



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

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|--|------------------------|
| Department: Microbiology | SOP No.: |
| Title: Preparation and use of Microbiological Media | Effective Date: |
| Supersedes: Nil | Review Date: |
| Issue Date: | Page No.: |

1.0 Objective

To lay down a procedure for the preparation and use of microbiological media.

2.0 Scope

This Standard Operating Procedure is applicable to Microbiology section at Quality Control Department.

3.0 Responsibility

Microbiologist : Shall be responsible for the Preparation and Use of Microbiological Media.

Head-QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

LAF : Laminar Air Flow

Pre-Incubated Culture medium : The culture medium which is used after incubation.

5.0 Procedure

5.1 General Instructions

5.1.1 Whenever fresh medium stock is received, check the quantity, Batch No, Lot No, Expiry date and Storage conditions.

5.1.2 Each lot of medium received shall be tested first for growth promotion test as per respective SOP before use.

5.1.3 Ensure that the medium shall used within 12 months after opening the bottle.

5.1.4 Any medium stock which is unopened shall be used within the shelf life of the container.

5.1.5 Verify batch No, lot No, expiry date etc. on the medium label before use.

5.1.6 After use, close the bottle tightly and preserve in a closed protected environment at temperature $25 \pm 5^{\circ}\text{C}$.

5.2 Preparation of Media

5.2.1 Prepare the media as per manufacturer's instructions mentioned on the containers.



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- 5.2.2 Ensure that before use the culture medium is not deteriorated physically and also check the expiry date.
- 5.2.3 Select a clean dry conical flask of appropriate size as per the quantity of the media required.
- 5.2.4 Allow glassware to dry in Laboratory oven or in air.
- 5.2.5 Weigh appropriate quantity of the medium as required.
- 5.2.6 Add the required amount of purified water from the side of the flask and shake to obtain a clear solution, If not clear, heat the medium for complete dissolution, using a water bath to avoid excessive heating. Record the quantity of the media prepared in the format as given in Annexure – 2
- 5.2.7 Cool the media to 25°C and check the pH on a calibrated pH meter and ensure that the pH of dissolved media shall within the specification as per manufacturer's instructions, given on the container. Record the pH in the format as given in Annexure - 2.
- 5.2.8 Assign the batch no of prepared media as MMXXX/YY
Where,
MM - Microbiological Media
XXX - 001, 002, 003, ----- to 999
/ - Slash
YY - Last 2 digits of the calendar year.
For example, first batch no. for year 2023 shall stand as MM001/23
- 5.2.9 Plug the flasks, tubes with non-absorbent cotton or S.S metallic caps followed by butter paper or aluminium foil and tighten with rubber band.
- 5.2.10 Sterilize the medium by autoclaving in Steam Sterilizer at 121°C to 124°C and 15 lbs pressure for 15 minutes.
- 5.2.11 After taking out from autoclave place the medium in thermostatically controlled water bath at 45°C to stabilize the temperature of medium. Culture media shall be



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dispensed into plates at about 45-50°C. The medium shall be mixed thoroughly, without bubble formation and aseptically pour into sterile Petri plates.

5.2.12 The sterile agar media plates and the broth culture tubes and flasks shall be incubated for 24h for bacteria and 48h for Fungi in respective temperature of incubation.

5.2.13 After pre-incubation, the agar plates, broth culture tubes and flasks shall be stored in refrigerator for further use.

5.2.14 Portion of the prepared agar media will be used on same day as per requirement.

5.2.15 The prepared media shall be used within the 3 days of preparation and its record shall be maintained as per Annexure-2.

5.2.16 Microbiological Media inward cum stock maintenance record shall be maintained as per Annexure - 1.

5.3 Safety Measures for Handling of Dehydrated Culture Media.

5.3.1 Most of the powders are fluffy in nature and should not be inhaled because irritation of the upper respiratory tract may occur. If skin rashes occur, avoid prolonged contact with the powder and ensure excessive dust is not produced. Any residue should be washed off with ample cold water.

5.3.2 Use masks while handling dehydrated media to prevent the risk of inhaling fine dust.

5.4 Handling of Hazardous and Toxic Products:

5.4.1 Culture Media may contain toxic substances as the constituents of the medium and therefore they should be handled considering the following precautions.

5.4.2 **Sodium Azide:** Generally, when sodium azide is used in any medium, the concentration is less than 1% which has low toxicity. Some persons are sensitive to this substance and therefore precautions must be taken to prevent ingestions or inhalation of the dust. Sodium azide has a property to react with heavy metals, particularly copper and lead which produce explosive metal azides.



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Sufficient water should be used to remove the powder remaining in contact with pipe line and drains. Such precautions are required to be observed in case of any biological solution which contains sodium azide as a preservative.

- 5.4.3 **Lithium Chloride:** Lithium chloride is harmful. Avoid body contact and inhalation of vapours. On contact with skin immediately wash with plenty of water.
- 5.4.4 **Cycloheximide:** Cycloheximide is highly toxic. Avoid skin contact or aerosol formation and inhalation.
- 5.4.5 **Basic Fuchsin:** Basic Fuchsin is a potential Carcinogen and care should be taken to avoid inhalation of the powdered dye and contact with the skin.
- 5.4.6 **Sodium Hydrogen Selenite:** Sodium Hydrogen Selenite is a highly toxic, teratogenic, corrosive agent. Hence it should be handled with great care, If there is skin contact wash immediately with plenty of water.
- 5.5 **First Aid Measures:** The following First Aid procedures should be taken in cases of accident while handling any of the toxic or hazardous products.
- 5.5.1 **Inhalation:** Person should be removed from the area of exposure. He should rest and keep warm and if required seek medical advice.
- 5.5.2 **Skin Contact:** It is suggested to remove all the contaminated clothing's immediately and wash the affected area thoroughly with soap and water. After completing the procedure, if any symptoms occur, seek medical advice.
- 5.5.3 **Ingestion:** In case of ingestion of material, wash the mouth thoroughly with plenty of water. One pint of water should be drunk immediately and if any symptoms occur, seek medical advice.
- 5.5.4 **Eye Contact:** Eyes should be washed thoroughly with plenty of water. If required, seek medical advice.
- 5.5.5 **Spillage:** Following precautions must be observed if material is spilled.



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5.5.5.1 For Large Quantities: Wear protective overalls, gloves, eye protection and face mask. Material should be collected in suitable container and then properly sealed. Disposal of such material is to be carried out according to local regulations. Wash away the residue with plenty of water.

5.5.5.2 For Small quantities. By using protective gloves, wash away the material with large volumes of running water.

6.0 Forms and Records

- 6.1 Microbiological Media Inward Cum Stock Maintenance Record : Annexure-1
- 6.2 Microbiological Media Preparation and Usage Record : Annexure-2

7.0 Distribution

- 7.1 Master Copy : Documentation Cell (Quality Assurance)
- 7.2 Controlled Copies : Quality Control, Quality Assurance

8.0 History

| Date | Revision Number | Reason for Revision |
|------|-----------------|---------------------|
| | 00 | New SOP |

