Medical Mycology

470 MBIO

Medical Mycology

In this course we will focus on:

- understanding the principle of some clinical techniques used for the identification and diagnosis of fungal infection.
- **fungal infections,** or mycosis, which are diseases caused by a fungus (yeast or mold

* Basic steps will be addressed before moving to the diagnostic techniques

- How to prepare fungus media (CZA and SDA)
- Isolation

Fungus media

- **A wide range of media are used for growing fungi**
- Media will affect **colony morphology and color**
- Media generally contain a source of carbon, nitrogen and vitamins.
- Glucose is the most widely utilizable carbon source followed by Fructose.
- Nitrogen sources include peptone, yeast extract.

Fungus media

•Two general types of culture media are essential to ensure the primary recovery of all clinically significant fungi from clinical specimens.

1-One medium should be **nonselective** (such as potato dextrose agar PDA) that is, one that will **permit the growth of all clinically relevant fungi**

2-The other medium **should be selective**, specially designed to isolate specific pathogenic fungi.

Fungus media

1-Czapek's Agar (CZA)

* This is a **defined medium** primarily for the cultivation of fungi (**saprophytic fungi**) that are **capable of using Sodium Nitrate as their main source of nitrogen**

Composition of the (CZA)

Ingredients	Benefits	Grams/L
Sucrose	a carbohydrate source	30
Sodium nitrate	Inorganic source of nitrogen	2
Dipotassium phosphate		**1**
Magnesium sulfate		0.5
Potassium chloride		**0.5**
Ferrous sulfate		0.01
Agar	**added as the solidifying agent**	**15**
Distilled Water		1000

Final pH
Final pH (at 25°C)
7.3±0.2

Fungus media

2- Sabouraud agar or Sabouraud dextrose agar (SDA)

*Type of agar growth medium containing **peptones**

*Commonly used for research and clinical diagnosis

*SDA is sufficient for the recovery of **dermatophytes from cutaneous samples**

Composition of the (SDA)

Ingredients	Benefits	Grams/L
Peptone	**provide the **nitrogen and vitamin source** required for fungal growth	10
Dextrose	a carbohydrate source which serves as a growth stimulant	40
Agar	added as the solidifying agent	15
Distilled Water		1000 ml

Final pH (at 25°C) 5.6±0.2

Fungus media

3-Potato dextrose agar (PDA)

- **A general-purpose media** for yeasts and fungus
- How to **inhibit bacterial growth in PDA**

* 1-Can be **supplemented with antibiotics to inhibit bacterial growth**

*2- **Use a specified amount of sterile tartaric acid (10%) to lower the pH of this medium which inhibits bacterial growth.**

Composition of the (PDA)

Ingredients	Benefits	Grams/L
Potatoes infusion	provides a nutrient base for the growth of most fungi	200
Dextrose	a carbohydrate source which serves as a growth stimulant	20
Agar	added as the solidifying agent	15
Distilled Water		1000 ml

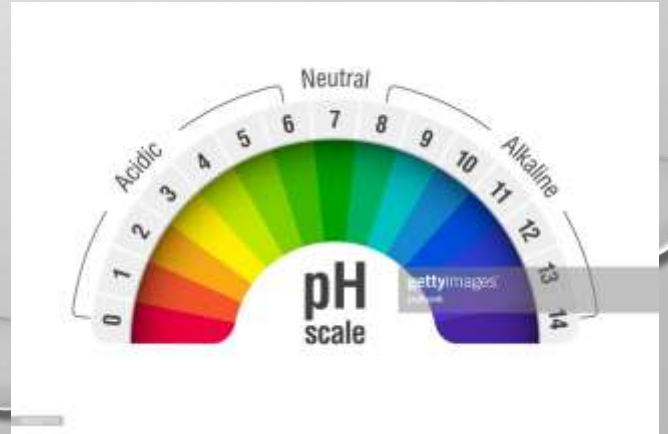
Final pH (at 25°C) 5.6±0.2

- Weigh all the ingredients (gram/L)
- Suspend all the ingredients in 1000 ml distilled water.

Even though we follow a precise protocol (recipe) to make any media, we still need to make sure that the of this media is suitable for fungal growth.

How to adjust the pH?

- Determine the pH level you have **using pH Meter**.
- Add either an acidic or alkaline substance to the media
- **To increase the pH use few drops of (0.1 M) Sodium Hydroxide(NaOH)**
- **To lower the pH use few drops of (0.1 M) hydrochloric acid (HCL)**



PH Adjustment - YouTube

- After adjusting the pH
- Use cotton plug and foil to close the flask (you can use Bottle with cap)
- **Label your flask with:**

A) Tape that has (your name- media type- date)

B) Autoclave tape that changes color upon appropriate sterilization

- If necessary, use warm water bath to dissolve the medium completely.
- **Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes.**

- **In specific clinical work, when pH 3.5 is required, the medium should be acidified with sterile 10% tartaric acid**

- **Antibiotics can be added to create selective culture**

- **In this case:**

- **Prepare the media as described , autoclave , THEN add acid/antibiotics**

And finally pour the media immediately.

DO NOT heat the media after adding the acid/antibiotics.

- Use a marker to briefly label the edge of petri dishes (name-date- experiment)

Example; Haya, 27/8, isolation/ or purification…etc

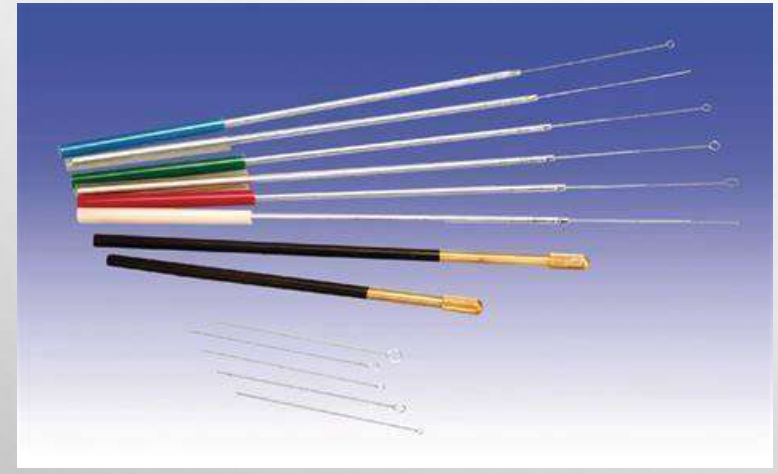
** **label the bottom of the plate only, NEVER label the lid**

- Pour the sterile media in petri dishes and wait until the media is completely solidify.

Isolation (from plants/fruits)

Under sterile conditions, use the appropriate tools to inoculate part of the fungal growth in suitable media

- In Microbiology labs, the most common tools used for this purpose is the inoculating needles.



- Needles can be used if there is visible mycelium/ spores

- **If you don’t see visible mycelium/spores, use sterile scalpel and forceps** to cut small section of the rotten tissue and plate it.

- **Incubate at 25-28^{o}C** for several days (keep tracking incubated plates to avoid overgrowth).

Fungus culture Purification

Disk method

- Label your plate (as you can't invert it post the purification process)
- Use sterilized glass Pasteur pipettes to cut out a disk from the **margin of the fungal** colony
- Use sterile forceps to invert the disk at the center of new plate (growth side should face the media)
- ** to make sure the fungus has access to all the nutrition required for an ideal growth
- Incubate as required

What is the purpose of the purification process (sometimes known as subculturing process)

A)-Characteristics (identification) of an infectious agent

1. Colony morphology (shape, margin, color)

** It has limited value in identification of fungal growth because most fungus show different morphological features on different media.

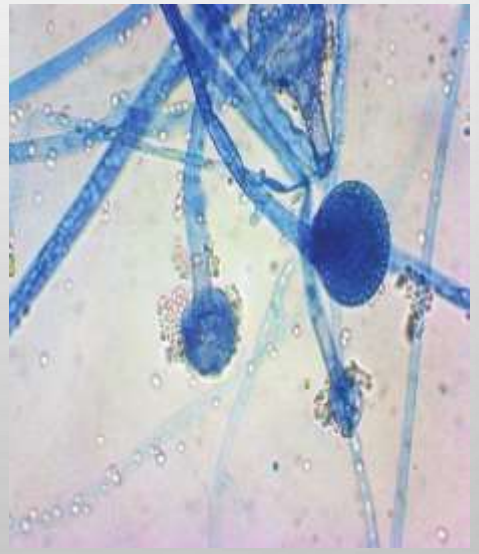
2. Microscopic observation

- using common staining reagent : Lactophenol cotton blue and use 40X objective to describe (septate /aseptate (non-septate hyphae), conidia shape..etc

B)-To maintain viability.

The following microscopic criteria are used to make a genus/species identification of the fungal isolate

1. Determine the structure of the hyphae. Are they : Septate or aseptate
2. Branching or not branching
3. Pigmented or not pigmented
4. The **size, shape, and arrangement of the spores**



(2) How to prepare a Scotch tape slide for fungal micromorphology assessment - YouTube