruginosa	**CTA/**	**30-35°C for 18-72hrs.**	**Observation**						
S. aureus	**MSA/**	**30-35°C for 18-72hrs.**	**Observation**						
SECONDARY TEST FOR SPECIFIED MICROORGANISM:									
For Bile Tolerant Gram Negative Bacteria **Test performed by/date:**									
VBA/		**30-35°C for 18-24hrs.**	**Observation**						
For *E. coli* Tested by/date:									
E. coli	**MCA/**	**30-35°C for 18-72 hrs.**	**Observation**						
Confirmatory identification test:			**Observation**						
For *Salmonella* Tested by/date:									
Salmonella	**XLD/**	**30-35°C for 18-48hrs.**	**Observation**						
Confirmatory identification test:			**Observation**						
For *Pseudomonas aeruginosa* Tested by/date:									
Confirmatory identification test:			**Observation**						
For *Staphylococcus aureus* Tested by/date:									
Confirmatory identification test:			**Observation**						

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P→ Characteristic growth observed, N→ No Characteristic growth observed

CONCLUSIONS:

Test Organism	**Drain point →**						NA
	Bile Tolerant Gram Negative Bacteria						
	E. coli						
	Salmonella spp.						
	P. aeruginosa						
	S. aureus						

Microbiologist:
Date:

Reviewed By:
Date:

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ANNEXURE – III
DRAIN POINT SAMPLING SCHEDULE

Location	Sampling Point Location	Sampling Point ID No.	Frequency (Monthly±5 days)					
			05	10	15	20	25	30
Dry Powder	Production Janitor		√					
	Dress Wash		√					
	Unit Preparation Room		√					
	Equipment Washing		√					
	Vial Washing		√					
Three Piece	CIP/SIP room			√				
	MFG -1			√				
	MFG -2			√				
	Unit preparation			√				
	Garment washing			√				
	Equipment washing			√				
Ampoule Line	Janitor				√			
	Ampoules washing area				√			
	Autoclave area				√			
	Equipment washing area				√			
	Garment washing area				√			
	CIP/SIP room				√			
	Manufacturing area				√			
	Terminal sterilizer				√			
FFS Line	CIP & SIP					√		
	Washing & Sterilization					√		
	Mfg. Area					√		
	Janitor Room					√		
LVP Line	Filling room 01							√
	Filling room 02							√
	Filtration room 01							√
	Filtration room 02							√
	Manufacturing area 01							√
	Manufacturing area 02							√
	Garment washing area							√
	Equipment washing area							√
	Janitor Room							√
	Disinfectant preparation room							√
	Unit Preparation Room							√