STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure For Identification Of Microbial Culture / Microbial Isolates	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down a procedure for identification of microbial culture/microbial isolates.

2.0 SCOPE:

This SOP is applicable to methods of isolation and identification of microbial cultures in Microbiology laboratory.

3.0 RESPONSIBILITY:

Microbiologist – Quality Control

Head – Quality Control

4.0 PROCEDURE :

4.1 General identification:

General identification of Micro-organisms is divided in to 2 steps.

4.1.1 Presumptive identification:

Identification is termed presumptive when it is deduced from the following criteria.

Colonial appearance on a general media (microscopic morphology).

Appearance of Gram-stained cultures by light microscopy.

Colonial appearance on selective diagnostic media.(microscopic morphology)

Other test as required (e.g. oxidase test, coagulase test)

Presumptive identification is limited to genus level.

4.1.2 Confirmed identification:

Identification is termed confirmed when it is obtained through detailed knowledge of the micro-organisms biochemical characteristics. These data can be obtained from the following approach.

From “traditional” biochemical or physiological tests.

In some instances, it is acceptable to perform some biochemical test or additional staining tests to exclude microorganisms. Examples include performing a coagulase test to exclude. Staphylococcus aureus and performing a spore stain to exclude Bacillus sp. These

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Issue Date:	**Page No.:**

exceptions, while not confirmed identification, can be reported as Coagulase negative Gram-positive cocci and Non spore forming Gram-positive rods.

Confirmed identification is also carried out using ready-made rapid identification kits.

Generally confirmed identification is done to species level (except moulds, in which case *genus* level is acceptable).

4.2 Source of Microbial isolates for which identification is carried out:

4.2.1 Environment Isolates.

Environmental monitoring of manufacturing facilities is the most critical monitoring done in microbiology. All micro-organisms isolated from this environment have a potential to contaminate product. All microbiologically different micro-organisms isolated from production areas presumptively identified.

4.2.2 Confirmed identification is usually carried out in case the same type of contamination is observed on a regular basis.

4.3 APPARATUS:

Nicrome wire loop

Glass slides

Microscope

Refrigerator

Incubators

LAF bench

4.4 Reagents / Kits:

Microbial identification kits for Micro organisms

4.5 Isolation And Identification :

4.5.1 Observe the colonies present on non-selective/selective media plates. Note down the colony characteristics on the assigned record. If two or more colonies are merged, then isolate the organism on fresh media plates by carrying out the ‘T’ method or four-quadrant method of

isolation.

4.5.2 With the help of a Nicrome loop, pick up the isolated colony and prepare a thin smear on clean glass slide. Fix the smear and stain by the Gram's staining method as per the procedures given in Biochemical tests.

4.5.3 Examine the stained smear under the microscope and note down the Gram's nature and the morphology of the organisms in the assigned record sheet.

4.5.4 Depending upon the Gram character and morphology of the organism, subculture on selective media, for further identification. Note down the nature of the colonies observed. (Alternatively, depending upon the gram character and morphology, use the appropriate identification system for identification of gram negative bacilli).

4.5.5 For detailed identification subculture the growth from the selective media plates to non-selective agar such as Nutrient Agar/Soyabean Casein Digest agar. Carry out biochemical tests, such as,

a) Sugar fermentation test
b) Indole production test
c) Oxidase test
d) Coagulase test

4.5.6 Refer to the procedure for common biochemical tests.

4.5.7 Record the results and interpret the identity of the organism by referring to the charts provided by manufacturer and data application in steps 4.7.

4.6 Use of Himedia Bio Chemical identification test Kit:

4.6.1 Under aseptic conditions remove the Himedia Bio Chemical identification test strip from its packet and place it in the tray.

4.6.2 With the help of a sterile Nicrome loop inoculate the isolate into 5 ml sterile purified water and shake to get a homogeneous suspension.

4.6.3 With the aid of a sterile pipette, fill both the tube and couple of tests CIP, VP, GEL, with the bacterial suspension. Fill only the tubes and (not the couples) of the other tests.

4.6.4 Create anaerobiosis in the tests ADH, LDC, ODC, URE, and H_2S by

Overlaying with sterile mineral oil.

4.6.5 Close the incubation tray and incubate at 35°C to 37°C for 18 to 24 hours.

4.6.6 Record the results of spontaneous biochemical tests by referring to the table provided with the identification table on the assigned record sheet.

4.6.7 If glucose is positive and/or 3 or more tests are positive reveal the tests which require addition of reagents, Refer the interpretation table supplied with Himedia Bio Chemical identification kit. Record the results on the record sheet

4.6.8 If glucose is negative and the number of positive tests is less than or equal to two, do not add the reagents. Instead reincubate for 24 hours and then add reagents. Record the results on the record sheet. In addition carry out the following supplementary test:

i) Confirm metabolism of glucose using of medium.

ii) Check growth on MacConkey agar plate

iii) Check for mobility

4.6.9 Record the results of the supplementary tests on the record sheet

4.6.10 Using the analytical profile index identification table (supplied with the kit), compare the results recorded on the record sheet with those given in the table.

4.6.11 Record the organism identified on the record sheet.

4.7 Applications of data from identification of microorganisms:

4.7.1 Determination of Most likely Source

The primary purpose of identification of micro-organisms is to determine the most likely source in order to be able to take appropriate preventive action to avoid or minimize future re-occurrence.

4.7.2 The following considerations should be borne in mind:

4.7.3 Micro-organisms of Environmental Origin

a) Bacillus Spp.

b) Moulds and yeast.

c) Micrococcus Spp.

4.7.4 Micro-organisms of Water Origin

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Procedure For Identification Of Microbial Culture / Microbial Isolates	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

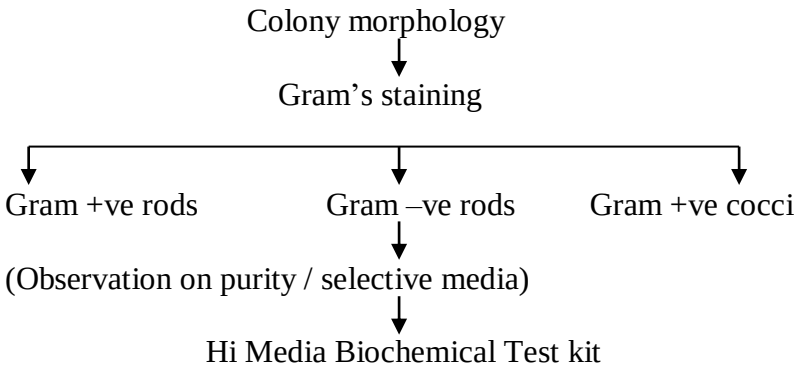
a) Pseudomonas Spp.
b) Enterobacteriace
c) Acinetobacter Spp.
d) Corynebacterium Spp.

4.7.5 Micro-organisms of Human Origin

a) Staphylococcus Spp.
b) Streptococcus Spp.
c) Micrococcus Spp.
d) Corynebacterium Spp.
e) Propionibacterium Spp.
f) Yeast and moulds
g) Enterobacteriace.

4.7.6 The typical micro flora of a particular facility must be defined by assembling a database of the species isolated and confirmed over at least one year's operation. The period of one year is to allow for any seasonal variations. The database should be reviewed annually and adjusted where necessary.

IDENTIFICATION OF MICROORGANISMS FLOW CHART



All morphologically different colonies (based on macro and microscopic examination and Gram stain) are presumptively identified.

STANDARD OPERATING PROCEDURE	
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Title: Procedure For Identification Of Microbial Culture / Microbial Isolates	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

5.0 ANNEXURE (S) :

Nil

6.0 REFERENCE (S):

SOP: Preparation, Approval, Distribution control, revision and Destruction of Standard operating Procedure (SOP).

7.0 ABBREVIATION (S) /DEFINITION (S) :

Nil

REVISION CARD

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
01	00	----	---	New SOP	-----