PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Maintenance of Cultures	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 **OBJECTIVE**

To lay down a procedure for maintaining of Bacterial cultures.

2.0 **RESPONSIBILTY**

Microbiologist / Executive.

3.0 **ACCOUNTABILITY**

Head - Quality Control

3.0 **PROCEDURE**

4.1 Bacterial cultures are very sensitive and if not sub-cultured they can change their morphological & biochemical characteristics. Sub-culturing or periodic transfer is a very delicate technique which has to be carried out by avoiding chances of contamination.

4.3 Prepare the media as mentioned in SOP**.**

4.2 Prepare slants in clean 18 mm diameter rimless test tubes and pre-incubate for 48 hrs at 30-35°C to check any contamination.

4.3 Boil the media till get completely dissolve. Cool to about 42 - 45°C and adjust the required pH. Fill each test tube with about 9 to 10ml of the medium & plug the tubes with non absorbent cotton & sterilize by autoclaving.

4.4 After autoclaving, prepare the solutions by slanting the test tubes to desirable angle.

4.5 After the medium get solidified, keep the prepared slants into the incubators (37°C for 48 hrs.) for pre incubation.

4.8 Prior to sub culturing incubation is necessary because to check for the contamination of slants occurs in process.

4.9 After incubation, clean the exterior surface of test tube with IPA 70% solution.

4.10 Mark each test tube with the name of organism & date of subculture.

4.11 At every passage from mother culture check the Gram character and morphology of the culture. In any case the passage from mother culture shall not exceed 5.

4.12 Subculture should be done in the Laminar flow clean air station & in between the two gas burners to avoid the chances of cross contamination. Take a loop full of culture from previous stock cultures or mother culture which ever is applicable, and inoculate in freshly prepared sterile slant

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4.13 Inoculate each organism in two slants (prepare two slants of each organism). After inoculating, incubate all the tubes at required temperature (i.e. 30-35°C for bacteria and 20-25°C for fungus).

4.13 After incubation check the cultures visibly for any contamination.

4.15 Mark the Newly prepared lot of cultures tubes as WORKING CULTURES and one as STOCK CULTURE.

4.16 After observing the growth, keep the newly prepared cultured tubes in clean double plain polythene bags and preserved the tubes in the refrigerator at 2 – 8 °C.

4.17 Prepare new slants from the STOCK CULTURE of previous month (at each month, as a new passage) and should proceed from more than four passage.

4.18 After completion of two passages from a single STOCK CULTURE, take the passage from MOTHER CULTURE slant, proceed for the next passage, by preparing STOCK CULTURE and WORKING CULTURE.

4.19 Enter the date of sub-culturing and passage number in the format as mentioned in Annexure I for sub-culturing of micro-organism.

4.20 After every one year procure the new culture from any national recognized institution with certificate for authenticity of the cultures.

4.21 The working cultures shall be used for preparing suspensions for positive control; for Growth Promotion test etc.

4.22 The STOCK CULTURE shall be used only for sub culturing.

4.23 After one month when the new cultures are ready for use destroy the old cultures.

4.0 **DISPOSAL OF CULTURES :**

5.1 Aseptically pipette 10ml of disinfectant solution in the culture tubes.

5.2 Sterilise at 121 °C for 20 mins.

5.3 After sterilization collect the media in the double polybag, pour sufficient disinfectant solution and tie it properly. Dispose this bag in Incinerator.

6.0 **MEDIA USED FOR PREPARING SLANTS**:

1. For Bacteria : Soyabean Casein digest Agar.
2. For Fungi : Sabaroud Dextrose Agar.

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7.0 ABBREVIATIONS: NIL

8.0 REFERENCE

USP and Recommendations by culture supplier.

9.0 ANNEXURE : Annexure I

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ANNEXURE I

CULTURE MAINTENANCE CARD

Name of the organism: **Strain No.:**

Maintenance Medium: **Incubation conditions:** ^{0}C for hours

Source:

Interval of transfer: One month. **Date of Receipt:**

Temp. of Preservation : 2 - 8 °C

Withdrawal From Mother Slant	Date Of Transfer	Gram Character	Shape	Contamination Checks		Next due on	Sign	Destroyed On	Sign
				Slant Observation	Microscopic				