PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Identification & Characterization of Environmental and Water Isolates	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1. **Purpose:** The purpose of this SOP is to describe for identification and characterization of environmental and water isolates.

2. **Scope:** This SOP is applicable for identification and characterization of environmental and water isolates in microbiology section of quality control department.

3. **References, Attachments & Annexures:**
   - 3.1. **References:**
     - 3.1.1. In-house
   - 3.2. **Attachments:**
     - 3.2.1. Attachment-1:Identification & characterization of environmental and water isolates worksheet
   - 3.3. **Annexures:** None

4. **Responsibilities:**
   - 4.1. **Microbiologist:**
     - 4.1.1. To perform the activity as per SOP.
     - 4.1.2. To maintain all the records as per SOP.
   - 4.2. **QC Head or designee:**
     - 4.2.1. To check the SOP.
     - 4.2.2. To give training to all concerned persons before implementation of SOP.
   - 4.3. **Quality Assurance:**
     - 4.3.1. To check the SOP.
     - 4.3.2. To ensure the implementation of system as per SOP.
   - 4.4. **Regulatory Affairs, Quality Head, Plant Head:**
     - 4.4.1. To approve the SOP.

5. **Distributions:**
   - 5.1. Quality Control
   - 5.2. Microbiology
   - 5.3. Quality Assurance

6. **Definitions of terms & Abbreviations:**
   - 6.1. **Definitions of terms:** None
   - 6.2. **Abbreviations:**

SOP	**:** Standard Operating Procedure
No.	**:** Number
QA	**:** Quality Assurance
QC	**:** Quality Control
NA	**:** Not Applicable
µl	**:** Micro liter
°C	**:** Degree Celsius
%	**:** Percent

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ml : Milliliter
SCDM : Soybean casein digest medium
SCDA : Soybean casein digest Agar

7. Procedure:

7.1. Colonies Selection:

7.1.1. Collect the exposed plates or water sample plates upon completion of specified incubation period.
7.1.2. Observe the colony forming units (CFU) on daily basis till the specified incubation period.
7.1.3. Select the colonies on the basis of morphological characteristics.
7.1.4. Select single well-isolated colony and inoculate into SCDM medium and incubate at 30-35°C for 18-24 hrs.
7.1.5. Streak a loop full of above suspension on SCDA & incubate at 30-35°C for 18-24 hrs. to get pure and fresh culture.
7.1.6. Repeat this activity for other selected bacterial colonies & perform Gram's staining.

7.2. Gram's character:

7.2.1. Prepare a smear on clean and grease free glass slide. Air dry and heat fix it.
7.2.2. Apply crystal violet for 1 min. Wash the slide with water.
7.2.3. Apply gram's iodine for 1 min. Wash the slide with water.
7.2.4. Apply ethanol/gram's decolorizer for 10-15 sec. Wash the slide with water.
7.2.5. Apply safranin/basal fuschin for 1 min. Wash the slide with gentle flow of water.
7.2.6. Air dry the slide and observe under microscope with oil immersion lens.
7.2.7. **Interpretation of results:**

Gram +ve cocci/Rods (Violet color)
Gram -ve cocci/Rods (Pink color)

7.3. For Gram +ve isolates (cocci):

7.3.1. Perform Catalase test as mentioned below.

7.3.1.1. **Catalase Test:**
The test demonstrates the presence of catalase, an enzyme that catalyses the release of O_2 from H_2O_2.
7.3.1.2. **Reagent:** 3.0% H_2O_2
7.3.1.3. **Method (Slide test) :**
Put a drop of 3.0% H_2O_2 solution on a clean glass slide. Pickup small amount of culture to be tested from the colony with sterile thin glass rod or sealed capillary tube.
7.3.1.4. **Interpretation of results:**
The production of gas bubbles indicates a positive reaction. A false positive reaction may be obtained if an iron wire loop is used.

7.3.2. If it shows Catalse test positive, then use identification kit for further identification.

7.4. For gram +ve isolates (Rods):

7.4.1. If it shows Catalse test positive, then use kit for further identification.
7.4.2. If it shows Catalse test negative, then use kit for further identification.

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7.5. **For gram -ve isolates (Cocci):**

7.5.1. Perform Oxidase test as mentioned below.

7.5.1.1. **Oxidase Test:**

The test demonstrates the presence of oxidase *Pseudomonas, which give positive reaction, and for* excluding the Enterobacteriaceae, all species of which give negative reaction.

7.5.1.2. **Reagent:** Oxidase disc or 1% Tetramethyl paraphenylene diamine dihydrochloride, stored in amber bottle.

7.5.1.3. **Method:**

Either take oxidase disc or place 2-3 drops of freshly prepared 1% Tetramethyl paraphenylene diamine dihydrochloride on filter paper. Pick up the colony to be tested with clean sterile glass rod and smear on the oxidase disc or filter paper.

7.5.1.4. **Interpretation of results:**

A positive reaction is indicated by change in color within 5-10 seconds as appearance of deep purple blue. A delayed positive reaction appears in 10-60 seconds, while a change in color later than 60 seconds or no color change at all is considered negative reaction.

7.6. **For gram -ve isolates (Rods):**

7.6.1. If gram negative rods showing Oxidase test positive reaction, use identification kit for further identification.

7.6.2. If gram negative rods showing Oxidase test negative reaction, use identification kit for further identification.

7.7. For Yeast, use identification kit.

7.8. Incubate this at 30-35°C for 24-48 hrs. for both bacterial and fungal (*Candida albicans*) isolates to be identified.

7.9. **Acceptance criteria:**

Environmental and water isolates should be non-pathogenic in nature.

7.10. **Frequency:**

7.10.1. When new microbial flora instead of pre isolated & pre identified flora is observed

7.10.2. If the action limits of source are crossed.

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Attachment-1

Identification & characterization of environmental and water isolates work sheet

Date of Testing:		**Source:** Environmental/Water	
Date of Result:		**Incubation Temperature:**	
S.No.	**Test Performed**	**Observation**	**Remarks**

Isolated Organism:

Results: The sample **Complies/does not Comply** the acceptance criteria.

Analyzed by :	**Checked by :**
Date :	**Date :**

8. History:

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