STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Culture Media	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE:

To lay down a procedure for Preparation of Culture Media.

2.0 SCOPE:

This SOP is applicable for Preparation of Culture Media at Microbiology Section in Quality Control area.

3.0 RESPONSIBILITY:

Officer / Executive – Microbiologist

4.0 ACCOUNTABILITY:

Head – QC

5.0 PROCEDURE:

5.1 Preparation of Culture Media:

5.1.1 Check and ensure that the Glassware is clean.

5.1.2 Before using the new containers / Bottles of media, check the GPT status of that media. If GPT not performed in such condition perform GPT along with test and release sample after completion of GPT. If GPT not passes the media invalidate analysis, Repeat the test with new lot of approved media

5.1.3 Weigh the required quantity of culture media on the butter paper/dry pan as per Manufacturer instruction.

5.1.4 Transfer appropriately weighed quantity of the culture media in to the cleaned conical flask or bottle containing 50% purified water of the total volume of media. Use the glassware in such a way that final volume of culture media to be prepared shall not exceed to the 70% of the volume capacity of glassware. For example if 350 ml of medium to be prepared then conical flask of 500ml will be required.

5.1.5 Gradually add the remaining quantity of the purified water.

5.1.6 Heat to dissolve the media if required, during heating shake the media regularly to avoid the overheating.

5.1.7 After volume making take about 10 to 20 ml of the reconstituted culture medium for pH measurement before sterilization.

5.1.8 Check the pH of Agar Culture media by using glass electrode or flat Probe pH meter at 25°C.

5.1.9 If the pH of medium goes out of limit as defined on media container, the whole media lot should be discarded as per the respective SOP.

5.1.10 After checking the pH dispense the media as per requirement in flasks/Bottles & Screw cap test tubes.

5.1.11 If heat labile supplements to be added into the culture medium then the temperature of media should be between 40 - 45°C or as per the direction of supplier /manufacturer, after adding the supplement adequate mixing shall be required to dissolve the medium into the culture medium.

5.1.12 Load the flasks/Bottles & Screw cap test tubes of media as per previously validated loading pattern in the chamber of autoclave.

5.1.13 Allocate the autoclave media reference No. to the each lot of culture medium as below of each and every autoclave media load with permanent glass marker.

SM/DD/MM/XX

Where,

SM = Sterilized media
DD = Date
MM = Month
XX = Serial no. how many time prepared in a Day
/ = Separator

e.g. SCA/23/06/10

5.1.14 After sterilization switch off the autoclave when the autoclave chamber pressure is fully released and pressure gauge display '0' kg /cm^2 pressure then open the autoclave and transfer the material in respective area through material transfer way.

5.1.15 For Broth culture medium, when the temperature of the medium get down up to 25°c then take small portion i.e.10-20 ml of each type of media of medium into separate empty cleaned beaker/test tube and check the pH by using Glass electrode or flat probe pH meter at 25°C.

5.1.16 For Agar media pour 15-20ml of culture agar media in pre sterilized petri-plates, after solidification check the pH by using Glass electrode or flat Probe pH meter at 25°C.

5.1.17 If the pH of sterilized medium goes out limit as defined on media container, the whole sterilized media lot should be discarded as per the SOP.

5.1.18 Transfer all the test tubes containing broth medium to the corresponding incubator for pre-incubation.

5.2 PREPARATION OF AGAR MEDIA PLATES:

5.2.1 Transfer the sterilized Agar media and sterilized petri-plates to the pass box provided to the aseptic area.

5.2.2 Enter in to the LAF Room as per current version of SOP**.**

5.2.3 Clean the LAF bench by 70% v/v IPA.

5.2.4 Transfer the sterilized agar media and sterilized petri-plates from pass box to the LAF bench.

5.2.5 Pour approx. 15-20ml medium in each petri-plate and allow solidifying.

5.2.6 The molten sterilized Agar medium can be held in water bath at 45-50^{0}C for maximum up to 8 hours if there is delay in pouring.

5.2.7 Sterilized Agar media can be stored at 2-8^{0}C for maximum up to 24 hours and solidified media can be re-melted only once by using water bath.

5.2.8 Transfer all the petri-plates containing Agar media after solidifying to the corresponding incubator for pre-incubation.

5.3 PREPARATION OF SLANTS:

5.3.1 Prepare the required quantity of media, slant of which to be prepared as per point no.5.1.4.

5.3.2 Heat to boil to mix the medium properly.

5.3.3 Dispense about 8-9 ml medium in glass test tubes (size 18 x 150 mm) and plugged with cotton and then wrap with aluminum foil.

5.3.4 Keep the slant tubes in autoclave and sterilize the slants as per Validated time and temperature

5.3.5 After sterilization cycle is over take out the slants from autoclave and allows solidifying by tilting it at about 30°C and placing in incubator for pre incubation 30-35°C for 24 hours.

5.3.6 Record the details of culture media in **Annexure–I**, Titled **"Culture Media Preparation Record"**.

5.4 STORAGE:

5.4.1 Pre incubate the media plates / tubes for NLT 24 hrs. For Media used in Environment monitoring, Microbial Limit Test (MLT), Water testing & sterility testing in Dedicated Pre-incbation Incubators.

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6.0 REFERENCES:

USP 37 Volume 1-General *Information* / <1117> Microbiological Best Laboratory Practices

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Culture Media Preparation Record	
Annexure – II	Culture Media Plates & Tubes Reconciliation Record	

ENCLOSURES: SOP Training Record

8.0 DISTRIBUTION:

- Controlled Copy No. 01 — Quality Assurance Department
- Controlled Copy No. 02 — Quality Control Department
- Master Copy — Quality Assurance Department

9.0 ABBREVIATIONS:

No.	Number
QA	Quality Assurance
QC	Quality Control
Ltd.	Limited
SOP	Standard Operating Procedure

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Details of Changes	Reason for Change	Effective Date	Updated By

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE-I
CULTURE MEDIA PREPARATION RECORD

Media Name:

Date	Name of Media	Media C. No.	Drown Qty.	Balance Qty.	Name of Supplement and Quantity to be added	Volume of Media prepare	Purpose	Sterilization Cycle No.	Media Ref. No.	Prepared By Sign & Date	Reviewed By Sign & Date	Remarks

PHARMA DEVILS

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ANNEXURE-II
CULTURE MEDIA PLATES & TUBES RECONCILIATION RECORD

Date	Name of Media	Media Ref. No.	No. of Plates/ Tubes Prepared	Prepared By Sign &Date	No. of Plates/tubes Withdrawal & date	Purpose	No. of Plates/Tubes in balance & date	Withdrawal By Sign &Date	Remarks