LAB DIAGNOSIS OF FUNGAL INFECTIONS

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INTRODUCTION

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- Mycology
- Eukaryotic
- Rigid cell wall
- Cell membrane
- yeast/molds
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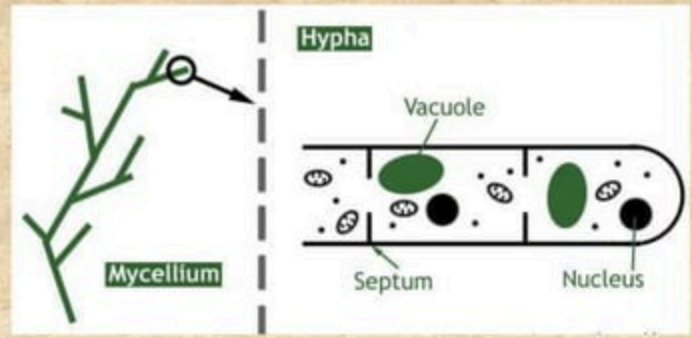


CLASSIFICATION OF FUNGI

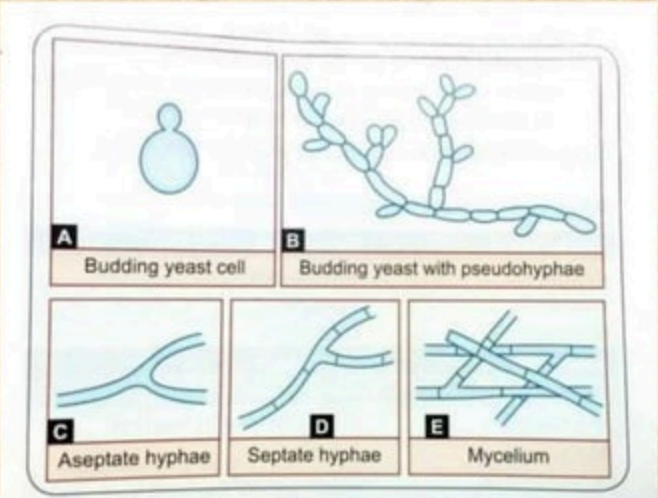
Based on morphology

1.Yeast

2.Yeast-like

3.Molds

4.Dimorphic fungi



Figs 52.1A to E: Morphological forms of fungi

CLASSIFICATION OF FUNGAL DISEASES

- Superficial mycoses
- Subcutaneous mycoses
- Systemic mycoses
- Opportunistic mycoses

COMMON ORAL INFECTIONS

- CANDIDIASIS
- ASPERGILLOSIS
- BLASTOMYCOSIS
- COCCIDIOMYCOSIS
- HISTOPLASMOSIS
- MUCORMYCOSIS

SPECIMEN COLLECTION AND TRANSPORT

Acc to Epstein and Pearsall et al. guidelines for specimen collection are

- Specimen should be collected from active lesion. Old burn out lesions don't contain viable organisms
- Specimen should be collected under aseptic conditions
- Collect sufficient specimen
- Use sterile collection devices and containers
- Specimen should be labelled appropriately

Methods of specimen collection

- Imprint
- Swab
- Impression
- Oral rinse
- Expectoration
- Paper points
- Smear

FUNGAL STAINS

WET PREPARATIONS

- KOH mount
- India ink stain
- Nigrosin stain
- Calcoflour white stain
- Lactophenol Cotton blue
- Neutral RED stain

DIFFERENTIAL STAINS

- Grams stain
- H and E stain
- Giemsa
- PAS
- Gomori's methamine stain
- Acridine orange stain
- Fluorescent antibody staining

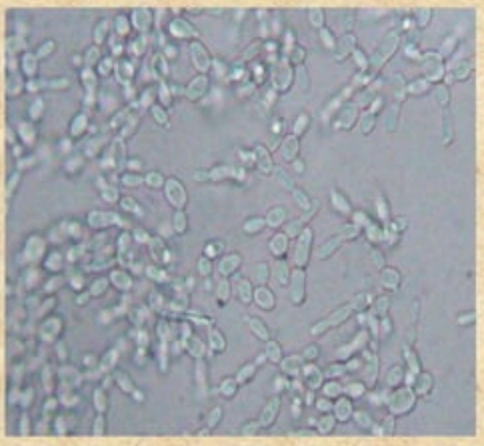
WET PREPARATIONS

KOH wet Mount

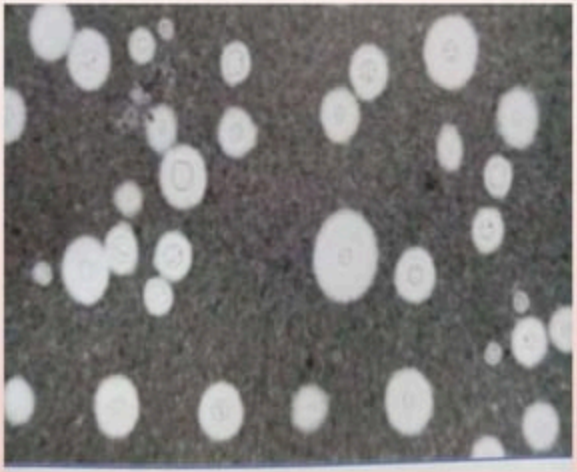
KOH 20 g
Glycerol 120 ml
Distilled water 80 ml

Dissolves protein debris
Addition of Glycerol
Slide is placed under
Brown walled hyphae and yeasts

Adv-
Dis adv-
Occasionally mixed with 10% KOH- Yeasts, hyphae & pseudohyphae are readily distinguished from background



CANDIDA

INDIA INK STAIN **(Negative stain)** **India ink 150 ml** **Merthiolate 3ml** **Tween 80 0.1 ml**	**Polysaccharide capsule repels opaque medium** **Distinct halo** **As it is a negative stain** **Ink should be free from**	
NIGROSIN STAIN Nigrosin granules 10g Formalin 100ml	Shelf life 1 yr	Irregular, encapsulated, spherical yeast cells

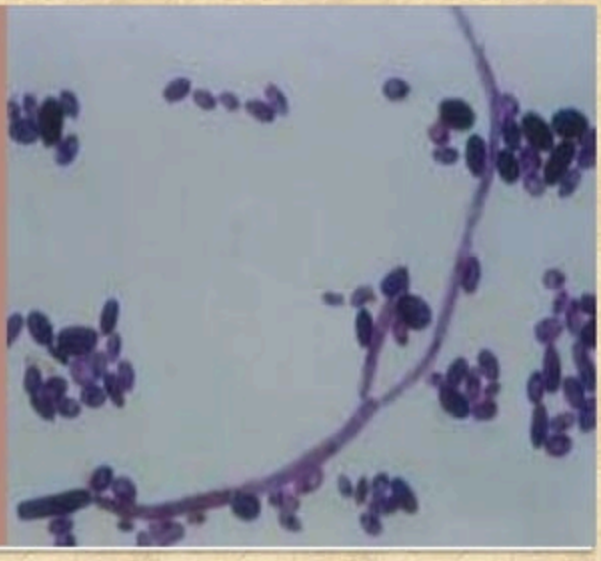
Calcofluor White Stain	Water soluble Selectively binds to cellulose and chitin
Calcofluor white 100mg Evans blue 50mg Distilled water 100ml	Superior to KOH
	Evans blue-↓non specific background florescence Light blue floresence
	Long UV and short visible Light source- Quartz halogen/mercury vapor lamp

Calcofluor white stained Candida albicans showing true hyphae (*) and pseudohyphae (+).
Citation: Journal of Oral Microbiology 2011, 3: 5771

LACTOPHENOL COTTON BLUE **Lactic acid 20 ml** **Glycerol 40ml** **Cotton blue 0.05gm** **Distilled water 20 ml** **Phenol 22ml**	**Stains outer wall of fungus** **Morphological features of fungal isolates**
NEUTRAL RED STAIN	Water soluble dye Through plasma membrane and stored in lysosome Evaluation of Viability

DIFFERENTIAL STAINS

Grams stain	Gram positive
Crystal violet Grams iodine Acetone/95%ethanol 0.5%safronine	Take up crystal Violet Yeasts –more darker Well stained morphology

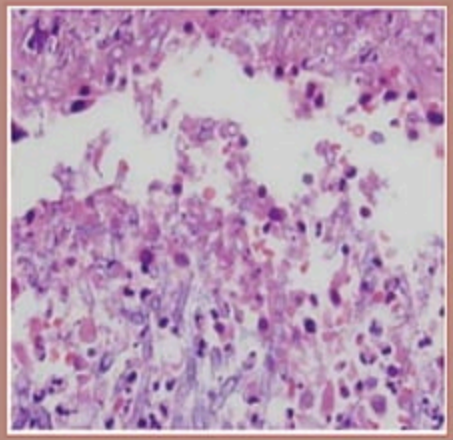


CANDIDA

H&E

Hematoxyllin 5g
Ethyl alcohol 50ml
alum 100g
eosin 1%

Does not disclose and distinguish fungal elements easily
small numbers-not located
Host response



RBC-Orange
Collagen, nerve ,amyloid-pink
Muscle, elastin , fibrin-bright red
Nuclei, RNA, Ca salts, bacteria-blue

GIEMSA

Compound stain-methylene blue and eosin

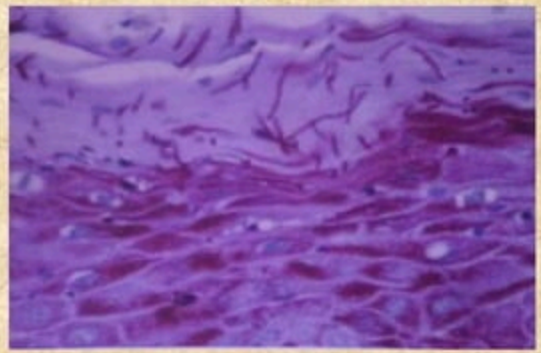
Intracellular yeast cells

Nuclei-purple

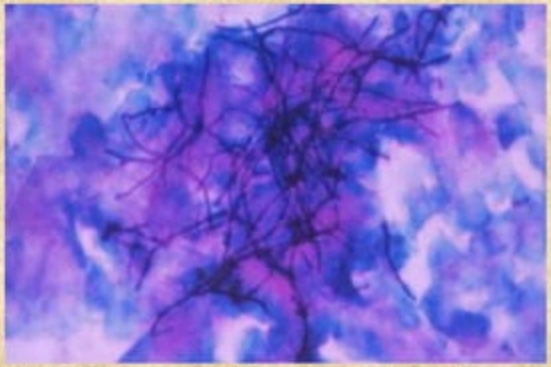
Cytoplasm-blue

RBC-Pink

PAS STAIN	1% Aqueous periodic acid Basic fuschin 1g Sod.meta bi sulphate 2g Conc.HCL 2ml	Nuceli -blue Fungi-magenta red For better result -



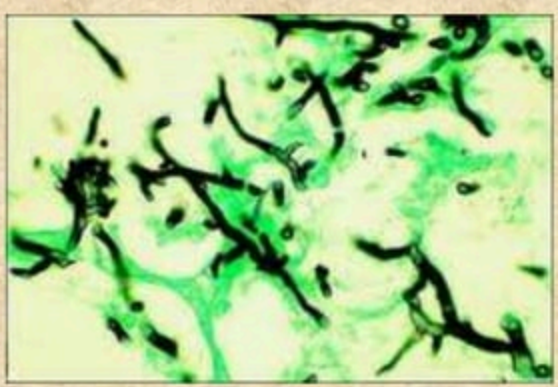
Candida-Tubular hyphae in parakeratin layer

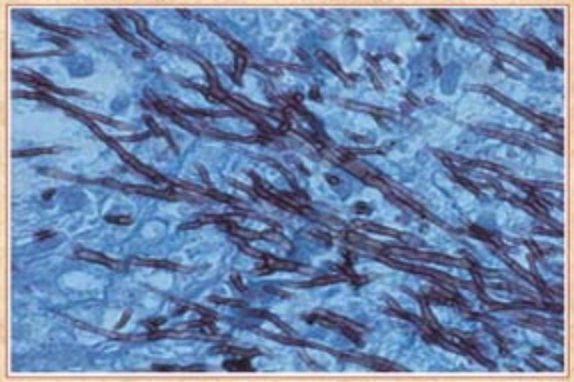


Candida-Tubular fungal hyphae and ovoid yeasts

ORAL AND MAXILLOFACIAL PATHOLOGY *Neville* 2nd edition

GOMORI-METHENAMINC SILVER STAIN	Methanamic silver nitrate solution Distilled water 50 ml Chromic acid 5% Sodium thiosulphate Alcohol,xylene,Gold chloride Tendolakar & colleagues	Deposition of reduced silver Fungi- brown to black Mucopolysaccharide-dark grey Tissue-pale green





ACRIDINE ORANGE

Affinity for nucleic acids-RNA,DNA
Bacteria and yeast – bright orange
WBC- pale apple green
RBC- may not stain or stain pale green

FLUORESCENT ANTIBODY STAINING

Tissue section or pus where organism is scanty(systemic infections)
Detects fungal antigen in smear and section
Antibody coated fungi can be demonstrated

FUNGAL CULTURE

- Basal Media
- Nutritional deficient media
- Enriched and selective media
- Differential agar media
- Media for stimulation of Ascospores
- Media used for biochemical tests

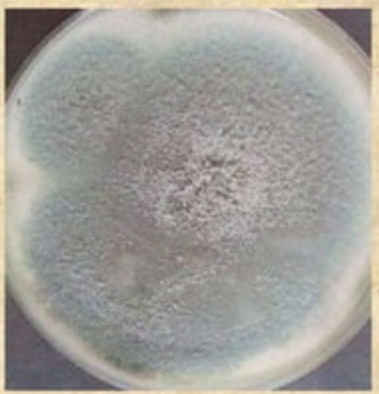
Basal Media

SABOURAUD DEXTROSE AGAR /SGA **pH -5.6**	**Peptone 10g** **Dextrose 40g** **Agar 20g** **Distilled water 1000ml** **Commonly used** **Primary isolation**
NEUTRAL SDA pH-6.8-7	neopeptone 10g Dextrose 20g Agar 20g Distilled water 1000ml
SDA+ANTIBIOTICS	SDA+ Cycloheximide 500mg Chloramphenicol 50mg Gentamicin 20mg Avoid bacterial contamination

CANDIDA

BLASTOMYCOSIS-wavy

ASPERGILLOSIS-,dryPowdery colonies



Nutritional deficient media

CORN MEAL AGAR/CORN MEAL TWEEN AGAR	**Corn meal 8g/zein 40g/100ml** **Tween80 2g** **Agar 4g** **Distilled water 200ml** **Large,highly refractile,thick walled**
RICE STARCH AGAR(RSA)	Cream of rice 4g Tween80 2g Agar 4g Distilled water 200ml pH 6.2

Enriched and selective media

BLOOD AGAR

Agar base 40g
Sheep blood 50ml
Distilled water 1000ml

Histoplasma capsulatum
Blastomyces dermatitides
Cryptococcus neoformans

Candida-moist opaque colonies

BRAIN HEART INFUSION AGAR

Brain heart infusion 37g
Glucose 20g
L-cysteine HCL 1g
Agar 20g
Distilled water 900g

Antibiotics
pH 6.7

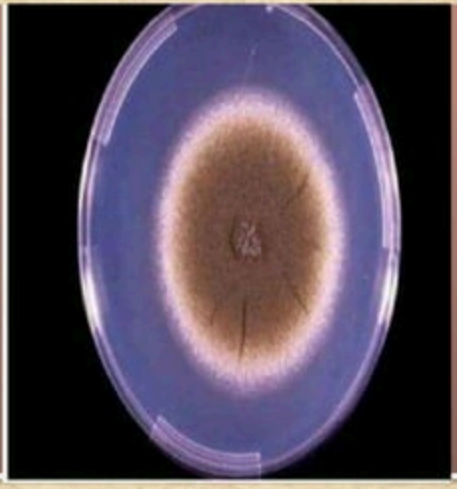
BIRD SEED AGAR/ NIGER SEED AGAR	**Niger seed extract 200 ml Glucose 1g Chloramphenicol 400mg Gentamicin 25mg Diphenyl solution 10ml Agar 20g Distilled water 800 ml**	**Primary isolation of Crytococcus neoformans**
CHOCLATE AGAR		**Candida appear as – yellow white colonies**
Sun flower seed agar PAL 'S MEDIUM	Pulverised sunflower seed 45g Chloramphenicol 100mg Agar 20 g Distilled water 1000ml	Cryptococcus neoformans Light to dark brown colored colonies

Czapek-Dox agar

NaNO33g
K2HPO41G
KCL 0.5G
MgSO4.7h20 0.5g
FeSO4.7H20.01G
Sucrose 30g
Agar 15g
Distilled water 1000 ml

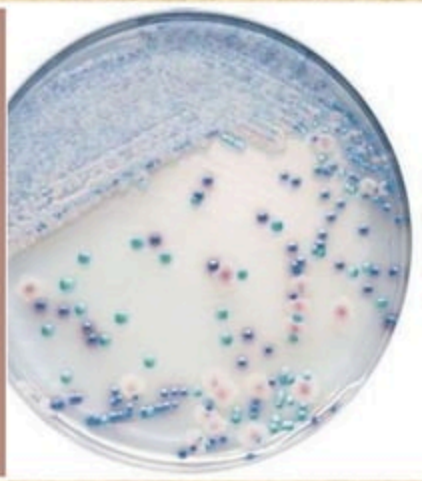
Aspergillus sps

Granular flat colonies
Radial grooves



Differential media

CHROMAGAR Ph-	Presumptive identification Direct detection of enzymatic activity Fluorochromes are added Multiple species in a specimen C.albicans –light green C.tropicalis-blue,pink halo C.Parapsilosis-cream C.Krusei-pink C.Glabrata-purple C.dubliensis-dark blue



Biggy agar :

Bismuth sulfite, glucose, glycine

c. albicans-

c. krusei-

c. tropicalis-

Pagano Levin agar

- Peptic digest – 10g/l, yeast extract – 1 gm, dextrose- 40gm, agar- 15gm
- Ph- 6.2
- Distinguishes candida sps based on ability to reduce triphenyl tetrazolium chloride
- C. albicans- cream to light pink
- C.parapsilosis- red to maroon
- C.tropicalis – red to marron
- C. krusei – white to cream spreading type of colonies

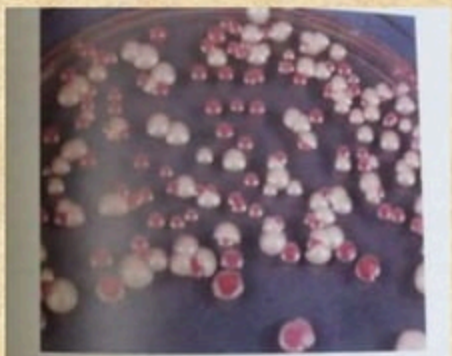


Fig. 22.3 *Candida albicans* and *C. tropicalis* growing side by side on a special medium (Pagano–Levin agar) which elicits differential colour reactions. Mixed oral candidal infections are not uncommon.

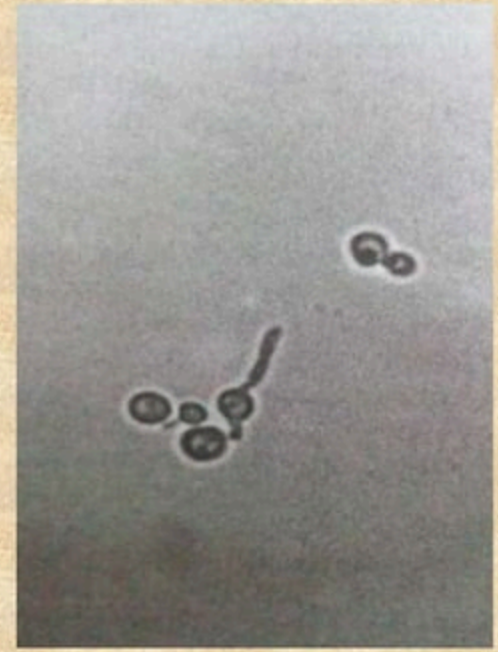
Media used for biochemical tests

TETRAZOLIUM REDUCTION MEDIUM	Tetrazolium is reduced in diferent gradients
Peptone 1g	C.albicans –pale pink
Glucose 4g	C.tropicalis –orange pink
Beef extract 0.1g	C.Parapsilosis-rose pink
Tetrazolium 20mg	C.Krusei-pink,dry
Neomycin 50mg	C.Glabrata-pale pink
Distilled water 100ml	
pH5.6-6.2	

Carbohydrate assimilation Media	**Sugar disk 4%** **KNO3 disk 1%** **Yeast nitrogen base1.17%** **Yeast carbon base 6.7%**	**Carbohydrate free medium** **Filter paper disks** **Growth around disc** **Utilization is determined** **Carbohydrate fermentation test can be**
UREASE TEST	Urea base 29g Agar 15g Distilled water 1000 ml Phenol red indicator	Ability to produce urease enzyme Urea to ammonia Amber to pinkish red Cryptococcus urease +ve Candida urease –ve
Rapid urease test	Christensen urea base 1% benzalkonium chloride	Urea to ammonia Yellow to purple Cryptococcus

GERM TUBE TEST

- Presumptive identification of candida albicans
- Reynaulds-braude phenomenon
- 5%-C.albicans negative, false positives
- Additional tests
- Human/sheep serum, incubated 2 hrs
- Long tube like process



SEROLOGICAL TESTS

- Monoclonal antibodies(MAB) are developed against mycelial cell wall protein.
- They react specifically in gel immunodiffusion test.
- Intraspecies antigenic relation ship can be elucidated

FUNGI	SPECIFIC ANTIGEN
H.capsulatum	H,M
B.dermatitidis	A

Skin tests

- Exposure and sensitization of individual to organism
- Fungal antigen injected→hypersensitivity reactions
- Epidemiological studies
- Histoplasmosis, Candidiasis, Coccidioidomycosis, Blastomycosis.
- Aspergillus-false positive results

MOLECULAR TECHNIQUES

- PCR –genome DNA is amplified and sequence typing is done
- Shorter period
- Genetic markers

RFLP (or Restriction fragment length polymorphism)
AFLP (or Amplified fragment length polymorphism)

ARTEFACTS

- KOH-crystals
- pollen, wbc, glove powder, fabric-fungi
- silver stained elastin

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