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

To lay down a procedure for Receipt, Approval and Preparation of Culture Media.

2.0 SCOPE:

This SOP is applicable for Receipt, Approval and Preparation of Culture Media at Microbiology Section in Quality Control.

3.0 RESPONSIBILITY:

Officer / Executive – Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

COA	Certificate of Analysis
GPT	Growth Promotion Test
IPA	Iso Propyl Alcohol
LAF	Laminar Air Flow
Ltd.	Limited
MLT	Microbial Limit Test
MSDS	Material Safety Data Sheet
NMT	Not More Than
No.	Number
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure

6.0 PROCEDURE:

6.1 RECEIPT OF CULTURE MEDIA:

6.1.1 Procure the dehydrated culture media or ready to use culture media required for microbiological testing either from HiMedia/Merck or other reputed media manufacturers.

6.1.2 Store Officer/Executive shall receive the Culture Media in store and intimate to Officer/Executive –Microbiology about the receipt of culture media.

6.1.3 QC Officer/Executive-Microbiology shall verify the received media COA/MSDS either from Vendor or Manufacturer Website.

6.1.4 QC Officer/Executive-Microbiology shall verify the received quantity, Lot No., Media code, Exp. Date, Pack size, Physical condition of container and same shall be recorded in **Annexure-I**.

6.1.5 After confirmation and inspection of all above points, assign the Media Container number to each container/pack as Follows - **MMM-XX/YY**.

Where,

MMM – Media Code; XX – Sequential number of Media being received.
YY- Total number of container
e.g. SCA-01/10

Where,

SCA – Soyabean Casein Digest Agar, 01/10- is the receipt sequential number

6.1.6 After assigning the Media Container number paste a sticker on media container as per **Annexure-III**.

6.1.7 Enter the details like Name of Media, Batch number, Media Container number, Date of Receipt, Received quantity and Physical condition etc. in **Annexure-I**, Titled **"Media Receipt Record"**.

6.2 APPROVAL OF CULTURE MEDIA:

6.2.1 Read the directions on the container and COA and perform tests as applicable by sampling required quantity of the media from one of the container.

6.2.2 Observe the contents for its description/ Appearance and compare with manufacturer's COA and appearance should comply as per manufacturer's COA.

6.2.3 Prepare the medium as per manufacturer's directions, by dissolving in purified water or WFI and boil in steam pot if required.

6.2.4 Observe the Prepared medium and compare with manufacturer's COA.

6.2.5 Check the pH above prepared medium after sterilization and it's should comply as per manufacturer's COA.

6.2.6 Growth promotion test performed on one container of each lot of media received, if lot number is same of all containers. In the case of different lot number of media container, perform Growth promotion test of each lot.

6.2.7 Carry out the growth promotion test of culture media as per Growth Promotion Test of Culture Media SOP**,** Title- **"Growth Promotion, Inhibitory and Indicative Properties of Culture Media Test"**, and Record the observations of media in **"Growth Promotion Test Report of Culture Media"** Format**.**

6.2.8 In case the media passes the growth promotion test, GPT details shall be filled on the container label and then the same should be used for analysis.

6.2.9 In case the media fails for the growth promotion test than a rejected label shall be affixed on the container then the same shall be rejected and accordingly the rejection entry should be made in the stock register.

6.2.10 The rejected media should be discarded or return back to the supplier.

6.2.11 If media is not used within one year after receipt; perform the requalification of culture media before use as per **Annexure-II** and opened media should also re-qualify before use.

6.2.12 Perform the GPT before use and label the container if the approved culture media container not in use.

6.2.13 Stored the media as per the manufacturer instructions.

6.2.14 Record the observation & details of culture media in **Annexure-II,** Titled- **"Media Approval Record".**

6.2.15 Abbreviation of all culture media are Listed in **Annexure-IV.**

6.3 GENERAL INSTRUCTIONS:

6.3.1 Use clean glassware and accessories for media preparation.

6.3.2 Before starting of media preparation, ensure that the Weighing Balance and pH Meter are calibrated.

6.3.3 Use Purified Water or WFI for preparation of media.

6.3.4 Check the dehydrated media container for its direction for preparation and use before date. Ensure that media used is within the use before date and no clumps in the media.

6.3.5 Do not keep the media container open for longer duration as dehydrated medium are hygroscopic in nature.

6.3.6 Store the dehydrated media and prepared media at recommended storage condition.

6.3.7 Wear gloves and nose mask while preparation of media.

6.4 PREPARATION OF CULTURE MEDIA:

6.4.1 Take required clean glassware and accessories before preparation of each type of media.

6.4.2 Before using the new containers / Bottles of media, check the GPT status of that media if media passes the growth promotion test, then the same should be used for analysis.

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

6.4.4 Transfer appropriately weighed quantity of the culture media in to the cleaned conical flask or bottle containing sufficient quantity of purified water/WFI. 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/bottle of 500 ml will be required.

6.4.5 Gradually add the remaining quantity of the purified water/WFI.

6.4.6 Heat to dissolve the media in steam pot if required, and dispense the media into required glassware (Test tube, conical flasks or bottles) with help of measuring cylinder.

6.4.7 Close the container with non-absorbent cotton plug or appropriate cap.

6.4.8 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 supplement into the culture medium.

6.4.9 Keep the prepared media containers in the autoclave as per the validated load pattern and sterilize the media as per sop of operation and cleaning of double door steam sterilizer.

6.4.10 Allocate the autoclave media reference Number to the each lot of culture medium as below of each and every autoclave media load.

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/20/03/01

6.4.11 After sterilization when the autoclave chamber pressure released then open the autoclave and transfer the material in respective area through material transfer way.

6.4.12 For Broth culture medium, when the temperature of the medium get down up to 25°C then take one tube of each type of medium and check the pH by using Glass electrode or flat Probe pH meter at 25°C±2°C.

6.4.13 For Agar media pour 20-25 ml of culture agar media in presterilized petriplates, after solidification check the pH by using flat Probe pH meter at 25°C±2°C.

6.4.14 If GPT of prepared media is not possible to perform same day with any reason than GPT shall be perform next working day.

6.4.15 If the pH of sterilized medium does not meet the limit defined on media container, the whole sterilized media lot should be discarded as per the SOP.

6.4.16 Transfer all the test tubes containing broth medium to the corresponding incubator for Pre-incubation at 30-35°C for 24-48 hrs. and after completion of pre-incubation transfer the pre-incubated media plates/tubes from pre-incubation room to storage room (20-25°C).

6.4.17 All prepared media sterilize in respective Sterilization Cycle Number and these sterilization cycle number Record in **Annexure–V**, Titled **"Culture Media Preparation Record"**.
H/ST/YY/MM/DD/XX

Where,

H = Horizontal Autoclave
V = Vertical Autoclave
ST = Sterilization
YY = Year
MM = Month
DD = Date
XX = Sterilization Cycle No.
/ = Separator

e.g. H/ST/17/04/11/04 or V/ST/17/04/11/04

6.5 PREPARATION OF AGAR MEDIA PLATES:

6.5.1 Transfer the sterilized Agar media and sterilized petriplates through the pass box provided to the aseptic area.

6.5.2 Enter in to the MLT and Microbial Assay Area as per current version of SOP **"Entry, Exit and Gowning for MLT and Microbial Assay Area"** and Clean the LAF bench with 0.22µ filtered 70 % v/v IPA.

6.5.3 Transfer the sterilized agar media and sterilized petriplates from pass box to the LAF bench.

6.5.4 Cool the media about 45 to 50˚C. If immediate pouring of agar medium is not possible with any reason, then keep the flasks/bottles in water bath / Steam Pot or incubator set between 50 to 60˚C till it is to be used. Do not keep the medium for longer duration (maximum up to 8 hours) and do not re-melt the medium.

6.5.5 For Preparation of 90 mm plates aseptically pour approximately 20-25 ml of media into sterile plates and allow the plates to solidify at room temperature.

6.5.6 For Preparation of 55 mm contact plates aseptically and gently pour approximately agar media into sterile contact plates such that raised convex agar surface will form. Ensure that medium do not overflow from the plate. Allow the plates to solidify at room temperature.

6.5.7 Transfer all the petriplates containing Agar media after solidifying to the corresponding incubator for pre-incubation.

6.5.8 Pre-incubate the agar media plates at 30-35°C for 24-48 Hrs. and after completion of pre-incubation transfer the pre-incubated media plates/tubes from pre-incubation room to storage room (20-25°C)

6.5.9 Record the details of culture media in **Annexure–VI**, Titled **"Culture Media Reconciliation Record"**.

6.6 PREPARATION OF SLANTS:

6.6.1 Prepare the required quantity of media, slant of which to be prepared as per point no.6.4.

6.6.2 Heat to boil to mix the medium properly.

6.6.3 Dispense about 10-12 ml medium in glass test tubes (size 18 x 150 mm) and plugged with cotton and then wrap with aluminum foil.

6.6.4 Keep the slant tubes in autoclave and sterilize the slants as per validated cycle.

6.6.5 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 24-48 hours.

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

6.7 STORAGE AND PRE INCUBATION OF MEDIA:

6.7.1 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 Pre-incubation Incubators.

6.7.2 At the end of the Pre-incubation, check the plates for following:

- Breakage of plate / lids.
- Less volume and dehydration.
- Cracks and presence of particles / Excessive bubbles.
- Microbial contamination.
- Raised surface in case of 55 mm contact plates.

6.7.3 Remove the plates showing breakage, less volume, dehydration/cracks and presence of particles or excessive bubbles and microbial contamination. In case of 55 mm contact plates in which there is inadequate raised surface or plates with agar overflowed to outside.

6.7.4 **Acceptance criteria for microbial contamination:** The contaminated plates should NMT 5% of prepared media plates. If more than 5% of contaminated plates observed, discard the particular lot and an investigation should be conducted.

6.7.5 **Acceptance criteria for liquid medium:** No contamination should be observed in pre Incubated containers representing the lot. If contamination is observed then discard the particular lot and an investigation should be conducted.

6.7.6 If Pre incubation results are satisfactory than media can be use for Environment monitoring, water testing or any other microbial activities.

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Media Receipt Record	
Annexure – II	Media Approval Record	
Annexure – III	Media Container Tag	
Annexure – IV	Media Index	
Annexure – V	Culture Media Preparation Record	
Annexure – VI	Culture Media Reconciliation Record	

ENCLOSURES: SOP Training Record.

8.0 DISTRIBUTION:

- Controlled Copy No. 01 Quality Assurance
- Controlled Copy No. 02 Microbiology
- Master Copy Quality Assurance

9.0 REFERENCES:

- USP 39 Volume 1-General *Information* / <1117> Microbiological Best Laboratory Practices.

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE-I
MEDIA RECEIPT RECORD

Name of Media:

Date	Manufacturer	Batch No. /Lot No.	Quantity Received	Date of Expiry	Physical Condition	Media Cont. No.	Received By Sign & Date	Issued On	Issued By Sign & Date	Reviewed By Sign & Date	Remarks

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ANNEXURE-II
MEDIA APPROVAL RECORD

Name of Media		**Batch No./ Lot No.**	
Name of Manufacturer		**Exp. Date**	
Date of Receipt		**Media Ref. No.**	
Date of Preparation		**Prepared By**	

S.No.	Test		Observations
1	Description		
2	Physical Nature		
3	Solubility		
4	Prepared media colour after sterilization		
5	pH of Media		
6	Growth Promotion Test	Promoting	
		Indicative	
		Inhibitory	
7	Storage Condition		
8	Any Abnormality		

Remarks :

_____

_____

Microbiologist:
Date:

Checked By:
Date:

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ANNEXURE-III

MEDIA CONTAINER TAG

Media Container No. : ----------
Received By/Date : ----------
Date of GPT : ----------
Release Date of GPT : ----------
Date of Opening : ----------
Valid up to : ----------
Issued By (Sign & Date) : ----------

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ANNEXURE-IV
MEDIA INDEX

S.No.	Media Name	Short Form
1.	Antibiotic Assay Medium No. 11	AAM
2.	B12 Culture Agar	BCA
3.	B12 Assay Agar	BAA
4.	Cetrimide Agar	CTA
5.	Columbia Agar Base	CLA
6.	Cooked Meat Medium	CMM
7.	De-Engley's Neutralizing Agar	DNA
8.	Enterobacteria Enrichment Broth Mossel	EEM
9.	Eosine Methylene Blue Agar	EMB
10.	Fluid Thioglycolate Medium	FTM
11.	GN Broth	GNB
12.	Glucose Yeast Extract Agar	GYA
13.	MacConkey Agar	MCA
14.	MacConkey Broth	MCB
15.	Mannitol Salt Agar Medium	MSA
16.	Mueller Hinton Agar	MHA
17.	Mitis Salviris Agar	MTS
18.	Nutrient Agar	NAM
19.	Peptone Bacteriological	PPW
20.	Pseudomonas Agar Medium (Pyocyanin)	PAP
21.	Pseudomonas Agar Medium (Fluorescein)	PAF
22.	PNY Agar	PNY
23.	Rappaport Vassiliadis Salmonella enrichment Broth	RVS
24.	Reinforced medium for clostridia	RMC
25.	R2A Agar	R2A
26.	Sabouraud Chloramphenicol Agar	SDA

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S.No.	Media Name	Short Form
27.	Sabouraud Dextrose Broth	SDB
28.	Sabouraud Dextrose Agar	SBD
29.	Soybean Casein Digest Agar	SCA
30.	Soybean Casein Digest Medium	SCM
31.	Triple Sugar Iron Agar	TSA
32.	Violet Red bile Glucose agar	VBA
33.	Vogel Johnson Agar	VGA
34.	Wilson Blair's WBS Agar	WBA
35.	Xylose Lysine Deoxycholate Agar	XLD
36.	Folic Acid Assay Medium	FAA
37.	Folic Acid Culture Agar	FCA
38.	Lactobacillus MRS Agar	MRS
39.	Glucose Yeast Extract broth	GYB
40.	Antibiotic Assay Medium H	AAH
41.	Antibiotic Assay Medium No.1	AA1

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ANNEXURE-V
CULTURE MEDIA PREPARATION RECORD

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

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ANNEXURE-VI
CULTURE MEDIA RECONCILIATION RECORD

Name of Media		**No. of Plates, Tubes & Bottles Prepared**	
Media Ref. No.		**Date of Preparation**	

Date	No. of Plates/tubes/ bottles Withdrawal	No. of Plates/tubes/ bottles Balance	Purpose	Withdrawal By Sign & Date	Received By Sign & Date

Reviewed By
Sign Date