# PRACTICAL MYCOLOGY

**(THE FIRST COURSE)**

**LAB-1**

# LABORATORY SAFETY
AND
# CULTURE MEDIA

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## Generalized sterile and safety techniques

To avoid hazards and contamination problems, each student must comply with the following steps:

1. Wear a clean lab coat to protect clothing and to reduce possible contamination of cultures.
2. Wear rubber gloves and protective mask for inhalation inside lab.
3. While in the laboratory avoid any hand to mouth operations, such as eating, or licking adhesive labels.
4. Wash hands thoroughly with soap and water, both before and after working with cultures.
5. Keep work surface clear of any unnecessary objects (e.g., books, purses, etc.)
6. Wash work surface with a capable disinfectant, such as 5% Lysol or 70% alcohol, both before and after working with cultures.

**Sterilization:**

Sterilization may be defined as the complete destruction of all organisms, or their removal from materials by means of heat ,chemicals, irradiation or filtration methods.

**Physical Method :-**

**Heat:** The usual methods employed for the sterilization of laboratory materials which involves:

1- **Dry heat** (oven): this is used for sterilizing all kinds of laboratory glassware, such as test tubes, pipettes, Petri dishes, and flasks, it may be used to sterilize other laboratory materials and equipment that are not burned by the high temperature of the sterilizer and this is operated at temperature of 160 to 180 °C for a period 1½ hr.

2- **Moist heat** (autoclave): this is used to sterilize most type of solid and liquid media with and without carbohydrate, gelatin media, distilled water, normal saline, rubber tubing and gloves. The autoclave is operated usually at 15 Ib. steam pressure for a period of 15 min., which corresponds to temperature of 121°C.

**Radiation sterilization:** Gamma rays are commonly used for sterilization of disposable medical equipment, such as syringes, needles Ultraviolet lite irradiation (UV, from a germicidal lamp) is useful only for sterilization of surfaces and some transparent objects

**Chemical Method:-**

**Chemical sterilization:** Chemicals are also used for sterilization. heating, it is not always appropriate, because it will damage heat-sensitive materials such as biological materials, fiber optics, electronics, and many plastics.

**Ethylene oxide:** (EO or EtO) gas is commonly used to sterilize objects sensitive to temperatures greater than $60^0$C such as plastics.

**Ozone:** is used in industrial settings to sterilize water and air, as well as a disinfectant for surfaces.

**Mechanical Method:-**

**Filtration:** some solution cannot be sterilized by heat without being greatly altered in their physical and chemical properties. Serum , antibiotics, enzymes and other preparation containing heat –sensitive compounds are best sterilized by process of filtration. The type of filters employed for this purpose include: Berke feld Filters, Fritted – glass Filters and Porcelain or Chamber land Filters.

## culture media

### Media

All organisms need nutrients for growth and bioactivity, so the FUNGI, require some form of carbon source for energy and nitrogen source for the synthesis of amino acids, purines, pyrimidines, nucleic acids, enzymes, vitamin. And other major elements (oxygen, hydrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron). The substance on which microorganisms are growth in the laboratory is called a medium and the microorganisms that growth on it, a culture. Culture media can be solid or liquid, depending on the sort of information the researcher to obtain. Such as For the aim of identification.

### Types of culture media according to (Physical State)

**Solid media**

Characterized this media wherein agar is added as the solidifying agent. Ex. PDA

**Liquid media**

Prepared without substances like gelatin or agar. Ex. PD broth.

**Semi-solid media**:

Such media are useful in bacterial motility test and separating motile from non-motile strains.

### Types of culture media according to (Chemical State)

**Synthetic media**

Are composed of substances of known chemical composition and concentration.

**Semi-synthetic media**

Are similar to Synthetic media in they contain a known group of substances but differ in that at least some of the substances have an unknown or variable composition.

**Natural media**

They are partly or completely composed of natural materials.

**Types of culture media according to USE**

**Simple media**

It is used to growth different types of microorganisms. (**PDA**)

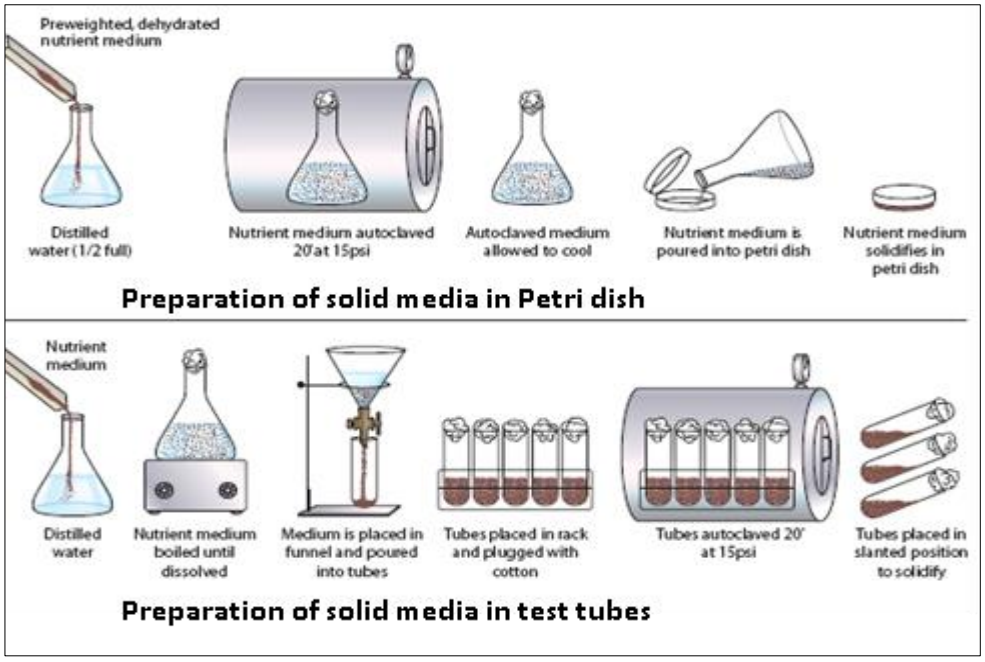
**Selective media:**

Many media contain selective components that inhibit the growth of non-target microorganisms. ( **Sabouraud's Agar**)

**Preparation of Media**

The substances in a medium are usually dissolved, and the medium is then sterilized. When agar is used as a solidifying agent, the medium must be heategently, usually to boiling, to dissolve the agar. In some cases where interactions of components, such as metals, would cause precipitates, solutions must be prepared and occasionally sterilized separately before mixing the various solutions to prepare the complete medium. The pH often is adjusted prior to sterilization, but in some cases sterile acid or base is used to adjust the pH of the medium following sterilization.

Many media are sterilized by exposure to elevated temperatures. The most common method is to autoclave.



**Common media:**

**1.Czapek's Solution Agar**

**2- Water agar (e.g. a 2% agar solution)**

**3- Potato Dextrose Agar**

| | |
|---|---|
| Thinly sliced, peeled white potatoes | 200 g |
| Dextrose | 20 g |
| Agar | 15 g |
| Distilled water | 1000 ml |

**Method:**

Scrub the potatoes clean, do not peel, cut into 12mm cubes. Weight out 200g, rinse rapidly in running water, place in 1 l water and boil until soft (1h), mash and squeeze as much of the pulp as possible through a fine sieve. Add agar and boil till dissolved. Add dextrose and stir till dissolved. Make up to 1l. agitate stock while tubing to ensure that each tube has a proportion of solid matter. Sterilize at 15 P.S.I. for 15 min.

**4- Sabouraud's Agar**

| | |
|---|---|
| Glucose | 40 g |
| Peptone | 10 g |
| Agar | 15 g |
| Distilled water | 1000 ml |

**5- Malt extract Agar**

| | |
|---|---|
| Malt extract | 20 g |
| Agar | 15 g |
| Distilled water | 1L |

**6- *Phytophthora* Isolation Medium No. 1**

**7- *Rhizoctonia* Isolation Medium**

**Note:** Antibiotic can be added to media to prevent the growth of bacteria. These are especially used for first planting, and also to purify cultures contaminated with bacteria. Useful antibiotics are e.g. chloramphenicol , novobiocin and tetramycin. They are used singly or in various combinations, usually at a concentration of 5 mg/L.