



## STANDARD OPERATING PROCEDURE

<b>Department:</b> Microbiology	<b>SOP No.:</b>
<b>Title:</b> Identification of In-House Isolated Microorganisms & Standard Microbial Culture	<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 the procedure for Identification of In-House Isolated Microorganisms & Standard Microbial culture.

### 2.0 SCOPE:

This SOP is applicable for Identification of In-House Isolated Microorganisms & Standard Microbial culture in Microbiology department.

### 3.0 RESPONSIBILITY:

Operating Person – QC Microbiology

### 4.0 ACCOUNTABILITY:

Head – QC

### 5.0 ABBREVIATIONS:

%	Percentage
A.R No.	Analytical Report Number
EM	Environment Isolate
ID	Identification
IHI	In-House Isolate
Ltd.	Limited
No.	Number
°C	Degree Celsius
Pvt.	Private
QA	Quality Assurance
QC	Quality Control
SCA	Soyabean Casein Digest Agar
SDA	Sabouraud chloramphenicol Agar
SOP	Standard Operating Procedure

### 6.0 PROCEDURE:

#### 6.1 Methodology for Isolation and Identification:

**6.1.1** In-house isolate library shall be maintained for the reference and new microbes found during environmental monitoring of Production, Microbiology, Water analysis plates, Media Fill sample and Sterility test etc shall be added in the library.



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- 6.1.2** Each colony, observed under grade A and in water for injection/ Pure Steam, Media fill containers & Sterility samples shall be identified. Every different, colony observed in grade B, C, D (of core area where product is being exposed or surroundings of product exposure area), Purified water samples shall be identified and same shall be acknowledged by quality assurance in **Annexure I**.
- 6.1.3** Streak from suspicious bacterial growth on pre-incubated SCA plate and incubate at 30-35°C for 24-48 hrs.
- 6.1.4** Streak from suspicious fungal growth on pre-incubated SDA plate and incubate at 20-25°C for 3-5 days.
- 6.1.5** When a proper growth is observed on the defined media plate; store the plate for further identification purpose. This plate shall be considered as mother isolate culture.
- 6.1.6** If proper growth is not observed then repeat the activity again to get isolated colonies.
- 6.1.7** Take the pre incubated SCA media slant and streak the loop full isolated colony from the incubated SCA media Petri dish for preservation.
- 6.1.8** Using following table observe the colony morphology and record into the **Annexure-I**.

Characteristics	Observations
Shape	Circular, irregular
Surface	Smooth, wavy, rough, granular, glistening etc.
Elevations	Low convex, concave, Flat
Edge	Even, curled, irregular
Colour	Colourless, pink, black, red, bluish green etc.
Structure	Opaque, translucent or transparent

#### 6.2 Assigning of In-House Isolate ID:

- 6.2.1** An individual number shall be assigned to each isolates as per the sequence of XX/YY/ZZ/NNN  
Where XX = Source of isolation like EM- Area Environment Sample, ML= MLT Sample, ST= Sterility Sample, LIA= Liquid Injection Area , HGMA= Hard Gelatin Mfg. Area, SGMA=Soft gelatin mfg. area, TBMA= Tablet Mfg. Area, PPA= Primary Packaging Area, RMSDA=Raw Material Sampling and Dispensing Area , OGFA= Ointment /Gel/Cream), Filling and applicator de – cartoning Are, MF= Media fill Container isolate, WS= Water Sample, FP= Finished Product, RM= Raw Material, DPI= Dry Powder Injection, TP= Three Piece.

YY= Last two digit of the Year

ZZ = Location or Sample ID/Batch No. /AR No.

NNN = Serial No. from 001-999



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For example- for the first isolate of the year from the Environment monitoring of Soft Gelatin Manufacturing Area settle plate's location SP-010 shall be assigned the number like EM-SGMA/17/SP-010/001.

#### 6.3 Identification of In House Isolate & Standard Microbial Culture:

- 6.3.1 Streak a single colony on a SCA media plate for Bacterial Culture and SDA plate for Fungal Culture.
- 6.3.2 Incubate the Petri plate in inverted position at 30 - 35°C for 24 to 48 hours for Bacteria and 20 - 25°C for 48 to 72 hours for fungus.
- 6.3.3 For the species level identification sample shall be send to outside laboratory and maintained the record of sample as per **Annexure- IV Titled "Outside Laboratory Analysis Record"**.
- 6.3.4 After identification preserve the microbial culture and store at 2-8 °C.
- 6.3.5 After identification; comment and recommendation shall be made by QA for further course of action.
- 6.3.6 Maintain the required Isolates for routine use as Standard Culture as per SOP titled **"Maintenance, Handling and Sub culturing of Microorganisms"** SOP.
- 6.3.7 Record the details of Environmental/Water isolates (In-house culture) in **Annexure-II**, Titled **"Identification of Standard Microbial Culture"**.
- 6.3.8 Identification details of In-house Isolates shall be recorded in the **Annexure -III**; Titled **"Index of In House Culture Library"**.
- 6.3.9 Representative/Predominant/Most frequent/Repeated(More than 3 times) in-house isolate from each group (Gram positive, gram negative, spore former, yeast and mold) shall be considered for disinfectant efficacy test.

#### 7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure-I	Identification of Environmental/Water Isolates	
Annexure-II	Identification of Standard Microbial Cultures	
Annexure-III	Index of In House Culture Library	
Annexure-IV	Outside Laboratory Analysis Record	

**ENCLOSURES:** SOP Training Record.



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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**

<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**  
**IDENTIFICATION OF ENVIRONMENTAL / WATER ISOLATE**

<b>Name of Area</b> :	<b>In-house Flora No.</b> :
<b>Room No.</b> :	<b>Sampling point/Plate Location</b> :
<b>Date of Monitoring</b> :	<b>Date of Observation</b> :
<b>Source of Isolation</b> :	

**Test Details:**

**Colony Morphology Characteristics**

<b>Morphological Characters</b>	<b>Observed Characteristics</b>	<b>Acknowledged by QA</b>
Shape		
Color (on SCDA/SDA)		
Surface		
Elevation		
Edge		
Structure		

**Microbiologist**  
**Date:**

**Reviewed by**  
**Date:**

**Identified Microorganism:**  
**Comment and Recommendation:**

\_\_\_\_\_  
\_\_\_\_\_

**Commented by**  
**QA**  
**(Sign & Date)**



**PHARMA DEVILS**  
MICROBIOLOGY DEPARTMENT

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**ANNEXURE – II**

**PURE & CURE HEALTHCARE PVT. LTD.**  
HARIDWAR  
MICROBIOLOGY

**IDENTIFICATION OF STANDARD MICROBIAL CULTURES**

<b>Name of Culture</b> :	<b>ATCC/NCIM No. /</b> :
<b>Passage No.</b> :	<b>Date of Sub -Culturing</b> :
<b>Date of Identification</b> :	

**Test Details:**

**1. Colony Morphology Characteristics:**

<b>Morphological Characters</b>	<b>Observed Characteristics</b>	<b>Remarks (If Any)</b>
Shape		
Color (on SCDA/SDA)		
Surface		
Elevation		
Edge		
Structure		

**Microbiologist**  
**Date**

**Reviewed by**  
**Date**



