

Preparation of Mycological Media & staining



Preparation of Mycological Media

- **Mycological media used to isolate, culture and then identify the fungi**
- **Some culture media used as preservation media**

Preparation steps:

- 1-Weigh the required amount of powder needed to dissolve in distilled water (based on the manufacturers specification in the container).
- 2- Dissolve the proper amount of the powder in flask with water. Label the flask with autoclave tape and cover it with an aluminum foil .
- 3- Sterilize it in the autoclave at 121 °C for 15- 30 min.
- 4- Leave the flask to cool till 45 °C.
- 5-Pore or dispense in appropriate container either Petri dishes or tubes
- 6- If we are not going to use it put it in the fridge (4 °C).

Preparation of Mycological Media

- The preparation of culture media from commercial dehydrated products is simple and straightforward. Each bottle of dehydrated medium has instructions for preparation on its label.

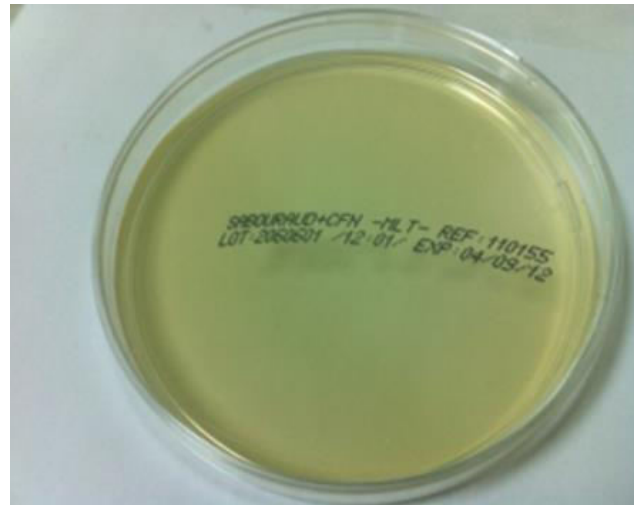


1- SDA (sabouraud dextrose agar)

- SDA is general, basic and the most common media used to isolate the fungi from clinical specimen.
- PH = 5.6 , This acidic pH inhibits many species of bacteria.

- Preparation:

D(+) glucose 40g
Peptone 10g
Agar 20g
D water 1000 ml



2- MSDA (Modified Sabouraud Dextrose Agar) or Emmons Media

- This type of media used to isolate systemic fungal infection
- Emmons modified the formula by adjusting the pH and reducing the glucose concentration . This neutral pH enhances recovery of some pathogenic fungi
- pH =6.9-7.0
- Preparation:

D(+)	glucose	20g
	Peptone	10g
	Agar	20g
	D water	1000 ml

3- SDA CC (Cycloheximide and chloramphenicol)

- It is a selective media (allow growth of some pathogenic fungi especially at RT).
- it is also called Mycobiotic or mycosel

- Preparation:

Glucose	10 g
Peptone	10 g
Agar	15.5 g
Cycloheximide	0.5 g
Chloramphenicol	0.05 g
D water	1000 ml

4- CMA(Corn Meal Agar)+tween 80

- This media is poor media, why ??
- It is used to see the sporulation of yeast, distinguish the different species of Candida, useful in slide culture technique.
- Tween 80 : used to maintain the surface tension (to keep the media intact)

- Preparation :

Corn meal agar 7 g
Tween 80 3 ml
D water 1000 ml

5- Bird Seed Agar

- It is a selective media that allow grow of *Cryptococcus neoformans* and inhibit other *Cryptococcus* species and yeasts.
- pH: 6.7

- Preparation:

Seeds	70 g
Creatinine	0.78 g
Dextrose	10 g
Chloramphenicol	0.05 g
Agar	20 g

7- Brain Heart Infusion Agar

- It is enriched media
- It is recommended for cultivation of fastidious pathogenic fungi such as *Histoplasma capsulatum*.

6- Czapek Dox Agar

- It is used for the general cultivation of fungi.
- pH: 7.3



8- PDA (Potato Dextrose Agar)

- This media used to:
 - 1-keep stock cultures .
 - 2-Inducing more sporulation of fungi.

- Preparation:

Potatoes 200g

Dextrose 10 g

Agar 20g

D water 1000ml

Mounting and staining fluids

- Mounting and staining used to facilitate the microscopic examination of fungus morphology.

Staining used in mycology lab:

- LPCB
- LP
- 10% KOH
- India ink

LPCB (Lacto Phenol Cotton Blue)

- It is the most widely used stain.
- It is useful for making permanent mount of the fungus which is in culture

LPCB stand of:

- L (lactic acid): used to preserve the morphological structure of fungus
 - P (phenol) :used to keep the stain sterile
 - CB (cotton blue) : is the stain
 - Glycerol : to prevent the dehydration of the slid
- **LP stain:** used to see the pigment of fungi

1- LPCB (Lacto Phenol Cotton Blue)

LPCB STAIN



Stain slid with LPCB



10% KOH (Potassium hydroxide)

- This stain used for direct microscopic examination of skin scraping, hair and nail for fungal infection.
- This stain digest the keratinized tissue without effecting the fungal cells.
- Sometimes we add DMSO to prevent dehydration of the slide and allow observation for up to 24-48 hrs.

India Ink Stain

- It is also called Negative stain
- Used in examination of CSF or other fluid for the presence of encapsulated fungi as *Cryptococcus neoformans*

Saline

- It is composed of NaCl 0.85 g dissolved in 100 ml of water>> autoclave for sterilization
- Uses:
 - To prepare fungal suspension
 - Suspending clinical specimens after collection to prevent drying before processing.