Bacterial Cultures Care Guide

Introduction

Use the following guide to learn how to properly care for bacterial cultures.

Safety Precautions

After use, agar plates will likely contain viable microbes. Although the bacteria are not likely to be pathogenic, do not open the plates unnecessarily. Use sterile techniques at all times when handling bacterial cultures. When plates are done being used, they should be autoclaved or opened under a 10% bleach solution and soaked for at least one hour. Bleach solution is a corrosive liquid that may discolor clothing and cause skin burns. Avoid contact of bleach with heat, acids and organic materials; chlorine gas will be generated. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Culturing and Maintenance

Prior to receiving bacterial cultures it is helpful to be aware of the required conditions for the particular strain of bacteria purchased. Each species has specific conditions that are necessary for optimal growth. Refer to the Bacterial Cultures Table below for incubation temperature and the appropriate general medium.

Bacterial Cultures Table

Description *(Genus species)*	**Incubation Temperature†**	**Medium**
Bacillus cereus	(20–35) 30 °C	Nutrient agar
Bacillus mycoides	30 °C	Nutrient agar
Bacillus megaterium	(25–35) 30 °C	Nutrient agar
Bacillus subtilis	(25–35) 25–30 °C	Nutrient agar
Enterobacter aerogenes	(30–37) 30 °C	Nutrient agar or MacConkey agar
Escherichia coli	37 °C	Nutrient agar or lysogeny broth
Lactococcus lactis	25–37 °C	Tryptic soy agar
Micrococcus luteus	25 °C	Nutrient agar
Micrococcus roseus	25–30 °C	Nutrient agar
Neisseria subflava	25–37 °C	Tryptic soy agar
Pseudomonas fluorescens	(25–30) 25 °C	Nutrient agar
Rhodospirillum rubrum	25–30 °C	Tryptic soy agar
Sarcina aurantiaca	25–28 °C	Nutrient agar
Sarcina subflava	25 °C	Nutrient agar
Serratia marcescens	(5–40) 25 °C or 37 °C*	Nutrient agar
Spirillum volutans	30 °C	Nutrient broth
Staphylococcus epidermidis	37 °C	Nutrient agar or tryptic soy agar
Streptococcus lactis	25–30 °C	Tryptic soy agar
Streptococcus salivarius	30–37 °C	Tryptic soy agar or blood agar
*Vibrio fischeri***	25 °C	Photobacterium agar

†The temperatures in parentheses are the range and the temperature not in parenthesis is the optimal temperature.
*Higher temperature expresses pigment formation.
**Reculture on fresh photobacterium agar upon arrival for optimal growth. A fresh slant is sent with order.

Always keep culture tubes sealed with caps or foam plugs. Sealing the tubes prevents cross-contamination of cultures and inhibits dehydration of the medium. Although all bacterial cultures sold from Flinn Scientific are considered non-pathogenic, always practice aseptic laboratory technique.

Bacterial cultures will need to be sub-cultured to fresh media every two to three weeks to ensure a thriving culture. New cultures require approximately 24 to 48 hours in order for colonies to fully develop. Refrigeration will retard growth rates,

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allowing more time to work with cultures long-term if desired.

Many bacterial cultures grow on nutrient agar but some require more specialized media. Mueller-Hinton agar is a solid growth medium commonly used for antibiotic susceptibility testing. MacConkey agar is a culture media used to isolate and differentiate organisms that are capable of fermenting lactose. It contains bile salts and crystal violet that inhibits growth of gram-positive bacteria while promoting growth of gram-negative bacteria. Lactose and neutral red are added to the agar to differentiate lactose fermenting bacteria. Lactose fermenting bacteria form pink colonies. Non-lactose fermenting bacteria form colonies that appear colorless.

Maintaining a pure culture is extremely important when working with bacterial cultures. It is essential to avoid all contaminants. Culture dishes should be incubated upside down, this will prevent excessive accumulation of moisture from flooding or diluting the surface of the medium. As a means of preventing the medium in dishes from drying and cracking, place a small container of water in the incubator with the cultures.

Flinn Scientific does not recommend that students randomly culture bacteria or fungi from biological sources (oral swabs, coughing, etc). The potential for culturing dangerous concentrations of pathogenic bacteria is too great. Culture dishes should always be taped shut following inoculation.

Medium Preparation

Prepared Media

Flinn sells a variety of prepared media that is ready for use immediately upon arrival. Try to order prepared media as close to the use date as possible to optimize results. If unable to utilize the media, refrigerate upon receipt and use within one month.

Dehydrated Media

Flinn offers a wide range of dry media that can be prepared prior to use. Each media has specific preparation instructions listed on the bottle. However, here are some important tips to keep in mind when preparing the dehydrated media. Agar-based media needs to be heated slowly and stirred frequently. Do not attempt to sterilize more than 1-liter in a single container. When sterilizing containers, make sure containers are covered with a loose cap or the mouth is covered with aluminum foil. Never attempt to sterilize a tightly closed container. Standard parameters for steam sterilization are 15 minutes at 15 pounds of pressure (121 °C). Preparation of 1-liter of medium should pour 50–60 standard (15 × 100 mm) Petri dishes or 130–140 standard (16 × 150 mm) culture tubes.

Specialty Agar Recipes

Lysogeny Broth

A standard recipe for 1-liter of Luria broth and Luria agar is to dissolve 10.0 g of Tryptone, 5.0 g of yeast extract and 10.0 g of sodium chloride in 1 liter of distilled or deionized water. For Luria agar (LA), add agar to a final concentration of 1.5%. Slowly heat the mixture to boiling, stirring frequently to dissolve ingredients. Test the pH and adjust to 7.0 using a 0.1 M solution of sodium hydroxide. Finally, sterilize the solution by autoclaving for 15 minutes at 15 psi, from 121–124 °C.

Photobacterium Agar

Photobacterium agar is used with *Vibrio fischeri*. *V. fischeri* should be cultured onto fresh photobacterium agar immediately upon arrival for optimal growth. To prepare the agar, place the following ingredients into 1 liter of distilled water, stirring after each addition. Use 1.5 g of calcium chloride, 5.5 g of magnesium chloride, 6.9 g of magnesium sulfate, 5.0 g of peptone, 0.7 g of potassium chloride, 28.2 g of sodium chloride and 3.0 g of yeast extract. Measure the pH of the solution and adjust to 7.4 with a 0.1 M solution of sodium hydroxide. Add 15 g of agar and mix well while slowly heating to a boil. Then, autoclave the solution for 15 minutes at 15 psi at 121 °C. Allow the solution to cool to 50–55 °C and pour into sterilized culture dishes.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The agar plates, inoculating loops, and pipets may be disposed of according to Flinn Suggested Biological Disposal Method Type I by sterilizing the agar plates with a freshly prepared 10% bleach solution or autoclaved at the culmination of the activity.

Bacterial Cultures materials are available from Flinn Scientific, Inc.

Catalog No.	Description
AP1051	Inoculating Loop, Nichrome Wire
N0019	Nutrient Agar, Dehydrated, 100 g
FB0526	Nutrient Agar, Prepared, 10 plates
FB0528	Tryptic Soy Agar, Prepared, 10 plates
M0203	Mueller-Hinton Agar, Dehydrated, 100 g
M0209	MacConkey Agar, Dehydrated, 100 g
AP1565	Incubator

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.