STANDARD OPERATING PROCEDURE				
TITLE : PREPARATION OF MEDIA				
SOP No.:		**Department :**	Microbiology	
Revision No.:	00	**Effective Date :**		
		Revision Date :		
Supersede SOP No.:	NIL	**Page No.:**	1 of 10	

1.0 OBJECTIVE

To lay down the procedure for the preparation of the media used by microbiology department for microbiological testing.

2.0 SCOPE

This SOP is applicable for the preparation of all the media used in Microbiology lab.

3.0 RESPONSIBILITY

Officer/Executive - Microbiology

4.0 ACCOUNTABILITY

Head-QC

5.0 ABBREVIATIONS

SOP : Standard Operating Procedure
QC : Quality Control
QA : Quality Assurance
SCDA : Soyabean Casein Digest Agar

6.0 PRECAUTIONS

6.1 Read the instructions given on the Bottle label for - ingredients, directions for use, expiry date, before use of commercial dehydrated media.

6.2 During weighing of media always wear gloves and mask so that the media is not inhaled.

6.3 Ensure that the dehydrated media powder has the following characteristics:-

a) Free flowing, uniform color, absence of lumps.

b) Do not use the media if not satisfied with reference to the above mentioned characteristics.

7.0 MATERIALS AND EQUIPMENTS

7.1 MATERIALS:

Dehydrated cultural media, dried spatula, Glass beaker, Glycerol, and Non absorbent cotton, Aluminum foil, Butter paper.

7.2 EQUIPMENTS:

Autoclave, Analytical Balance, pH meter

8.0 PREPARATION OF MEDIA:

8.1 Always ensure that the Growth Promotion test of each lot of medium should be done before or parallel of the test, for each autoclaved load or each lot of medium for its growth promoting qualities by separately inoculating container of each medium with less than 100 viable microorganisms of each of the strains and incubating according to the conditions specified as per SOP.

8.2 Place a piece of butter paper on the weighing pan of the analytical balance large enough to accommodate the quantity to be weighed.

8.3 Open the bottle containing the dehydrated media.

8.4 With the help of a clean and dried spatula/spoon add the dehydrated media powder on the butter paper.

8.5 Weigh the media as given in Table I

TABLE I

S.No.	MEDIA	SUPPLEMENT TO ADD PER LITRE OF STERILISED MEDIA	SHORT NAME	pH AFTER STERILIZATION
1.	Soyabean Casein Digest Medium	None	SCDM	7.3 ± 0.2
2.	Fluid Thioglycollate Medium	None	FTM	7.1± 0.2
3.	Enterobacteria Enrichment Broth Mossel	None	EEBM	7.2 ± 0.2
4.	Bacteriological Peptone	None	PW	7.1 ± 0.2
5.	MacConkey Broth	None	MCB	7.3 ± 0.2
6.	Buffered Sodium Chloride Peptone Water	None	BPW	7.2 ± 0.2
7.	GN Broth	None	GNB	7.0 ± 0.2
8.	Rappaport Vassiliadis Salmonella Enrichment Broth	None	RVSEB	5.2 ± 0.2
9.	Columbia Agar	None	COA	7.0 ± 0.2
10.	Cetrimide Agar	None	CA	7.2 ± 0.2
11.	Mannitol Salt Agar	None	MSA	7.4± 0.2
12.	Eosin Methylene Blue Agar	None	EMB	7.1 ± 0.2
13.	Soyabean Casein Digest Agar	1% Glycerol	SCDA	7.3 ± 0.2
14.	Pseudomonas Agar Medium for	None	PAF	7.2 ± 0.2

S.No.	MEDIA	SUPPLEMENT TO ADD PER LITRE OF STERILISED MEDIA	SHORT NAME	pH AFTER STERILIZATION
	Detection of Fluorescin			
15.	Pseudomonas Agar Medium For Detection of Pyocyanin	None	PAP	7.2 ± 0.2
16.	Sabouraud Dextrose Agar	None	SDA	5.6 ± 0.2
17.	MacConkey Agar	None	MCA	7.1 ± 0.2
18.	Reinforced Media	None	RFM	7.2 ± 0.2
19.	R_2 A Agar	None	R_2 A	7.2 ± 0.2
20.	Xylose Lysine De-oxycholate Agar	None	XLDA	7.4 ± 0.2
21.	Violet Red Bile Glucose Agar	None	VRBGA	7.2 ± 0.2

8.6 After weighing the required amount take it slowly from the pan without spilling transfer it into a Clean glass conical flask and cover it with aluminum foil.

8.7 SCDA media shall be supplemented with 1% glycerol media before sterilization to avoid dehydration during exposure.

8.8 Add a required volume of Water for Injection and mix to dissolve, if necessary heat the medium to dissolve completely by using a hot plate/ water bath at temp range of 80-90°C. Do not over heat the medium.

Table II

TEST TO BE PERFORMED	TYPE OF GLASSWARE FOR DISPENSING	QUANTITY OF THE MEDIA	TEMPERATURE OF MEDIA DURING DISPENSING
Swab Test (Peptone water)	Bottles	500 ml / bottle	At ambient temperature
Sub culturing (Slant preparation)	Test Tubes	10 ml / test tube	Approximately 40-50°C

TEST TO BE PERFORMED	TYPE OF GLASSWARE FOR DISPENSING	QUANTITY OF THE MEDIA	TEMPERATURE OF MEDIA DURING DISPENSING
Microbial limit test	Test Tubes, Conical flask/ Roux bottle, Plates	10ml/test tube, 100ml/conical flask, 20ml/plates	At ambient temperature/40-50°C

8.9 Close the test tubes and conical flasks with autoclavable caps or cotton plug of non absorbent cotton and wrap them with aluminum foil.

8.10 Before sterilization always check the pH of the media if the pH is not within the specified limits, it should be adjusted by adding 1N or 0.1N Hydrochloric acid or Sodium Hydroxide solution.
After that autoclave the media as per SOP No.

8.11 Check the pH of the media after sterilization if the pH of the media is not within the specified limits as per the details, discard the media.

8.13 Frequency-Daily during the activity.

9.0 PRE-INCUBATION OF MEDIA:

9.1 Pre-incubation of agar media:

9.1.1 Pre incubate 100% of all agar media plates at 30-35^{o}C for 24 hours for all media with following exception:-

9.1.2 Soyabean casein digest agar shall be incubated at 20-25^{o}C for 48 hours and then incubated at 30-35^{o}C for 24 hours.

9.1.3 R_2A agar shall be incubated at 30-35^{o}C for 72 hours.

9.1.3 Fore pour plate media shall be prepared freshly and shall be used, in such case keep negative control plate up to the test completion.

9.2 Pre incubation of liquid media:

9.2.1 Pre incubate 100% of all media tubes at 30-35^{o}C for 24 hours for all media with following exception:-

9.2.2 Soyabean casein digest medium shall be incubated at 20-25^{o}C for 72 hours and Fluid thioglycollate medium shall be incubated at 30-35^{o}C for 72 hours.

9.2.3 Mac Conkey Broth shall be incubating at 42-44^{o}C for 24 hours.

9.3 Checking of media plates/liquid media for the following after Pre-incubation

9.3.1 Breakage of plate or lids.

9.3.2 Less volume or unequal filling of plates/tubes.

9.3.3 Dehydration resulting in crakes or dimpled surfaces on solid medium.

9.3.4 Excessive number of bubbles and Microbial contamination.

9.3.5 Excessive darkening or colour change of the medium.

9.3.6 Microbial contamination of the liquid medium.

9.3.7 Raised surface in case of 55 mm contact plate.

9.3.8 Visually checks remove the all defected plates and discard.

9.4 Acceptance Criteria for Solid Media plates after Pre-Incubation:

9.4.1 There should not be any deficiency in media plates as given under point no. 9.3 and the contaminated plates should not be more than 5% of the prepared plates. If more than 5% of contaminated plates observed, discard the particular lot.

9.5 Acceptance Criteria for Liquid Media after Pre-Incubation:

9.5.1 No contamination should be observed in pre incubated containers representing the lot. If contamination is observed then discard the particular.

10.0 ENCLOSURES:

Annexure I – Media Preparation and consumption record
Annexure II _ Media Receipt and Issue record
Annexure III _Sterile Media Reconciliation Record
Annexure IV –Media Preparation Label
Annexure V _ Media Receipt Label

11.0 DISTRIBUTION

11.1 The SOP shall be distributed to respective department by QA. The QA department Shall authorize for the distribution of SOP and shall sign for "Issued by: "
Follow the distribution of SOP as per following:
Master Copy - QA Department
Control Copy - User Department

12.0 HISTORY OF REVIEW:

PREVIOUS EFFECTIVE DATE	PREVIOUS REVISION NO.	CCF No. IF ANY	REASON FOR REVIEW
	00	NIL	New SOP

Annexure I

MICROBIOLOGY DEPARTMENT MEDIA PREPARATION AND CONSUMPTION RECORD

Name of Media……..

Date	Preparation		Media B. No	Stock	pH before sterilization	pH after sterilization	Preparation B. No	Use before	Used on	Media used for	Destruction		Checked by
	Qty in g	Volume ml									On	By	

Annexure II

MICROBIOLOGYDEPARTMENT MEDIA RECEIPT AND ISSUE RECORD

Media Name: …… Code No: ……..

Date	RECEIPT				ISSUE				Balance Quantity	Checked by
	Qty	Batch No.	Exp.Date	Received by	Qty	Batch No.	GPT No.	Issued by		

Annexure III

MICROBIOLOGY DEPARTMENT STERILE MEDIA RECONCILATION RECORD

Name of Media: .....

Date of Preparation	Preparation Batch no.	No. of Plates/Tubes /Bottles prepared	No. of Plates/Tubes /Bottles used	Used for (Purpose)	Used on	Used By	Remaining Stock	Destruction On	Destruction By	Checked by

Annexure IV

MEDIA PREPARATION LABEL:

	MEDIA PREPARATION LABEL
Name of the Media	
Autoclave lot no.	
Date of preparation	
Use before	
Prepared by	

Annexure V

MEDIA RECEIPT LABEL

Name of the Media	
Qty. Received	
Bottle No.	
Date of Opening	
Date of Release	
Done by	