

Establishment and Application of Ideal Media Room in Microbiology Laboratory

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Abstract

When microorganisms are tested, the laboratories provide good conditions for the growth of microbes. Therefore, there is a need to develop techniques to manage areas of high microbial populations and mechanisms to prevent those entering aseptically protected areas like media preparation room. So the basic design of a microbiology laboratory needs to include the physical separation of spaces to carry out different functions and requirements. Media room is the basis of a microbiology laboratory, so it needs special attention. The present paper reviews the design and proper functioning of an ideal media preparation room.

Key words: Dehydrated media, Dispensing, Equipments, Glassware, Storage, Sterilization, Sterility check.

Introduction

Media preparation room is the basis and backbone of a microbiology laboratory. It is a place where all the media i.e. the nutritional components required for growing and visualizing microorganisms is prepared. An ideal media room should be designed in such a way that it should be always free of moisture, humidity and exposure to direct sunlight. It should be well ventilated and supplied with electricity and water all the 24 hours. Adjacent to the media room should be the washing area containing sinks.

Equipment, Tools & Glassware used in a media preparation/ storage room: Various equipment's, tools and glassware used in a media preparation room are Dehydrated media or media components, Chemicals, Stains and dyes, Calibrated digital weighing balance, Calibrated pH-meter, Autoclave, a laminar air flow bench for pouring of media, Distillation apparatus, Refrigerators for storing the prepared media, Hot plate, Spatula, Petri dishes, beakers-flasks (high quality, borosilicate glass (all sizes), Test tubes (small, medium and large), Pipettes, NaOH solution, HCl solution, Dispensing bottles, Liquid media dispenser, Identification labels, Distilled and double distilled water, Marker pens, Cotton, Syringes and Protocols for all types of media preparation.

Receiving and Storing of dehydrated media: After receiving a new lot of dehydrated medium, it should be recorded in the log book of microbiological media receipt for the following information: Name of media, Lot number, Quantity received, (i.e., size and number of containers), Date received, Expiration date, Storage location, Date opened etc.

1. The dehydrated media should be properly stored, sealed tightly, safely, away from humidity or moisture, light and heat, which are the most

frequent causes of their alteration and on the specific temperature indicated on the box.

2. The dehydrated medium should be discarded if the container is expired, if the color of media has changed or if the powder is not free flowing.

Reconstitution of Dehydrated Media: It is important to properly follow the directions for the preparation of culture media on the label of each container. Distilled water should be used to prepare dehydrated culture media. All the glassware should be properly washed and rinsed before use. The media should be prepared in a flask that holds twice the final volume of the medium to avoid boiling out of media at the time of autoclaving. Appropriate amount of dehydrated medium should be weighed out accurately. The container should be tightly closed to prevent contamination. The accurate volume of distilled water should be added to the flask, followed by dehydrated medium. Culture media containing agar or gelatin must be agitated and boiled for few minutes to completely dissolve the medium. The media should be allowed to cool and reach the room temperature before adjusting its pH. After adequate cooling, the pH of the media should be adjusted using HCL 1M or NaOH 1M to the specified limit according to the manufacture recommendation.

Dispensing of dissolved media for sterilization: The liquid media (broth) should be dispensed in the glass bottles, flasks or test tubes according to the required volumes. The media should be dispensed into the specified bottles or flasks ensuring gentle mixing during dispensing to maintain homogeneity of the agar. The bottles or flasks should be either capped tightly or fitted with cotton plug at the mouth for sterilization.

Sterilization of prepared media and glassware: The autoclave set-temperature should be 121°C at 15 psi for 15- 20 minutes. Some microbiological media are thermo labile so they should not be autoclaved, but only

heated on hot plate and immediately poured. Autoclaved should never be overloaded. Bottles and flasks containing media should be carefully handled at the time of taking out from autoclave to avoid thermal shock to self. All glassware like flasks, bottles, test tubes etc. should be sterilized in hot air oven at 160°C for 2 hours prior to use for media pouring.

Dispensing of prepared Media (Plate or tube dispensing): Medium should be cooled to 50-55°C prior to dispensing in petriplates or test tubes. Media should be gently swirled before and during dispensing to ensure that it is evenly mixed. Plates should be immediately recovered to reduce the chance of contamination. Petriplate covers should be slightly ajar, for some time to reduce moisture build-up on lids.

Storage of prepared Media: The recommended expiration date of prepared culture media varies greatly. Screw-capped tubes can be stored for 6 months or longer at low to ambient temperatures. Plated media may be stored inverted in a plastic bag or other container in a refrigerator for 1–2 weeks. All media should be stored away from light and heat. Prepared media should be stored away from bio hazardous materials in the laboratory. Older lot of prepared media and reagents should be used first.

Inspection of Prepared Media: After preparation and during storage of media, the medium tubes or plates should be inspected for the following conditions

1. Uneven distribution of media in petridishes or tubes
2. Shelf-life of prepared media
3. Discoloration or hemolysis
4. Storage location
5. Integrity of packaging
6. Broken or cracked petriplates or tubes.
7. Quality, physical appearance of prepared media and accuracy of labeling
8. Any kind of contamination in prepared plates or tubes in the form of visible growth or turbidity.
9. Split or retracted , dry surfaced medium
10. Slanting or uneven filling of petridishes
11. Gel strength of the medium
12. Pitted surface or large bubbles on medium surface
13. Presence of leakage from ready petriplates or tubes
14. Prepared media should not be used if any of the above stated conditions was deviated

Disposal of expired-deteriorated and contaminated media: Prepared Media should be disposed after use or upon expiration date whichever is earlier. It should be disposed of into a trash receptacle for disposal of biological waste.

Sterility check of media: Samples of microbiological media prior to use from a lot should be incubated at 30-35°C for 24 hours, the media must not appear with any type of growth.

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