PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbial Identification Program	**Effective Date:**
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1.0 OBJECTIVE

To lay down a procedure for Isolation, Identification and handling of the Isolates.

2.0 SCOPE

This procedure is applicable for isolation, identification and handling of the Isolates obtained from Microbial Limit Testing of In-process bio-burden samples and finished products, Microbial Monitoring of manufacturing and testing areas and Water testing at all the Formulation Units.

3.0 RESPONSIBILITY

Microbiologist is responsible for adhering to the procedure.
Head-Microbiology is responsible for implementation and technical compliance.
Head-Quality Control or his designee is responsible for overall compliance.

4.0 DEFINITION

Microbial Identification Program: Microbial Identification Program (MIP) is defined for the purpose of this SOP as an overall approach towards the isolation, identification and handling of the isolates during various stages of manufacturing.

Specified Microorganisms: A minimum list of organisms that shall be absent in the pharmaceutical products as per the Unites States Pharmacopeia (USP). They include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* species, *Candida albicans*, *Clostridium* species and the organisms belonging to the Enterobacteriaceae group.

5.0 PROCEDURE

5.1 Isolates from Water

5.1.1 Isolate all the morphologically different colonies and identify them up to the genus preferably up to species level. Create a database for the cultures obtained from the water.

5.1.2 Update the data base as and when a new isolate is identified.

5.1.3 Identify the predominant flora when the total microbial counts exceed alert and action limits in water. The characterization of the predominant flora shall be useful in investigating the excursions.

5.1.4 Due to the physiological stress on the isolates, the organisms inherently may show up slightly different morphology. In this context, if an isolate is found morphologically different, the isolate shall be picked and identified. In case this organism is already identified (repeat isolate), then the database shall be updated accordingly, but the culture need not be preserved.

5.1.5 Verify the growth promotion ability of the media using the Isolate initially.

5.1.6 The identified isolates shall be preserved for a period of one year using the seed lot technique. Prepare the required number of seed lots as per the procedure detailed in the current version of SOP.

5.2 Isolates from the In-Process Bioburden Samples

5.2.1 Isolate and identify the colonies if the total microbial counts exceed the action limits (Out of Specification results) in case of the in-process samples.

5.2.2 Evaluate the isolates, summarize the identification profile and the recommendations to the Quality Assurance through an addendum to the OOS document.

5.3 Isolates from the non-sterile products (Oral Solid Dosage Forms)

5.3.1 Isolate and identify the colonies if the total microbial counts exceed the action limits (Out of Specification results) in case of the finished products.

5.3.2 Evaluate the isolates, summarize the identification profile and the recommendations to the Quality Assurance through an addendum to the OOS document.

5.4 Isolates from the Manufacturing and Testing Environment

5.4.1 Isolate all the morphologically different colonies and identify them up to the genus preferably up to species level. Create a database for the cultures obtained from the manufacturing and testing environment.

5.4.2 Update the database as and when a new isolate is identified.

5.4.3 Identify the predominant flora when the total microbial counts exceed alert and action limits in the manufacturing environment. The characterization of the predominant flora shall be useful in investigating the excursions.

5.4.4 Due to the physiological stress on the isolates, the organisms inherently may show up slightly different morphology. In this context, if an isolate is found morphologically different, the isolate shall be picked and identified. In case this organism is already identified (repeat isolate), then the database shall be updated accordingly, but the culture need not be preserved.

5.4.5 In case any of the isolates is identified as Specified Microorganisms, notify the Quality Assurance and Production department through a Quality-impacting incident. Proceed as per the procedure detailed in the current version for investigation and disposal of the incident.

5.4.6 Verify the growth promotion ability of the media using the Isolate initially. Further, use any one of the isolates for growth promotion testing of media on routine basis.

5.4.7 Use the isolates to challenge the efficacy of the existing set of the disinfectants.

5.4.8 In case any of the isolates is found resistant to the existing set of the disinfectants, notify the Quality Assurance and Production departments through an incident. Proceed as per the procedure detailed in the current version for investigation and disposal of the incident.

5.4.9 The identified isolates shall be preserved for a period of one year using the seed lot technique. In case the new isolates are not obtained, the isolates shall be stored till the availability of the new isolates. Prepare the required number of seed lots as per the procedure detailed in the current version of SOP.

5.4.10 In case of fungi obtained from monitoring, these isolates may not be identified always. However, the fungal isolates shall be used to verify the growth promotion ability of the media used for monitoring and to verify the efficacy of the existing set of disinfectants against these isolates.

5.5 Isolation procedure

5.5.1 Pick the colony to be isolated and streak with the help of loop aseptically on to the pre-incubated Soya bean casein digest agar plate.

5.5.2 Incubate the plate at 30-35°C for 18-72 hours. In case of fungal isolates, incubate the plate for 5 days.

5.5.3 Sub-culture the colony by streaking second time so as to obtain a single isolated pure colony with its original phenotypic expression. Inoculate the culture in to a slant also for further identification and seed lots preparation. Incubate the plate at 30-35°C for 18-72 hours. In case of fungal isolates, incubate the plate for 5 days.

5.5.4 Note down the colony characters of the isolate after second sub-culturing as per the specimen format given in Annexure.

5.6 Identification of Bacterial Isolates

5.6.1 Conduct the orientation checks like Gram nature and the shape and arrangement of the cells on the isolate. Perform the Gram staining as below.

5.6.2 Prepare a thin smear of suspension on a clear, dry glass slide.

5.6.3 Allow it to dry and fix by gentle heat.

5.6.4 Flood with Gram's Crystal Violet for 1 minute.

5.6.5 Wash with tap water.

5.6.6 Flood the smear with Gram's Iodine and allow it to remain for 1 minute.

5.6.7 Decolourize with Gram's Decolourizer until the blue dye no longer flows from the smear.

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5.6.8 Wash with tap water.
5.6.9 Counter stain with 0.5% w/v Safranin. Allow it to remain for 1 minute.
5.6.10 Wash with tap water.
5.6.11 Allow the slide to air dry or blot dry and examine under oil immersion objective.
5.6.12 Gram positive organisms should stain bluish purple colour.
5.6.13 Gram negative organisms should stain pinkish red colour.
5.6.14 Run the positive control during the Gram staining. For the positive control, use a reference culture of known Gram reaction.
5.6.16 Note down the observations as per the specimen format given in the Annexure.
5.6.17 Identify the isolate using a suitable identification method.
5.6.18 Preserve the culture on a slant or plate till the identification is obtained.
5.6.19 Record the results of identification in Annexure.

5.7 Identification of Fungal Isolates

5.7.1 Note down colony characteristics of fungal colonies developed on the media plates.
5.7.2 Perform staining of the colonies using Lactophenol Cotton Blue as below.
5.7.3 Place a drop of Lactophenol Cotton Blue reagent on a clean and dry slide.
5.7.4 By using an inoculating wire, carefully tease the fungal culture in to a thin preparation.
5.7.5 Place a cover slip on the preparation and wait for 5 minutes.
5.7.6 Observe under microscope with low power for hyphal arrangement and morphology.
5.7.7 Based on the Microscopy, identify the organism up to genus level by comparing it with characteristics given in the literature.

5.8 Extent of Characterization of Isolates

5.8.1 In all the above cases, the microbial isolates shall be identified based on the phenotypic characteristics as listed below.
5.8.1.1 Culture- Colony morphology, colour, shape and size, pigment production
5.8.1.2 Morphology- Cellular morphology, Gram reaction.
5.8.1.3 Biochemical- Carbon utilization, carbohydrate oxidation or fermentation, enzyme patterns.
5.8.1.4 For the biochemical characteristics, the isolates may be identified using conventional tests or with the readily available commercial identification kits.
5.8.2 In case of any of the below cases, the isolates shall be identified by genotypic methods based on the investigation need.
5.8.2.1 Media fill failure, Sterility test failure
5.8.2.2 Any market complaint related to microbial quality of the product
5.8.2.3 Significant adverse trends in environmental and water monitoring.
5.8.2.4 To support any investigation related to the product quality.
5.8.3 For the genotypic identification, an outside contract laboratory shall be used for these services. Send the cultures to the outside contract laboratory as per the procedure described in the current version of SOP.

5.9 Allotment of Number to Isolates

5.9.1 Allot the number to the environmental isolates as below.
5.9.2 First 3 letters “ENI” denote Environmental isolate.
5.9.3 Next alphabet denotes the year.
5.9.4 Next 3 numeric characters indicate serial number of the isolate starting from 001 and increasing by 1 for next isolate.

5.10 Maintenance of Isolates

5.10.1 Follow the procedure detailed under ‘Seed lot preparation’ in the current version for preparation and maintenance of the seed lots.
5.10.2 Revive seed lots of environmental isolates as and when required.

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5.10.3 Follow the procedure detailed in the current version for culture suspension preparation of the isolates.
5.10.4 Prepare Culture suspension of isolates as and when required and use within its validity.

6.0 REFERENCE(s)

SOP No. (Current Version)	Title
	Procurement, Identification, Revival, Preparation, Maintenance of Reference Cultures.
	Reporting, Investigation and Disposition of Incidents

7.0 ABBREVIATION(s)

Abbreviation	Full Description
SOP	Standard Operating Procedure
QC	Quality Control
No.	Number
USP	United States Pharmacopeia
°C	Degree Centigrade

8.0 FLOW CHART(s)

Not Applicable.

9.0 ANNEXURE(s)

Annexure No.	Details/Title of Annexure	Format No. (Current Version)
	Colony Characteristics of Isolates	
	Identification Summary Report	

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ANNEXURE I

Colony characteristics of Isolates

Date of Isolation:		
Source of Isolate:		
Medium Used for Isolation:		**Isolate ID.:**

Colony Characteristics	**Observations**
Colour	
Size	
Shape	
Margin	
Consistency	
Opacity	
Elevation	
Arrangement of cells	
Gram Nature	
Positive Control	Satisfactory/ Not Satisfactory

Observed By:
Sign& Date:

Checked By:
Sign& Date:

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ANNEXURE II

Identification Summary Report

Isolate Id.:	
Identification System Used:	

Summary:

Summarized By:
Sign & Date:

Checked By:
Sign & Date: