| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

**PURPOSE:**

To lay down a procedure for the preparation of media plates, slant and tubes for Microbiological analysis and sterilization and storage of media.

**SCOPE:**

This procedure is applicable to microbiology laboratory.

**RESPONSIBILITY:**

Officer/Executive - Quality Control.

**ACCOUNTABILITY:**

Head –QC department.

**PROCEDURE**:

**1.0 Receipt of dehydrated media:**

1.1 On receipt of dehydrated media, the manufacture and expiry date of the media shall be verified.

1.2 If the dehydrated media is nearing its expiry date the same shall be sent back to the supplier for replacement with the new stocks.

1.3 As and when the dehydrated media is opened for usage, the media shall be visually inspected for normal texture of that particular media type, for e.g., if unusual lumps are observed, the dehydrated media shall be discarded and the same shall be recorded in media stock record, Annexure-I.

1.4 COA of the respective media shall be received from the manufacturer and filed when ever is required the same shall be produced.

1.5 The date of opening of the dehydrated media containers shall be written on the label and sticked to the container as mentioned below.

| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

| |
|---|
| Received on :-_____<br>Use Before :-_____<br>Sign/ Date :-_____<br>Opened on :-_____<br>Sign/ Date :-_____ |

1.6 All freshly received dehydrated media shall be put to Growth Promotion Test (GPT) as per SOP.

1.7 If the GPT is satisfactory, the dehydrated media shall be used for media preparation.

1.8 The dehydrated media shall be discarded after nine (09) months of opening the container or on its expiry date which ever is earlier.

**2.0 Preparation of media;**

2.1 Use purified water for media preparation.

2.2 Before dispensing of dehydrated media wear nose mask and gloves.

2.3 Calculate the quantity of dehydrated media required for the volume of media to be prepared as per manufacturers label claim

2.4 Weigh calculated quantity of media on a calibrated weighing balance and add into the conical flask containing half quantity of the required water.

2.5 Add the remaining quantity of water into the flask. Dissolve the media by swirling the flask or boil the media if required on heating mantle to dissolve completely.

2.6 While swirling make sure that the content does not spill.

2.7 After weighing the media, tightly secure the lid of the dehydrated media container. If pouch pack is being used, fold the mouth of pouch and place a U-clip over the folding and keep the pouch in a self sealing poly bag.

2.8 Carry the different Media to the pH meter.

2.9 Switch off the pH meter.

2.10 Check the pH of the unsterilized media as per the SOP "Operation and Calibration of pH Meter". Record the Verify the pH of media with the Table-I mentioned below, if the pH varies, adjust it by addition of 0.1N HCl or 0.1N NaOH.

2.11 If the pH is satisfactory cotton plug the flask / tube.

2.12 Before sterilization, white paint on the media flask shall be labeled with marker and cello tape shall be pasted. For test tubes, test tubes stand shall be labelled for name of the media, date of sterilization and cello tape shall be pasted.

2.13 Keep the prepared media and sterilize the media in steam sterilizer ID by operating the autoclave as per Cycle No.2 (validated Gravity Cycle for media sterilization). Follow current SOP for operating the autoclave.

2.14 After completion of sterilization cycle take the cycle print from the printer and write the sterilization load number and record in media preparation and sterilization details, Annexure-II.

2.15 Unload the sterilized media from autoclave, by pressing the Door 2 Open button of autoclave from the buffer room side and transfer the sterilized media into static pass box.

2.16 Allow the Liquid media to come to room temperature. Check the pH of the sterilised Liquid media as per the SOP "Operation and Calibration of pH Meter".

2.17 Verify the pH of media with the Table-I mentioned below. Record the observed pH in media preparation and sterilization details, Annexure-II. If the pH of media is out of limit, discard the media as per SOP.

2.18 Like wise check the pH for all the Liquid media.

2.19 **For checking the pH of the Agar media:**

2.19.1 Switch off the pH meter. Gently detach the wire of the electrode from the pH meter board at the back. Fix the wire of the Flat bottom Electrode to the pH meter board at the back.

2.19.2 Eliminate air bubbles in the membrane chamber by shaking. (Like a fever thermometer).

2.19.3 Switch on the pH meter.

2.19.4 Remove the cap of the electrode containing KCl solution. Rinse 2-3 times with Milli-Q water. Remove the water at the bottom of the electrode by gently touching the tip of the electrode with dry tissue paper

2.19.5 Carry the calibration of the pH meter as mentioned in SOP "Operation and Calibration of pH Meter".

2.19.6 After calibration, Rinse 2-3 times with Milli-Q water. Remove the water at the bottom of the electrode by gently touching the tip of the electrode with dry tissue paper.

2.20 Place the Flat bottom Electrode on to the surface of the media. Gently hold the electrode on media, wait until the pH reading stabilizes.

2.21 Record the observed pH in media preparation and sterilization details, Annexure-II. If the pH of media is out of limit, discard the media as per SOP.

2.22 Like wise check the pH for all the Agar media.

2.22.1 Switch off the pH meter. Gently detach the wire of the electrode from the pH meter board at the back. Fix the wire of the bulb Electrode to the pH meter board at the back.

2.22.2 Switch on the pH meter.

2.22.3 Replace the electrode in the cap containing KCl solution.

2.22.4 Record the usage of pH meter in the Annexure-I, "Format for pH meter usage log book".

2.23 Discard the media which has been used for checking of the pH as per SOP.

**3.0 Preparation of Petri plates [90 mm diameter]**

3.1 The Petri plates shall be prepared for the following activities as listed below.

- Plate exposure in the sterile and non sterile manufacturing facilities.
- Plate exposure in the microbial limit testing area and general Microbiology laboratory.
- Bioburden of primary packaging material samples by Membrane filtration method.
- Selective media for the analysis of pathogens in water.
- Selective media for the MLT of raw materials and non sterile products.

| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

3.2 Sterilized Petri plates shall be transferred to the Laminar Air flow work station. The Petri plates shall be removed from the petricans in the work station and kept aside on the trolley. The Petri plates shall be arranged in the work station so as not to obstruct the laminarity of the air flow.

3.3 The cover of the Petri plates shall be lifted from one side just enough to permit the entry of the neck of the flask of sterilized media.

3.4 Media shall be poured onto the sterilized Petri plates either directly from the sterilized media flask or if the flask is of higher volume, the media shall be dispensed in small sterilized beakers and then shall be poured.

3.5 20-25 mL of the respective media shall be poured to the Petri dishes and ensure that the media shall be dispensed at the temperature range of 40-45$^{o}$C to avoid condensation in the cover of the Petri plates and without any air bubbles.

3.6 After pouring to the plate, the Petri plates shall be covered and allowed to solidify in the laminar air flow station.

3.7 After solidification, the plates shall be incubated at 30-35$^{o}$C for 24 hours as a pre incubation. The incubated plates shall be observed for any growth and shall be used for the microbiological analysis.

3.8 Parallely, Petri plates with the solidified media shall be randomly selected and tested for growth promotion tests.

3.9 The media used for the plate exposure shall be SCDA / PDA / SDA. 1% Glycerol [To avoid desiccation of the media during the exposure] shall be added for the media used preparing petriplates used for plate exposure in sterile and non sterile manufacturing facilities, Microbiology laboratory and bioburden checking of packaging materials.

3.10 The media used for the pathogen testing of water samples and MLT of non sterile products shall be of selective media as required by the testing procedure.

3.11 Microbiologists shall take all the necessary precautions like gowning, wearing gloves, nose mask etc and sanitization practices to avoid contamination during the plate preparation.

| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

**4.0 Preparation of Contact plates [65 mm diameter]**

4.1 The contact plates shall be prepared for the following activities as listed below.

- Surface monitoring in the manufacturing facilities, Microbial limit testing area of the Microbiology laboratory.

4.2 Pre-sterilized contact plates packs shall be sanitized with 70% IPA and shall be transferred to the Laminar Air flow work station. The outer wrap of the contact plates and the individual inner wrap shall be removed in the laminar air flow station.

4.3 The pre-sterilized contact plates shall be arranged in the work station so as not to obstruct the laminarity of the air flow.

4.4 The cover of the contact plates shall be lifted from one side just enough to permit the entry of the neck of the flask of sterilized media.

4.5 Media shall be poured onto the pre-sterilized contact plates either directly from the sterilized media flask or if the flask is of higher volume, the media shall be dispensed in small sterilized beakers and then shall be poured.

4.6 Respective media shall be poured to the pre-sterilized contact plates so as to form a convex surface of the media at the top and ensure that the media shall be dispensed at the temperature range of 40-45$^{o}$C to avoid condensation in the cover of the contact plates and without any air bubbles.

4.7 After pouring to the plate, the contact plates shall be covered and allowed to solidify in the laminar air flow station.

4.8 After solidification, the contact plates shall be incubated at 30-35$^{o}$C for 24 hours as a pre - incubation. Observe the incubated plates for any growth and shall be used for the microbiological analysis.

4.9 The media used for the preparation shall be SCDA for the surface monitoring in manufacturing facilities and Microbial Limit testing area of the Microbiology laboratory.

4.10 Microbiologists shall take all the necessary precautions like gowning, wearing gloves, nose mask etc and sanitization practices to avoid contamination during the contact plate preparation.

| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

**5.0 Preparation of media slants**

5.1 The media slants shall be prepared for the following activities as listed below.

- For the sub culturing of the ATCC cultures and In house environmental isolates.
- For the confirmative tests in the pathogen testing of water samples & MLT of non sterile products.

5.2 The test tubes along with the respective media shall be plugged with cotton and shall be sterilized.

5.3 In case of stabs, the sterilized test tubes with the media shall be allowed to solidify in an upright position. In case of slants, the sterilized test tubes with media shall be allowed to solidify in a slanting position by maintaining a butt of not less than 1 inch.

5.4 After solidification, the test tubes shall be incubated at 30-35ºC for 24 hours as a pre incubation. The incubated tubes shall be observed for any growth and shall be used for the microbiological analysis.

5.5 The media used for the pathogen testing of water samples and MLT of non sterile products selective media as required by the testing procedure.

5.6 The media used for the sub culturing shall be of SCDA / SDA / PDA / FTGA Vitamin $B_{12}$ culture and assay agar or any selective media as required by the testing procedure.

**6.0 Preparation of medium for rinsing.**

- For the rinsing of the products during the bioburden testing of primary packaging materials. The rinsing fluid used shall be 0.1% Peptone water.

6.1 The sealed conical flask along with the medium [0.1% Peptone water] shall be sterilized and unloaded in the Microbial Limit testing area.

6.2 The cooled 0.1% Peptone water medium shall be tested for colour .The medium shall be used only after the tested parameters passes for the lot of the autoclaved medium.

**7.0 Storage of media:**

7.1 Dehydrated media shall be stored either in the refrigerator or rack as per the recommendation of the manufacturer.

**STANDARD OPERATING PROCEDURE**

| | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

7.2 The ready to use preincubated media in petriplates (90mm diameter), contact plates (65 mm diameter) shall be stored at 20-25°C and shall be used within the period of 2+1 day.

7.3 The Peptone water in conical flask shall be stored at < than 25°C and shall be used within a period of 2+1 day.

7.4 The preincubated tubes shall be stored at 2-8°C and shall be used within a period of 02+ day.

**TABLE - I**

| S.No. | Name of Medium | Instructions if any | Sterilization details | Final pH & Acceptance Criteria |
|---|---|---|---|---|
| 1 | Buffered Peptone Water (BPW) | ------ | 15 lbs, 121°C for 15 mins. | 7.0 ± 0.2 |
| 2 | Soyabean Casein Digest Agar (SCDA) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.0 ± 0.2 |
| 3 | Sabouraud Dextrose Agar (SDA) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.3 ± 0.1 |
| 4 | Sabouraud Glucose Agar (SGA) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 5.6 ± 0.2 |
| 5 | Fluid Casein Digest Soya Lecithin Medium (FSM) | Warm to dissolve | 15 lbs, 121°C for 15 mins. | --- |
| 6 | Nutrient Broth (NB) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.4 ± 0.2 |
| 7 | Fluid Lactose Medium (FLM) | ------ | 15 lbs, 121°C for 15 mins. | 6.9 ± 0.2 |
| 8 | Mac Conkey Broth (MCB) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.3 ± 0.2 |
| 9 | Peptone, Bacteriological | ----- | 15 lbs, 121°C for 15 mins | --- |
| 10 | Mac Conkey Agar (MCA) | Heat to dissolve | 15 lbs, 121°C for 15 mins. | 7.1 ± 0.2 |
| 11 | EMB Agar (EMBA) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.1 ± 0.2 |
| 12 | Fluid Selenite broth (Selenite F broth) (SCB) | Warm to dissolve and distribute in sterile test tubes. Sterilize in a boiling water bath for 10 minutes. | Do not autoclave | 7.0 ± 0.2 |
| 13 | Tetrathionate Brilliant green bile broth (Fluid Tetrathionate medium (TBGBB) | Boil to dissolve | Do not autoclave | 7.0 ± 0.2 |
| 14 | Bismuth sulphite Agar (BSA) | Boil to dissolve | Do not autoclave | 7.6 ± 0.2 |
| 15 | Brilliant Green Agar (BGA) | Heat to dissolve | 15 lbs, 121°C for 15 mins. | 6.9 ± 0.2 |

**STANDARD OPERATING PROCEDURE**

| | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

| S.No. | Name of Medium | Instructions if any | Sterilization details | Final pH & Acceptance Criteria |
|---|---|---|---|---|
| 16 | Deoxycholate Citrate Agar (DCA) | Boil to dissolve | Do not autoclave | 7.3 ± 0.2 |
| 17 | Xylose-lysine Deoxycholate Agar (XLDA) | Boil to dissolve | Do not autoclave | 7.4 ± 0.2 |
| 18 | Triple Sugar Iron Agar (TSIA) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.3 ± 0.2 |
| 19 | Urea broth (UB) | Sterilize by filtration | Do not boil and heat the medium | --- |
| 20 | Soyabean Casein Digest Medium (SCDM) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.3 ± 0.2 |
| 21 | Mannitol Salt Agar (MSA) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.4 ± 0.2 |
| 22 | Vogel-Johnson Agar (VJA) | Boil to dissolve. After autoclave, add 20ml of sterile 1% Potassium Tellurite. | 15 lbs, 121°C for 15 mins. | 7.2 ± 0.2 |
| 23 | Baird-Parker Agar (BPA) | Boil to dissolve. After autoclaving cool to 50°C add aseptically 50ml of conc. egg yolk emulsion and add 3ml of sterile Potassium Tellurite. | 15 lbs, 121°C for 15 mins. | 6.8 ± 0.2 |
| 24 | Cetrimide Agar(CA) | Heat to dissolve | 15 lbs, 121°C for 15 mins. | 7.2 ± 0.2 |
| 25 | Pseudomonas Agar For Fluorescein (PAF) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.2 ± 0.2 |
| 26 | Pseudomonas Agar For Pyocyanin (PAP) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.2 ± 0.2 |
| 27 | $R_2A$ | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.2 ± 0.2 |
| 28 | EE Broth, Mossel (EEB) | Heat in boiling Water for 30 min | Do not autoclave. | 7.2 ± 0.2 |
| 29 | Violet Red Bile Agar (VRBA) | Heat with stirring to boiling to dissolve | Do not autoclave. | 7.4 ± 0.2 |
| 30 | Antibiotic Assay Medium (AAM) | Heat to dissolve | 15 lbs, 121°C for 15 mins. | 7.0 ± 0.2 |
| 31 | Nutrient Agar (NA) | Boil to dissolve | 15 lbs, 121°C for 15 mins. | 7.2 ± 0.2 |
| 32 | Columbia Agar | Boil to dissolve | Do not autoclave. | 7.3 ± 0.2 |
| 33 | Reinforced media for clostridia | Boil to dissolve | Do not autoclave. | 6.8 ± 0.2 |
| 34 | Vitamin $B_{12}$ Culture agar | Boil to dissolve | 15 lbs, 121°C for 15 mins | 7.0 ± 0.2 |
| 35 | Vitamin $B_{12}$ Assay agar | Boil to dissolve | 15 lbs, 121°C for 15 mins | 7.2 ± 0.2 |

**STANDARD OPERATING PROCEDURE**

| | |
|---|---|
| **Department:** Microbiology | **SOP No.:** |
| **Title:** Preparation, Sterilization and Storage of Media | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

| S.No. | Name of Medium | Instructions if any | Sterilization details | Final pH & Acceptance Criteria |
|---|---|---|---|---|
| 36 | Cooked Meat medium (R.C.Medium) | Mix thoroughly & allow to stand for 15 minutes until all the particles are thoroughly wetted. | 15 lbs, 121°C for 15 mins | 7.2 ± 0.2 |
| 37 | Fluid Thioglycollate Medium(FTM) | Boil to dissolve | 15 lbs, 121°C for 15 mins | 7.1 ± 0.2 |
| 38 | Thioglycollate Agar(TGA) | Boil to dissolve | 15 lbs, 121°C for 15 mins | 7.1 ± 0.2 |

**ANNEXURE(S):**

Annexure -I: Media Stock Record.

Annexure -II: Media preparation & Sterilization details.

Annexure-III: Visual inspection Record of Dehydrated Media.

**REFERENCE(S):**

Nil.