

# Use of Dehydrated Culture Media

## Receipt of Dehydrated Culture Media

Upon arrival in the laboratory, dehydrated culture media should be labeled with the date of receipt. Dehydrated culture media are hygroscopic and sensitive to heat, light, and moisture.

1. Store media as directed on label, usually below 30°C in a dry area, away from direct sunlight, autoclaves, drying ovens, or other heat sources. When indicated, store at 2 - 8°C.
2. Check expiration date on the label. Expiration dates vary depending upon the culture media.
3. If possible, use inventory in lot/batch number order. When the container is opened for the first time, note this date on the label. After use, make sure the container is tightly closed and returned to the proper storage area.
4. Discard medium if the powder is not free flowing, or if its appearance has changed from its original color or consistency.

## Reconstitution of Dehydrated Culture Media

Directions for the preparation of culture media are provided on the label of each bottle. Follow these directions carefully, using the guidelines below.

1. Dehydrated culture media should be prepared with purified water, having a neutral pH.
2. Rinse all glassware before use to remove any trace substances, especially detergents. Prepare the medium in a flask that holds twice the final volume of the medium to allow adequate mixing.
3. Weigh out the appropriate amount of dehydrated medium quickly and accurately. Avoid creating dust. Do not inhale powder. Close the container as soon as possible to prevent contamination.
4. Add half of the required volume of water in the flask, followed by the weighed quantity of medium. Agitate briskly for a few minutes until a homogeneous suspension is obtained. Add the remaining water, ensuring any medium that has adhered to the wall of the flask is added to the liquid.
5. Culture media without agar or gelatin (broth formulations) can usually be dissolved with gentle heat and agitation. Culture media containing agar or gelatin must be heated with frequent agitation and boiled for one minute to completely dissolve the medium. **Caution:** Overheating culture media containing agar will cause it to boil over very rapidly.
6. After adequate cooling, media that should not be autoclaved are ready to pour into petri dishes or tubes. Most culture media require sterilization in an autoclave. Refer to label directions.

## Sterilization

The recommended steam sterilization cycle is 15 minutes at 121°C for 1 liter or less. Larger volumes may require longer cycles. Autoclaves vary in performance and thermocouple tests using volumes of media should be carried out to verify heating and cooling times.

## pH Adjustments

Commercial dehydrated culture media are prepared so the final pH conforms with the label specification. The pH should be tested after the medium has cooled to 25°C and solidified. For filter sterilization, adjust the pH if necessary, prior to filtering. Avoid excessive pH adjustments because this can alter the chemical composition of the medium.

## Supplements

Add supplements to media cooled to 45 - 55°C, because they are frequently heat sensitive. Swirl after addition to ensure adequate mixing. Sterile broths may be cooled to room temperature before adding supplements. Sterile blood used for the preparation of blood agar should be fresh and stored at 2 - 8°C. Warm blood in an incubator (35 - 37°C) before adding to sterile medium. Defibrinated blood is recommended for use rather than blood containing an anticoagulant.

## Dispensing Media

1. Medium should be cooled in a water bath to 50 - 55°C prior to dispensing.
2. Gently swirl medium before and during dispensing to ensure that it is evenly mixed. Dispense quickly.
3. Immediately recap or recover tubes to reduce the chance of contamination. Petri dish covers should be slightly ajar for 1 – 2 hours to reduce moisture build-up on lids.

## Storage

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 or longer. Store all media away from light.

It is good laboratory practice to establish expiration dates for all prepared media formulations, record the information for future lot numbers and documentation, and date the containers. Internal environments vary greatly and can affect media performance and appearance. Dispose of prepared media if contamination occurs, appearance changes, hemolysis is present, or signs of dehydration including shrinking or cracking appear.

## Quality Control

Consistent, documented quality control procedures and results are essential for every laboratory. Quality control tests ensure culture media are prepared according to label directions, and performance characteristics are within specification. Each lot / batch number of medium that is prepared in-house should be tested for the following parameters:

1. pH value: Check pH of prepared medium (after cooled to 25°C) to ensure the pH falls within the range on the product label.
2. Sterility: A representative sample of each lot / batch number of medium should be incubated for 2 – 5 days at 35°C. There should be no evidence of microbial growth after incubation.
3. Growth performance: Test each lot / batch number of medium to ensure it will support the growth of appropriate microorganisms. Inoculate medium with appropriate stock cultures, and if required, perform necessary biochemical tests. Comparing old / new lot numbers is extremely helpful.
4. Stability: Periodically perform the above procedures on stored prepared media to determine that storage conditions will give optimal results.

Certificates of Analysis are obtainable for every formula and lot number of dehydrated culture media available from Acumedia. Certificates of Analysis contain the minimum quality control specifications for each lot number of dehydrated culture media.

If a prepared medium does not perform as expected please inform Technical Service. Record the nature of the problem and method of preparation. Note the lot / batch number and the date prepared and received.

## Precautions

Dehydrated culture media from Acumedia are for Laboratory Use or for Manufacturing Use as labeled. Follow usual precautionary measure for handling chemicals when working with dehydrated culture media. Keep all culture media away from food and beverages. Immediately remove soiled and contaminated clothing. Avoid contact with skin and eyes.

Observe aseptic techniques and follow all laboratory precautions against microbiological hazards when performing any procedure. All samples should be treated as infectious. The use of a biohazard cabinet is recommended when working with pure cultures or samples suspected of containing fungi, brucellae, or mycobacteria. Follow universal precautions when handling blood or blood products.

## Disposal

After use, prepared plates, samples, sample containers, or other contaminated materials must be sterilized or incinerated before discarding. Directions for use of the incinerator or autoclave must be followed carefully. Dispose of all autoclaved biohazards in accordance with applicable federal, state, and local environmental regulations.