MEDICAL EDUCATION RESOURCE

Medical Mycology

? GENERAL PROPERTIES AND CLASSIFICATION OF FUNGI

? DEFINITION OF FUNGI

- Eukaryotic, heterotrophic, non-photosynthetic organisms
- Absorb nutrients ? saprophytic or parasitic
- Reproduce by spores
- Important human pathogens

? EUKARYOTIC STRUCTURE

? Key Features

- True nucleus with nuclear membrane
- Membrane-bound organelles present
- Larger than bacteria

? ULTRASTRUCTURE (VERY HIGH-YIELD)

? Cell Wall

- **Outer layer ? Mannan**
- **Middle layer ? Glucan**
- **Inner layer ? Chitin**
- Provides rigidity and antigenicity

? Cell Membrane

- Contains **ergosterol (NOT cholesterol)**
- Target for antifungal drugs

? Cytoplasmic Organelles

- Nucleus
- Mitochondria
- Endoplasmic reticulum
- Vacuoles

? Capsule

- Seen in **Cryptococcus neoformans**
- Polysaccharide capsule
- Anti-phagocytic

? MORPHOLOGY

? YEASTS

- Unicellular, oval/round
- Reproduce by **budding**
- Example ? Candida

? MOULDS

- Multicellular, filamentous
- Composed of hyphae
- Example ? Aspergillus

? DIMORPHIC FUNGI (VERY HIGH-YIELD)

- Exist in 2 forms:
  - **25°C ? mould**
  - **37°C ? yeast**
- “Mould in cold, yeast in heat”

? HYPHAE

? Types

FEATURE	SEPTATE HYPHAE	ASEPTATE HYPHAE
Septa	Present	Absent
Appearance	Segmented	Continuous
Examples	Aspergillus	Mucor

? MYCELIUM

? Types

- **Vegetative mycelium** ? nutrient absorption
- **Aerial mycelium** ? spore formation

? SPORES CLASSIFICATION (VERY HIGH-YIELD)

? Asexual Spores

- **Conidia** ? Aspergillus
- **Sporangiospores** ? Mucor
- **Arthrospores** ? fragmentation
- **Chlamydospores** ? thick-walled (Candida)

? Sexual Spores

- **Ascospores**

- **Basidiospores**
- **Zygospores**

? REPRODUCTION

- Asexual ? budding, fission, spores
- Sexual ? fusion of nuclei

? CLASSIFICATION OF FUNGI

? Morphological Classification

- Yeasts
- Moulds
- Dimorphic fungi

? Clinical Classification

- Superficial
- Cutaneous
- Subcutaneous
- Systemic (endemic)
- Opportunistic

? TABLES (VERY HIGH-YIELD)

? Yeast vs Mould vs Dimorphic Fungi

FEATURE	YEAST	MOULD	DIMORPHIC
Structure	Unicellular	Multicellular	Both
Form	Oval	Filamentous	Temp dependent
Reproduction	Budding	Spores	Both
Example	Candida	Aspergillus	Histoplasma

? Septate vs Aseptate Hyphae

FEATURE	SEPTATE	ASEPTATE
Septa	Present	Absent
Branching	Acute angle	Right angle
Example	Aspergillus	Mucor

? Spore Types Classification

TYPE	SUBTYPE	EXAMPLE
Asexual	Conidia	Aspergillus
	Sporangiospores	Mucor
	Arthrospores	Dermatophytes

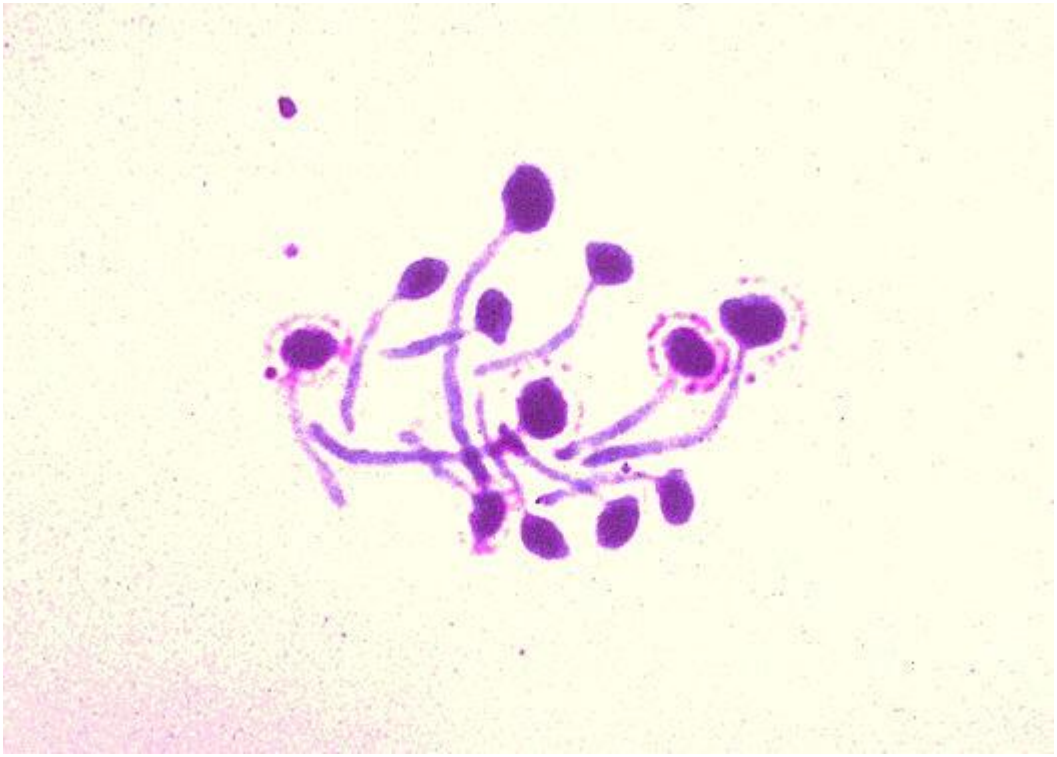
TYPE	SUBTYPE	EXAMPLE
	Chlamydospores	Candida
Sexual	Ascospores	Yeasts
	Basidiospores	Mushrooms
	Zygospores	Mucor

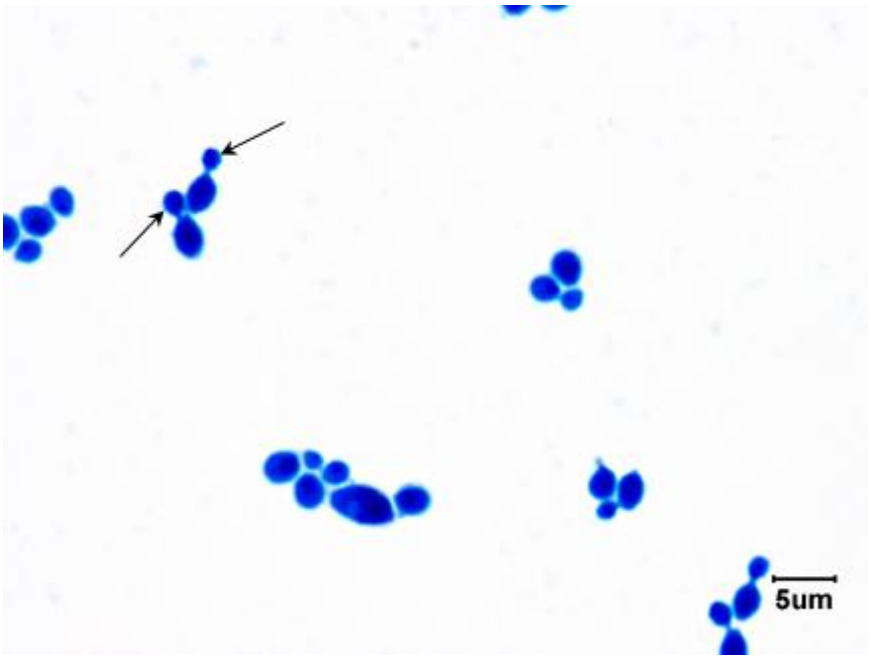
? Clinical Classification of Fungi

TYPE	SITE	EXAMPLE
Superficial	Stratum corneum	Malassezia
Cutaneous	Skin, hair, nails	Dermatophytes
Subcutaneous	Dermis	Sporothrix
Systemic	Internal organs	Histoplasma
Opportunistic	Immunocompromised	Candida

? SLIDES (EXAM FAVORITE)

? Budding Yeast (Candida)

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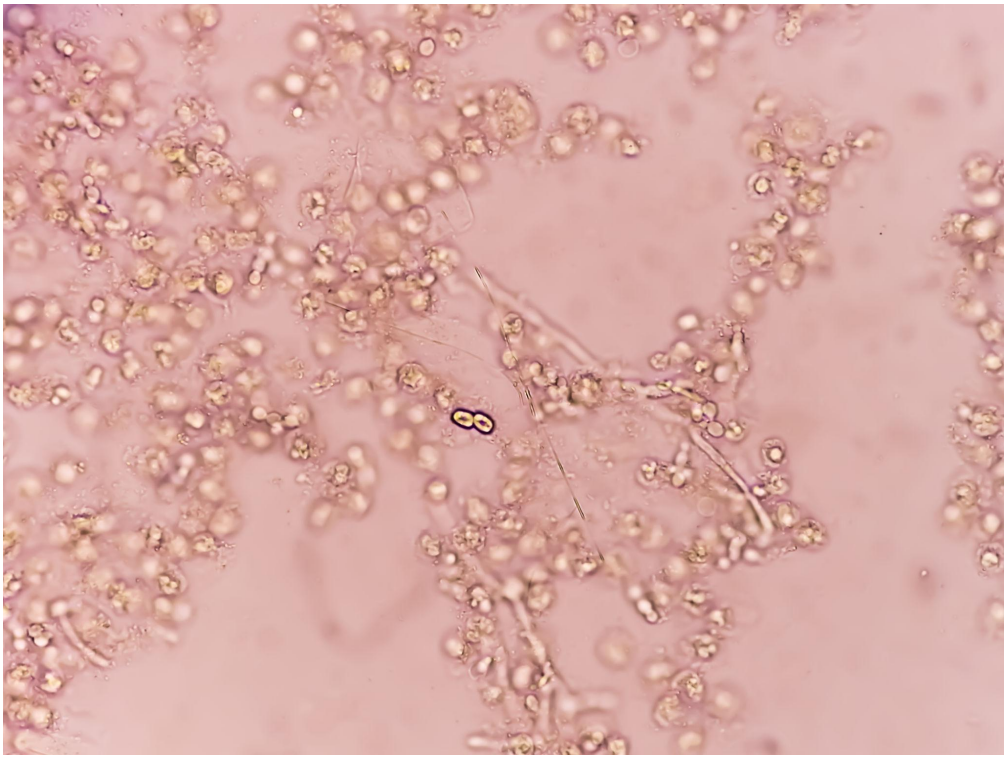
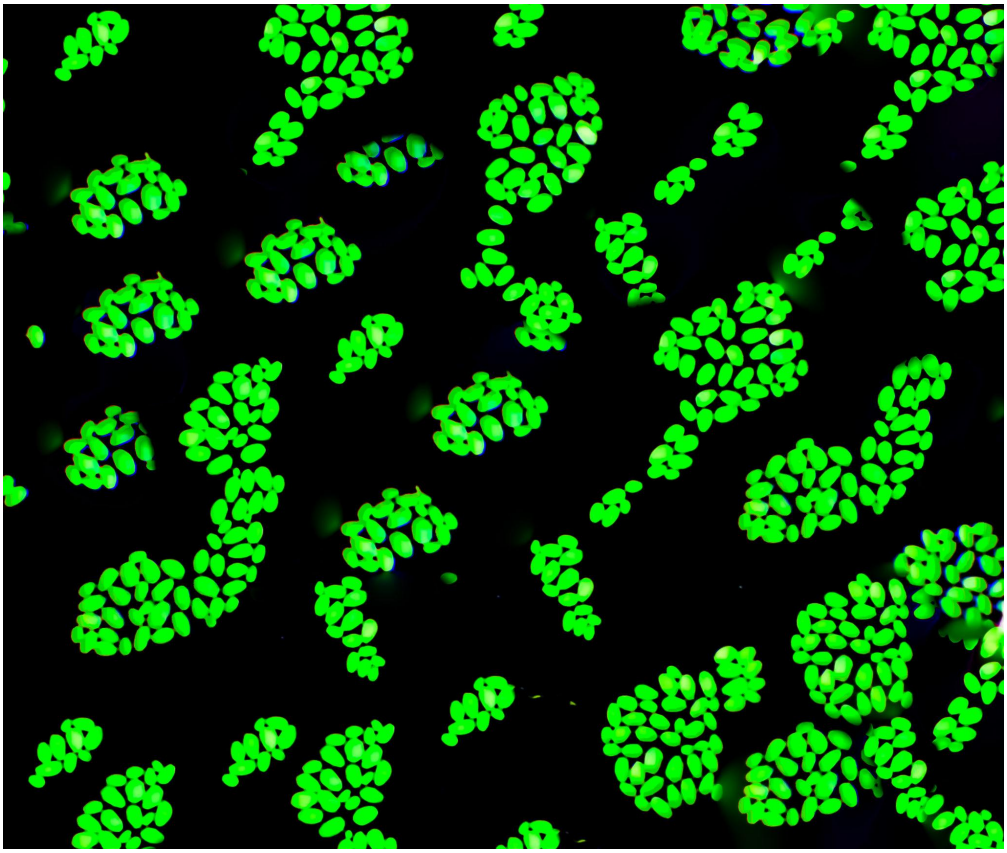
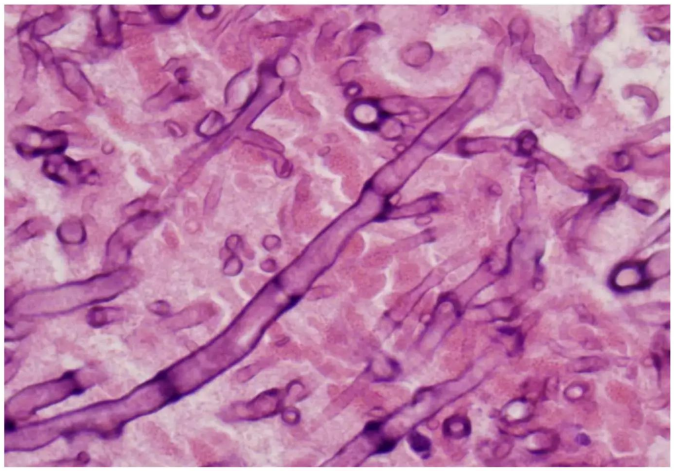
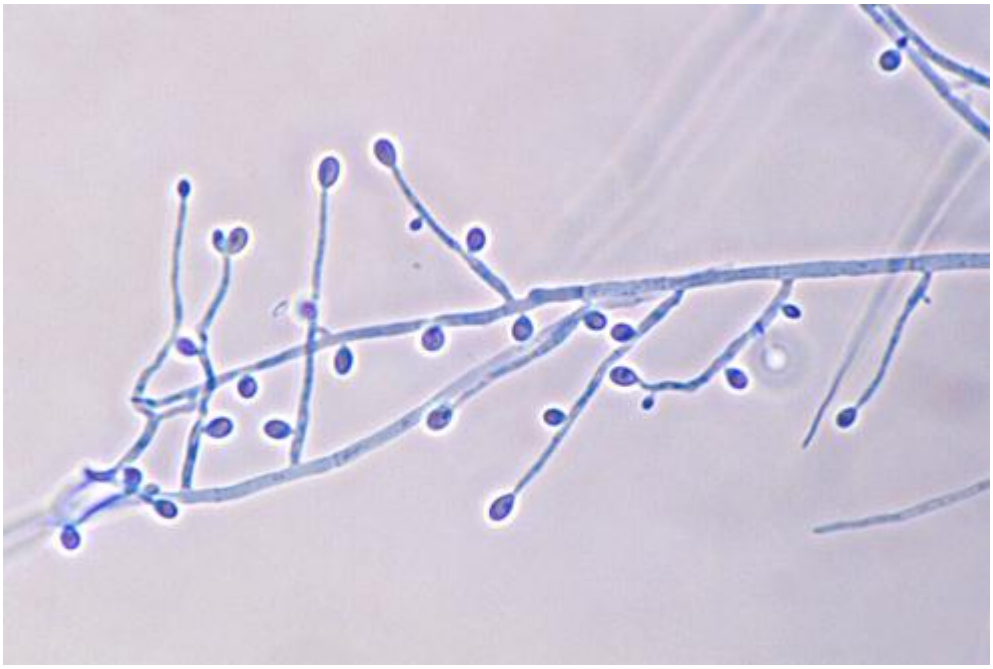


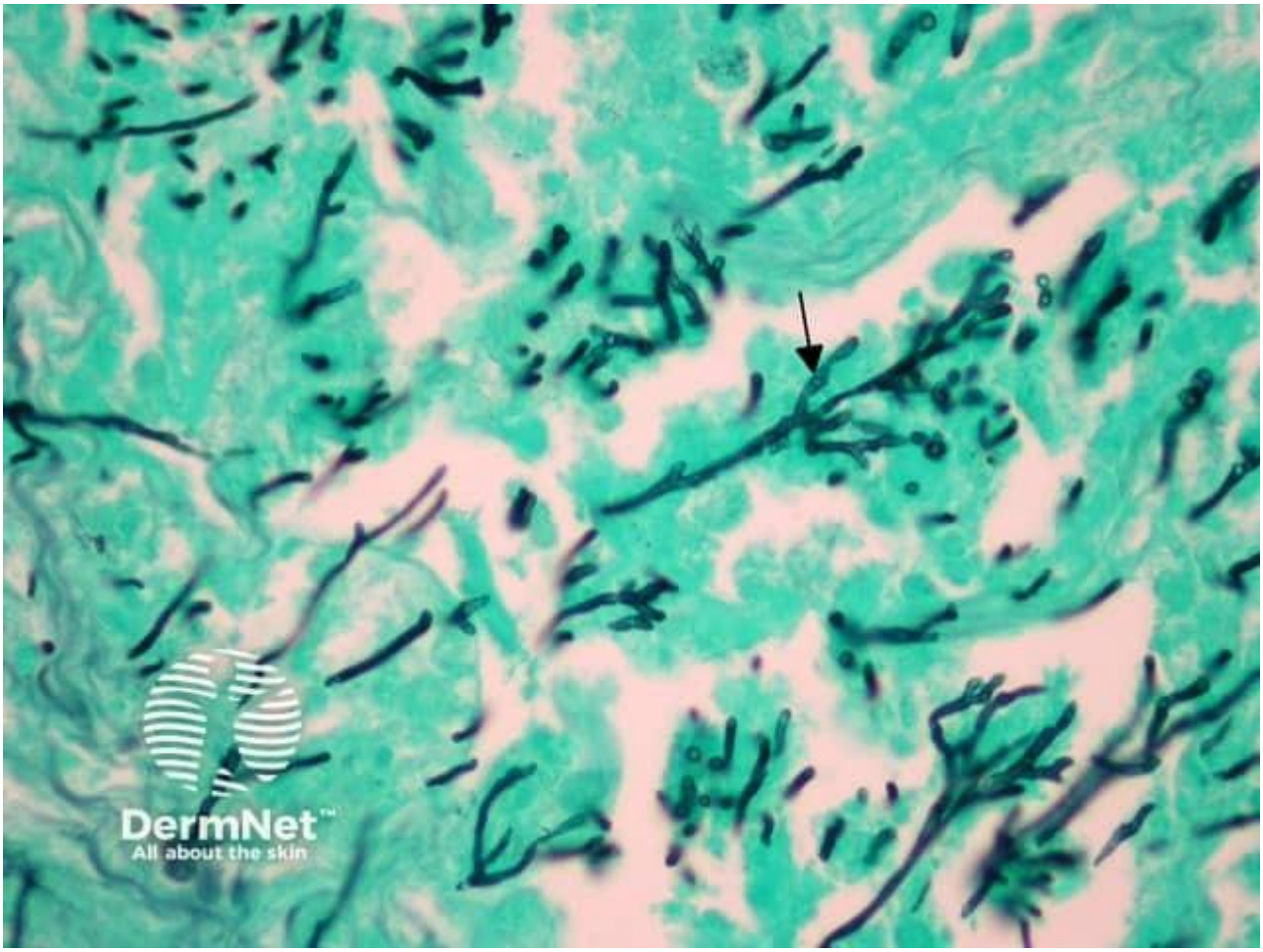


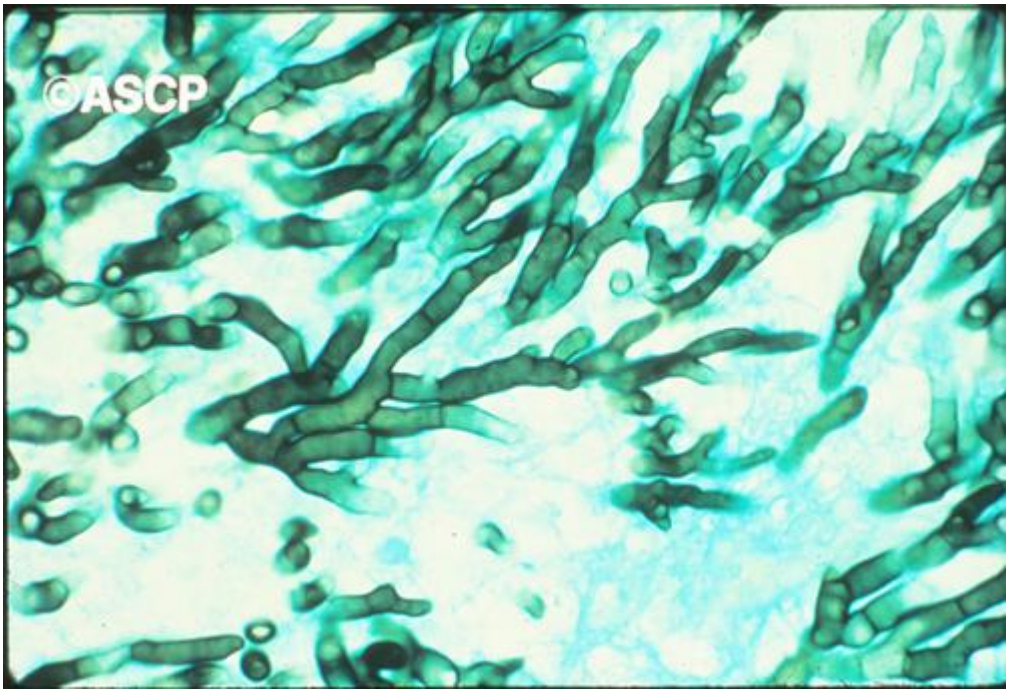
? Septate Hyphae (Aspergillus)

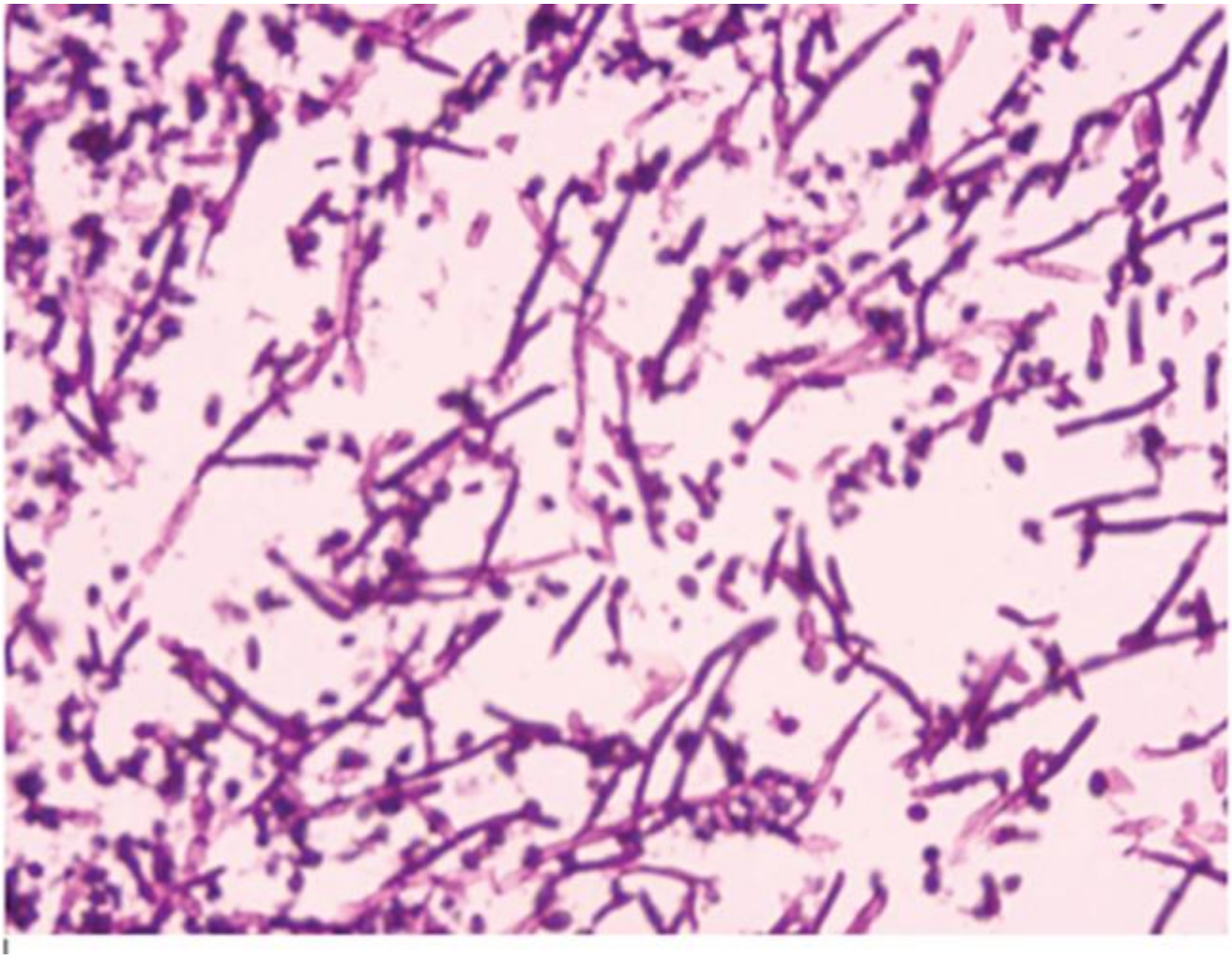
H &E staining of tissue section



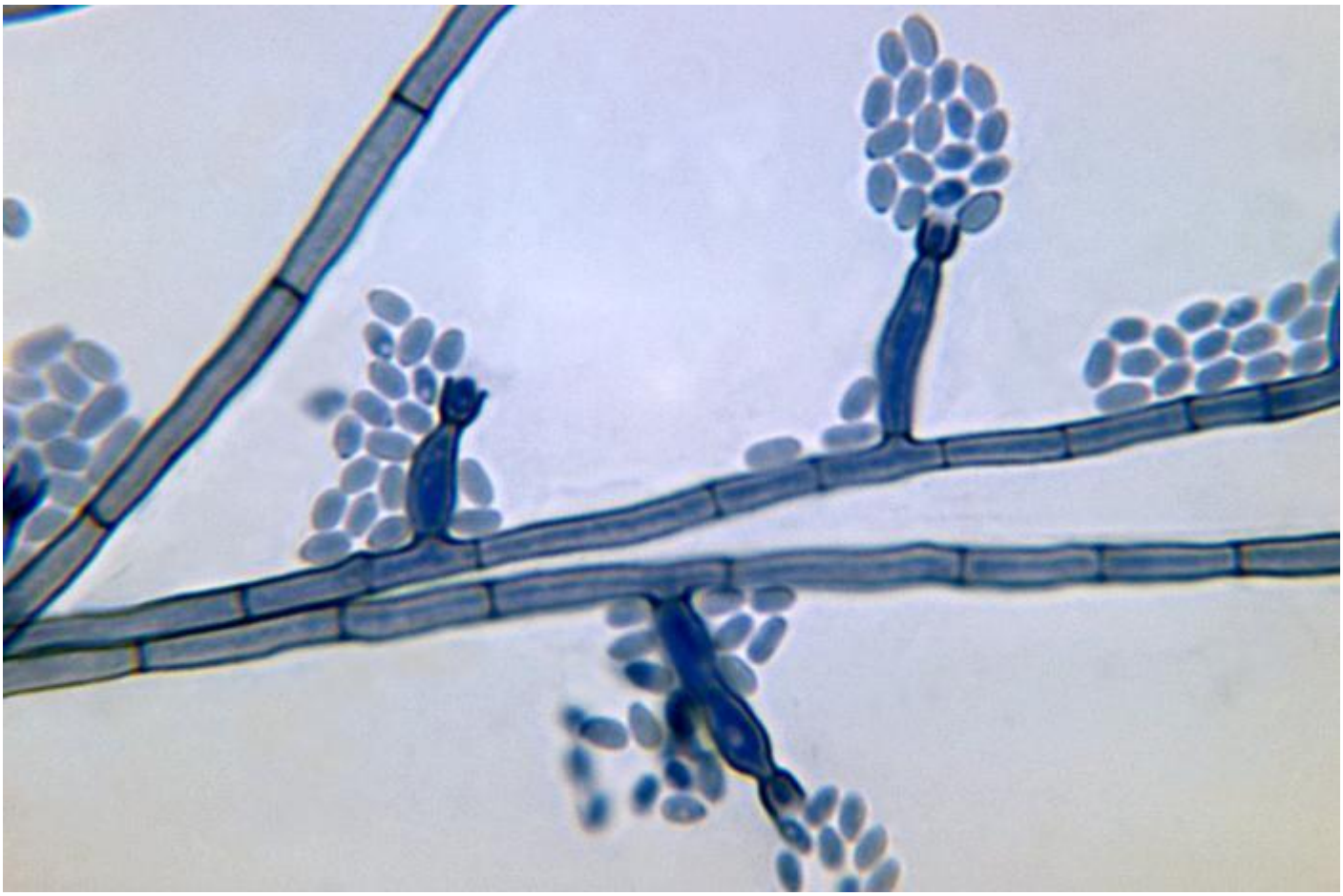


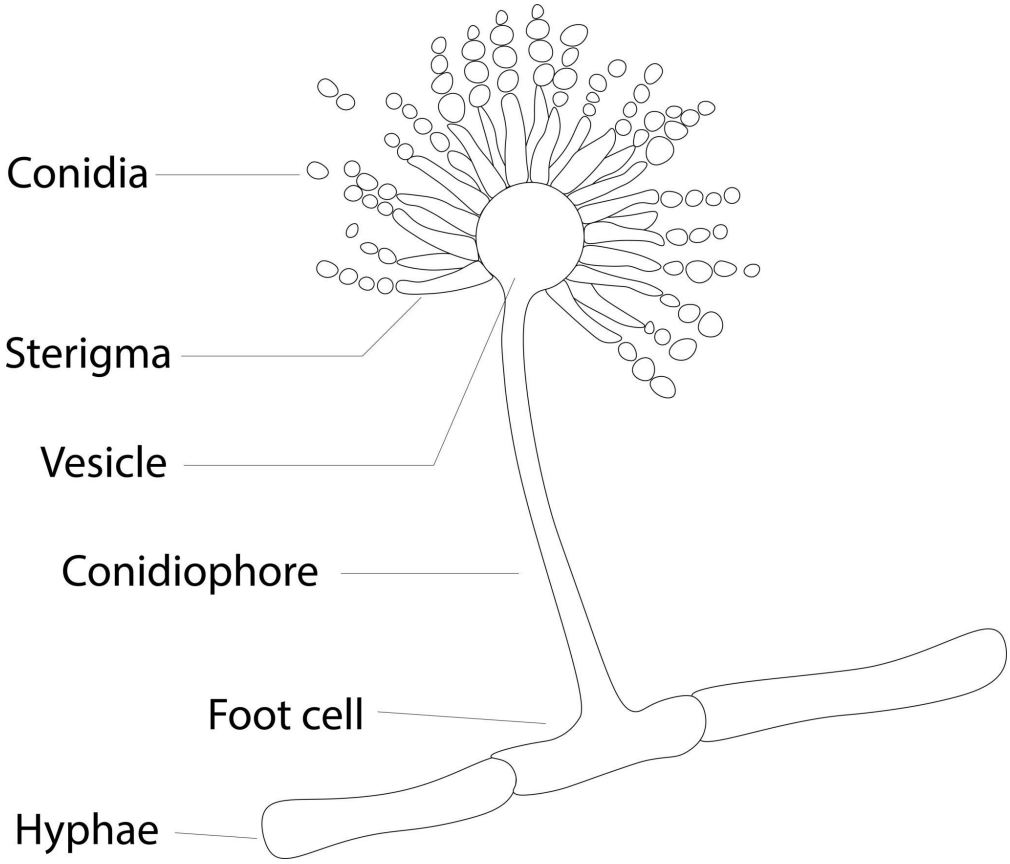

DermNet™
All about the skin

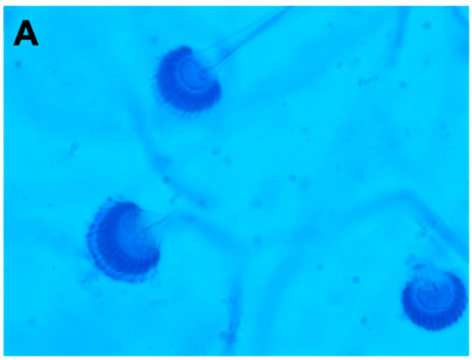




? Aspergillus Conidial Head




Conidia
Sterigma
Vesicle
Conidiophore
Foot cell
Hyphae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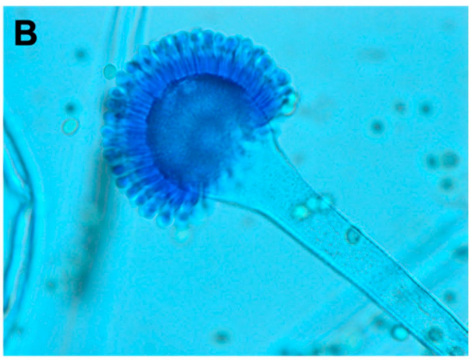
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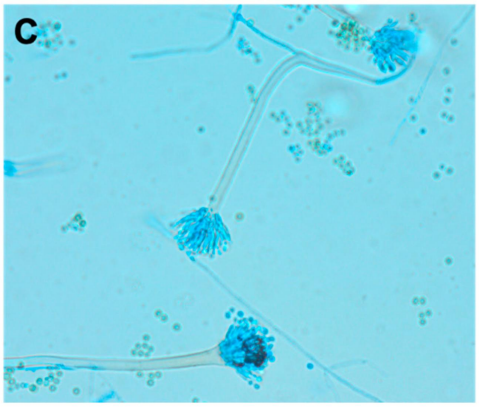
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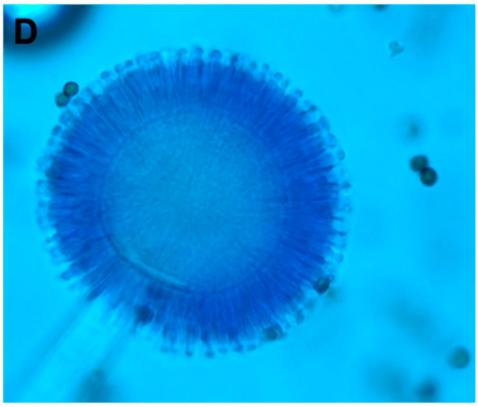
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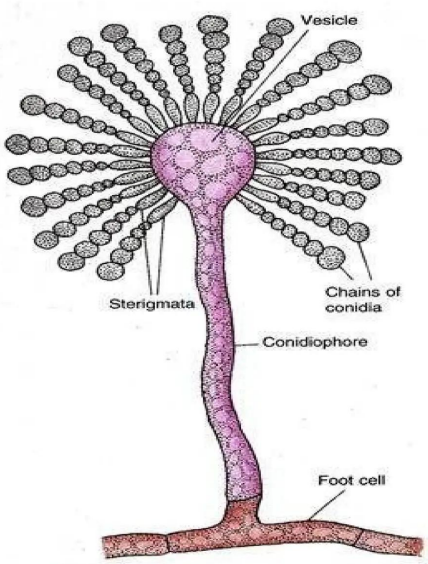


Fig. 10.3. *Aspergillus*. A mature conidiophore bearing sterigmata and chains of conidia.

? Candida Pseudohyphae

? DIAGRAMS / FLOWCHARTS

? Fungal Cell Structure

Fungal cell
?
Cell wall (mannan + glucan + chitin)
?
Cell membrane (ergosterol)
?
Cytoplasm (organelles)

? Fungal Classification Flowchart

Fungi
?
Morphology
? Yeast
? Mould
? Dimorphic

?
Clinical
? Superficial
? Cutaneous
? Subcutaneous
? Systemic
? Opportunistic

? Spore Formation Diagram

Hyphae
?
Spore formation
?
Asexual ? conidia / sporangiospores
?
Sexual ? ascospores / basidiospores / zygospores

? EXAM PEARLS

- Ergosterol ? target of antifungals
- Dimorphic fungi ? very high-yield
- Septate vs aseptate ? image-based questions
- Chlamydospores ? Candida identification
- Capsule ? Cryptococcus (India ink test)

? GROWTH AND ISOLATION OF FUNGI

? CULTURE MEDIA

? Sabouraud Dextrose Agar (SDA) (VERY HIGH-YIELD)

- Standard medium for fungal culture
- pH ? **5.6 (acidic)** ? inhibits bacteria
- Contains:
  - Dextrose
  - Peptone
- Variants:
  - SDA + antibiotics (chloramphenicol)
  - SDA + cycloheximide (selective)

? Selective Media

- **Cycloheximide-containing media**
  - Inhibits saprophytic fungi
  - Allows pathogenic fungi growth
- **CHROMagar Candida**
  - Differentiates Candida species by color

? GROWTH CONDITIONS

- Temperature:
    - **25°C ? mould form**
    - **37°C ? yeast form (dimorphic fungi)**
- Aerobic growth
- Slow-growing organisms (may take weeks)

? COLONY MORPHOLOGY

- Texture:
    - Cottony
    - Velvety
    - Powdery
- Pigmentation:
    - Surface color
    - Reverse pigmentation (VERY IMPORTANT)
- Growth rate

? IDENTIFICATION METHODS

? Lactophenol Cotton Blue (LPCB) Mount

- Stains fungal elements
- Components:
  - Lactic acid ? preserves
  - Phenol ? kills fungi
  - Cotton blue ? stains chitin
- Used to visualize:
  - Hyphae
  - Conidia

? Slide Culture Technique

- Maintains natural fungal structure
- Used for:
  - Identification of moulds
- Prevents distortion of hyphae

? STAINING METHODS

? KOH Mount (VERY HIGH-YIELD)

- **10–20% KOH**
- Dissolves keratin ? clears background
- Used for:
    - Skin
    - Hair
    - Nail samples

? Gram Stain

- Yeasts ? Gram-positive
- Limited role for moulds

? PAS Stain

- Stains fungal cell wall **magenta**
- Used in tissue sections

? GMS (Gomori Methenamine Silver)

- Fungi appear **black**
- Background green
- Highly sensitive

? India Ink Preparation

- Demonstrates **capsule of Cryptococcus**
- Negative staining

? SPECIAL TESTS (VERY HIGH-YIELD)

? Germ Tube Test

- Identifies **Candida albicans**
- Formation of germ tubes in serum at 37°C

? Urease Test

- Positive in **Cryptococcus**
- Differentiates from Candida

? CHROMagar Candida

- Differentiates species:
  - C. albicans ? green
  - C. tropicalis ? blue
  - C. krusei ? pink

? TABLES (VERY HIGH-YIELD)

? Culture Media and Uses

MEDIUM	USE
SDA	General fungal culture
SDA + antibiotics	Prevent bacterial growth
SDA + cycloheximide	Select pathogenic fungi
CHROMagar	Candida differentiation

? Stains Comparison

STAIN	USE	APPEARANCE
KOH	Direct microscopy	Clears debris
Gram	Yeast	Gram-positive
PAS	Tissue fungi	Magenta
GMS	Tissue fungi	Black fungi
India ink	Capsule	Clear halo

? Special Tests Comparison

TEST	ORGANISM	RESULT
Germ tube	Candida albicans	Positive
Urease	Cryptococcus	Positive
CHROMagar	Candida spp.	Color differentiation

? SLIDES (EXAM FAVORITE)

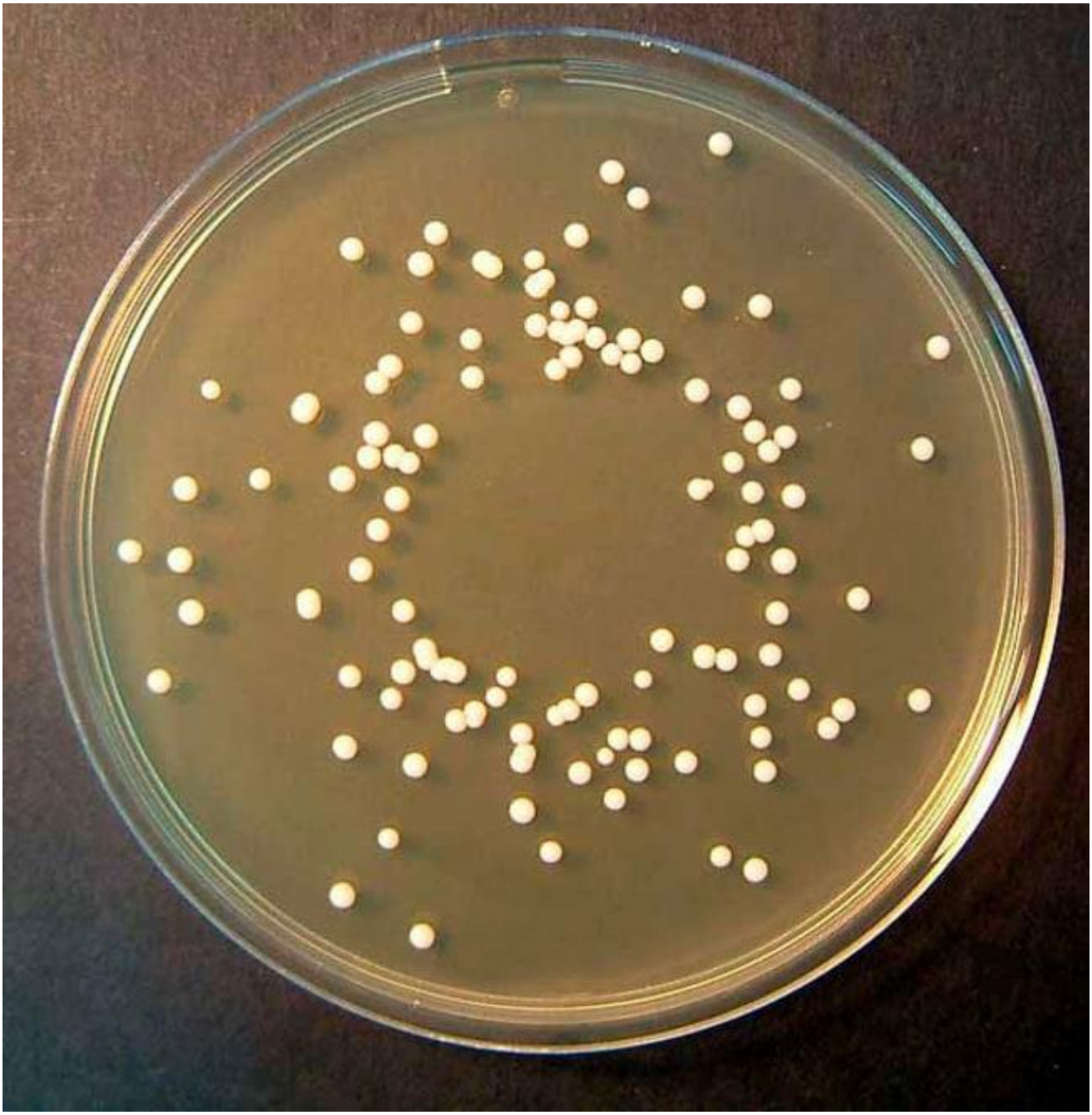
? SDA Colonies

Candida albicans in SDA

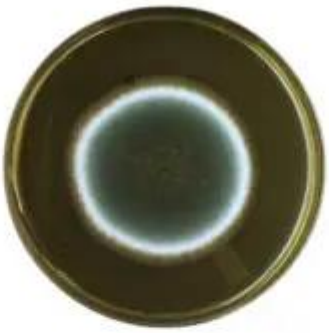
Source: Wikipedia

Trichophyton terrestre in SDA

Source: Wikipedia



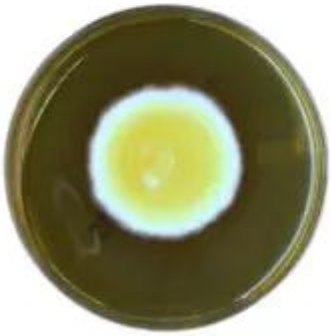
Trichophyton rubrum



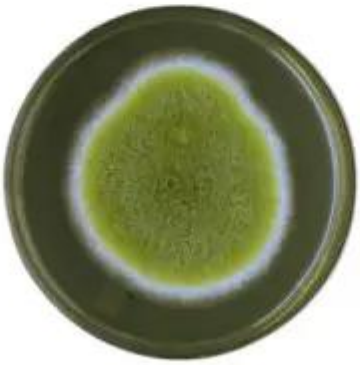
Aspergillus fumigatus

Candida albicans

Colony Morphology in SDA



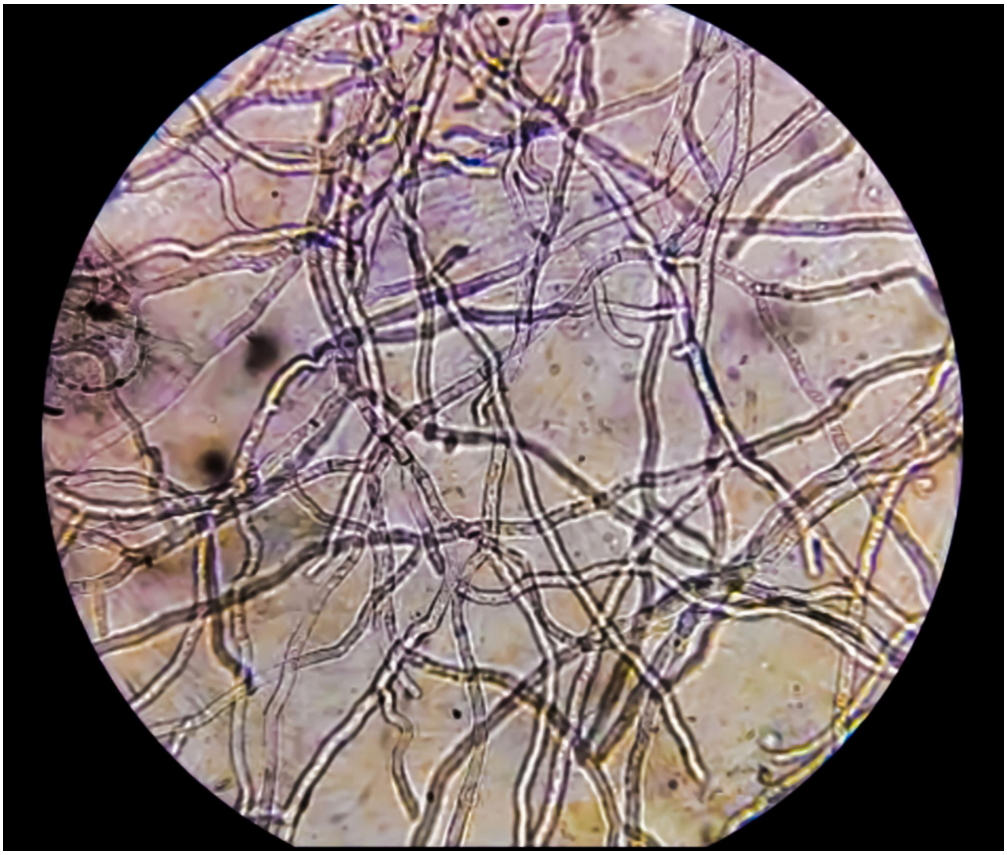
Blastomyces dermatitidis



Aspergillus flavus

Aspergillus niger

? KOH Mount



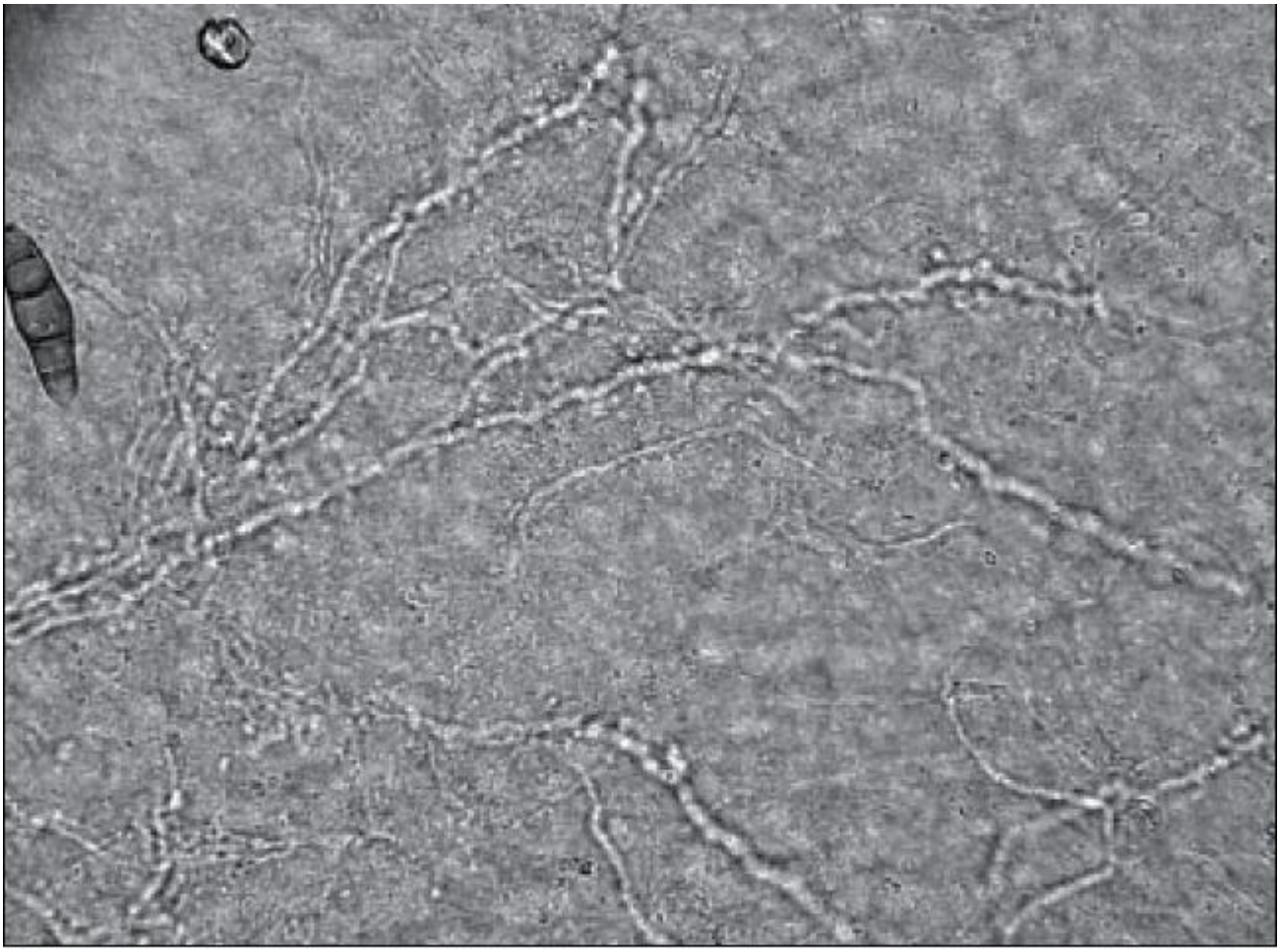
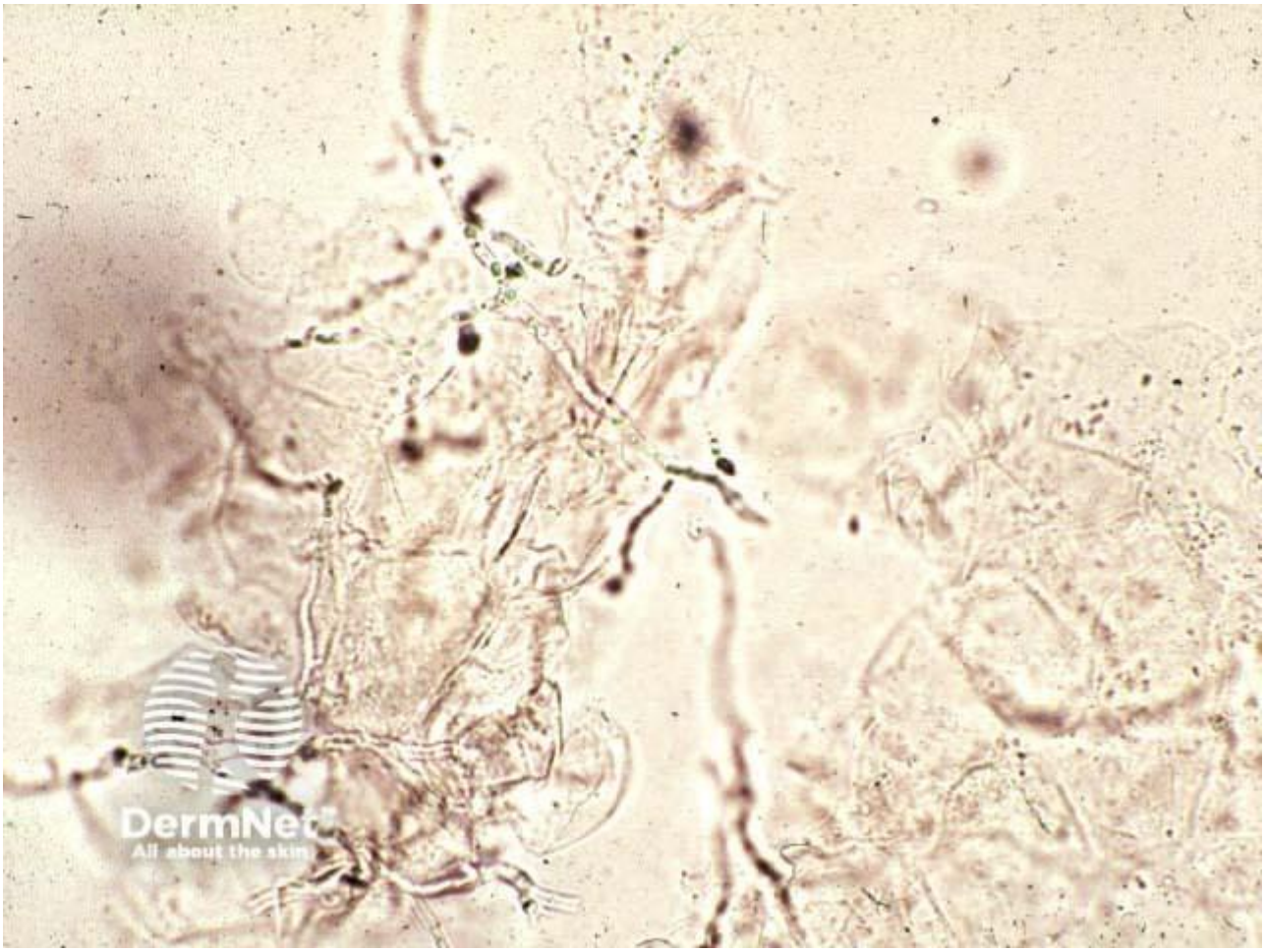


Figure 1: Skin scraping and KOH mount showing branching fungal hyphae in dermatophyte infection

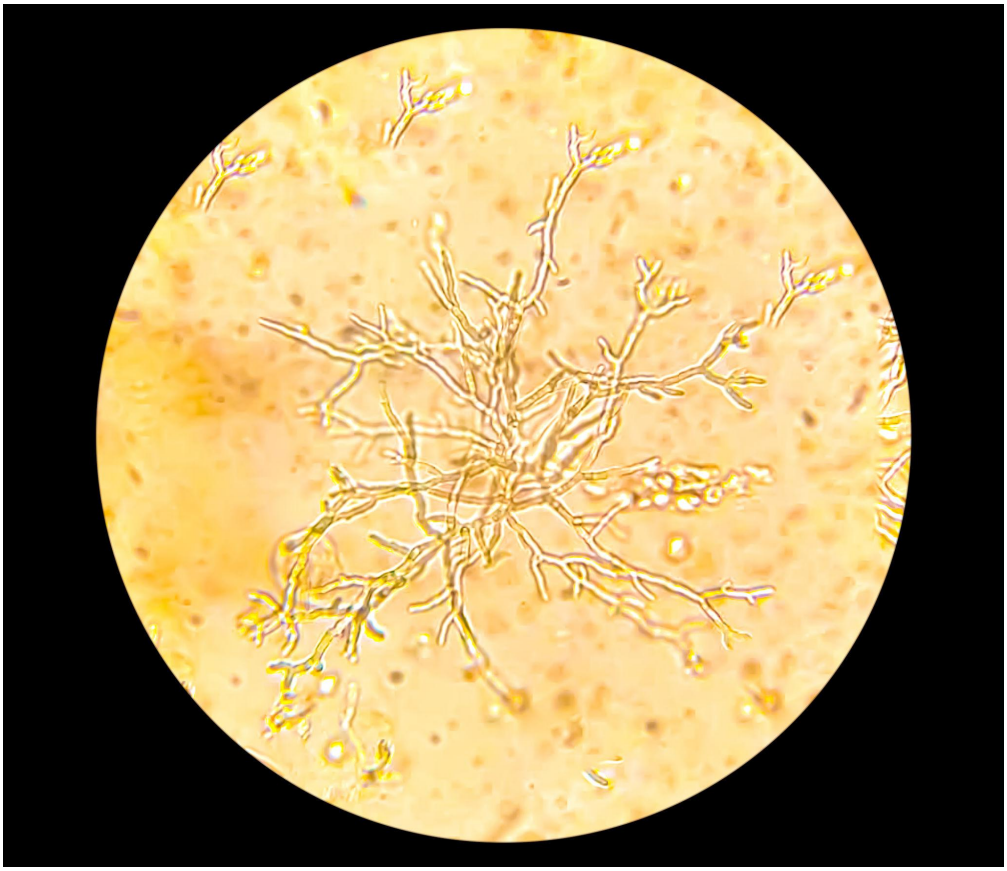


Preparation of KoH

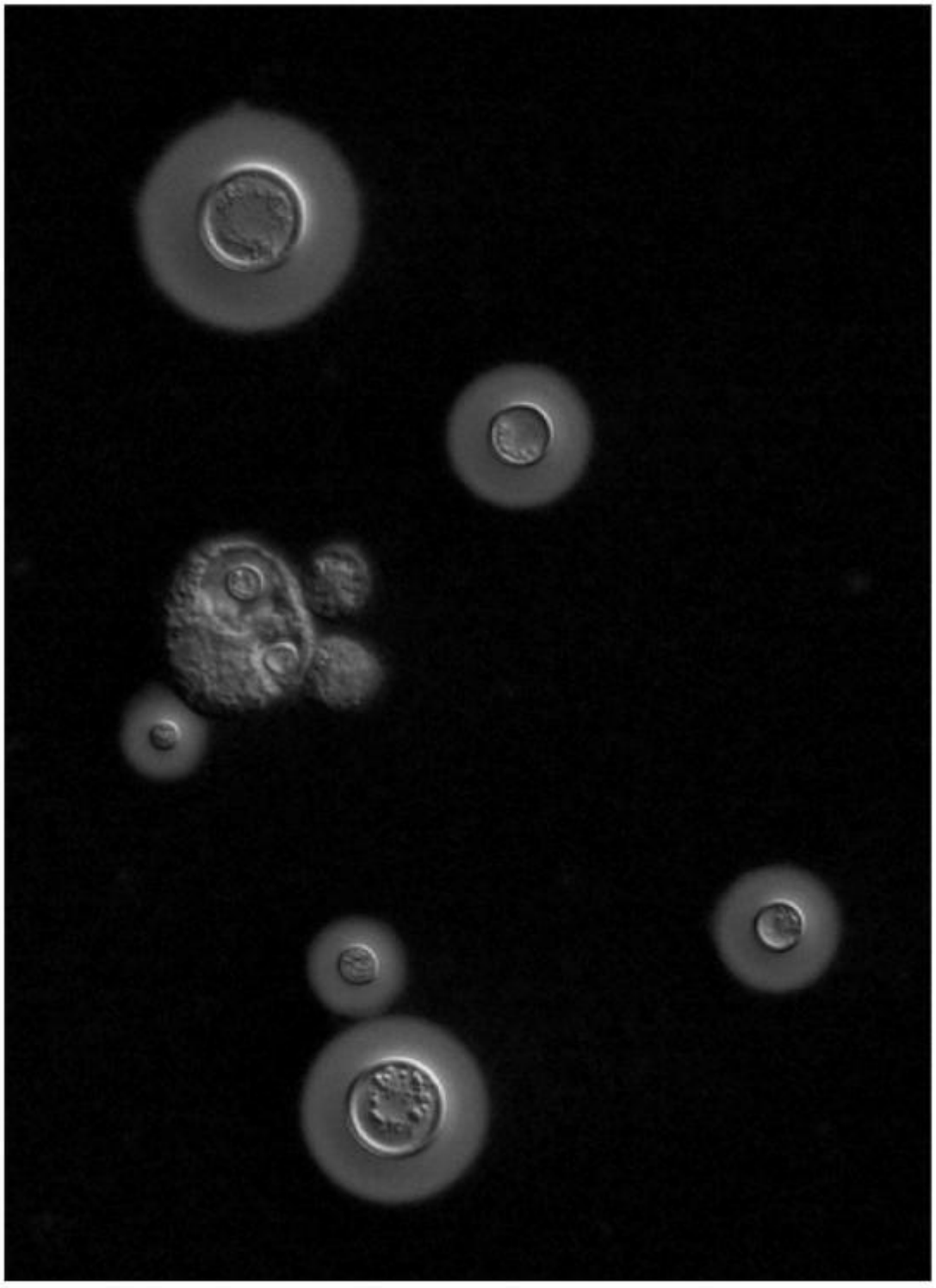
Potassium hydroxide (KOH) preparation: Keratinized tissue specimens such as skin scrapings and plucked hair samples are treated with 10% KOH which digests the keratin material so that the fungal hyphae will be clearly seen under the microscope.

- 1O% is the usual concentration of KOH used.
- 20-40% KOH is needed for the specimens such as nail and biopsy tissues that take longer time to dissolve.
- **Glycerol** (10%) can be added to prevent drying
- **DMSO** (dimethyl sulfoxide) - help in tissue digestion

Image



? India Ink Capsule (Cryptococcus)



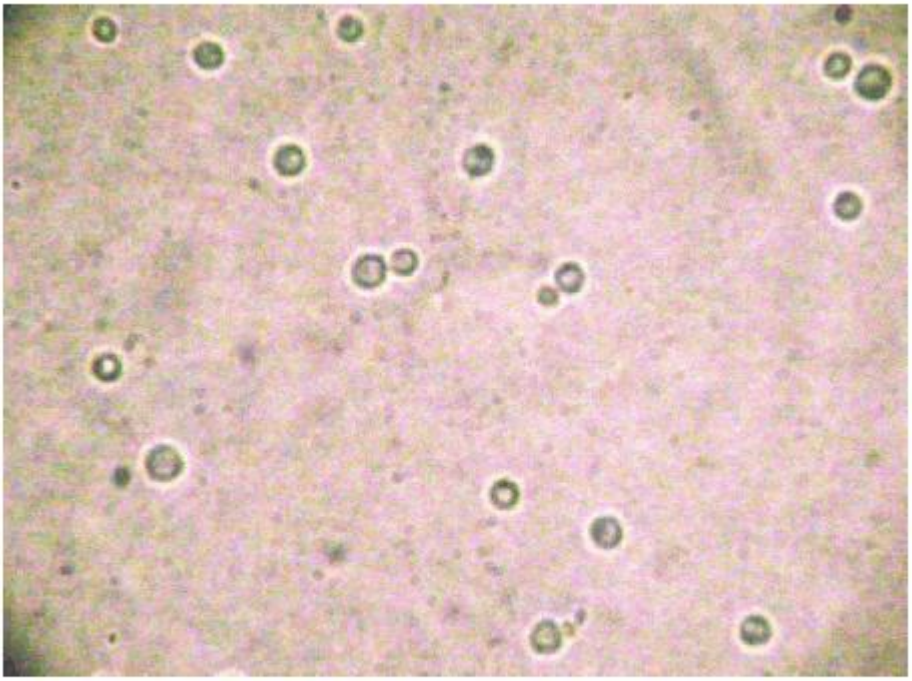
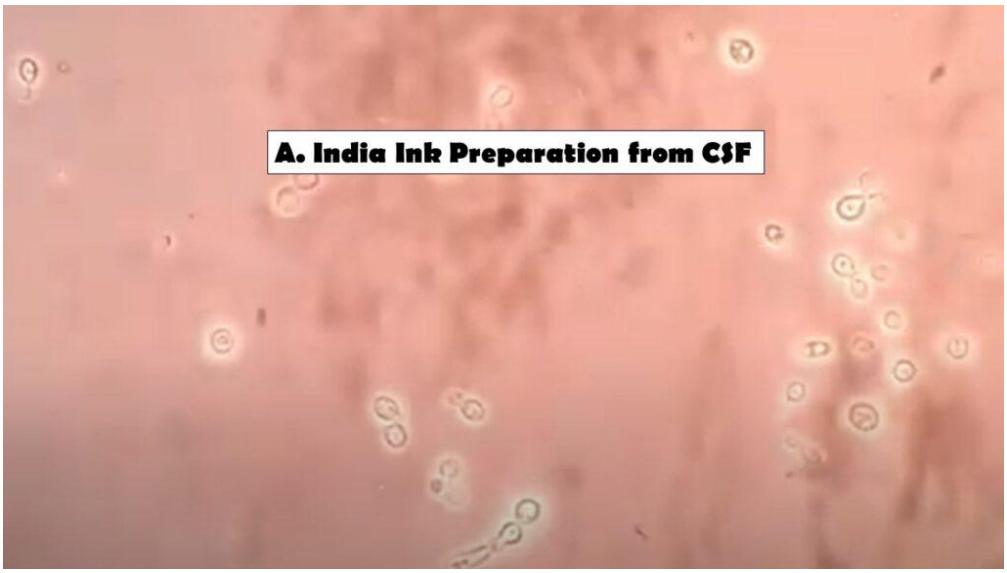
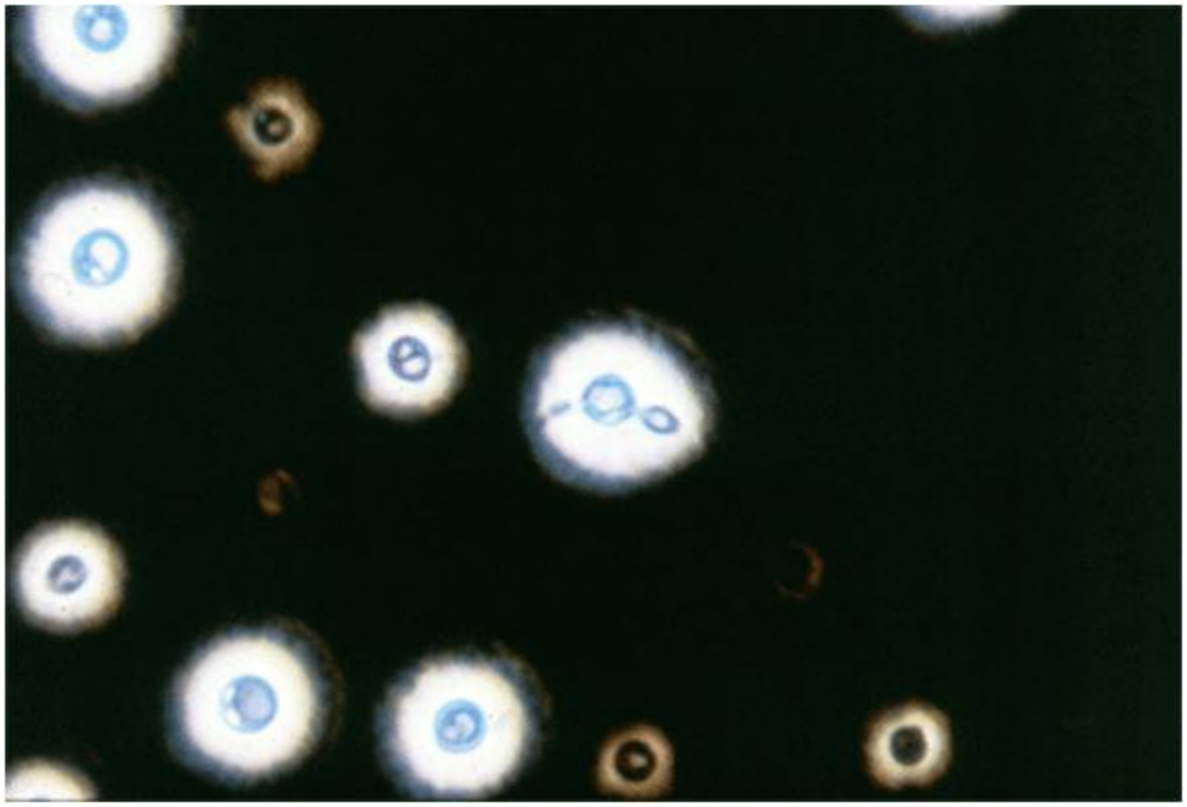
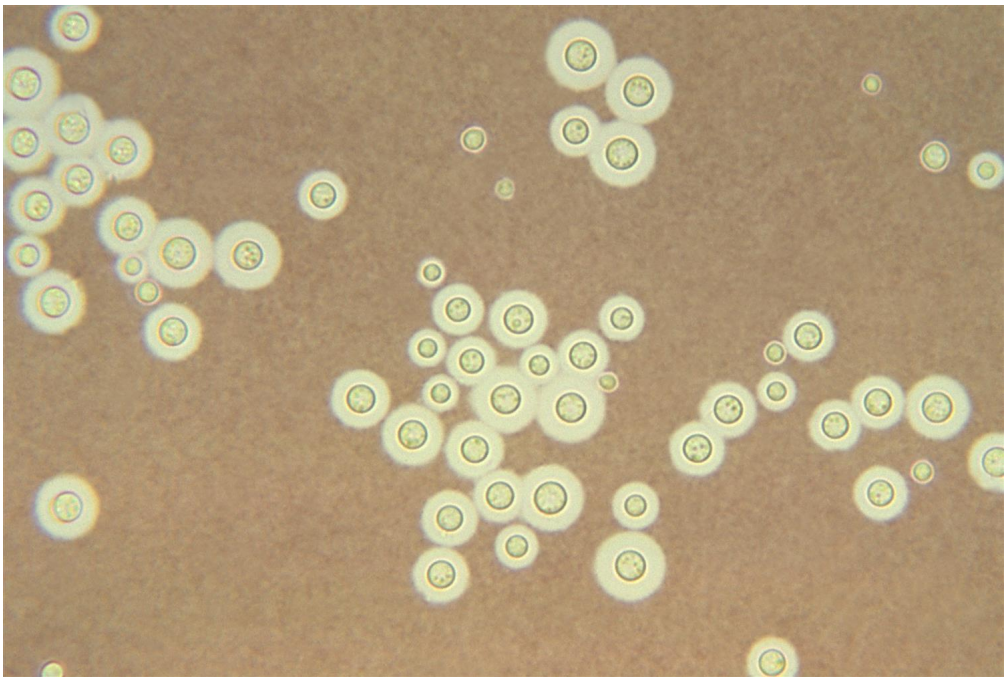
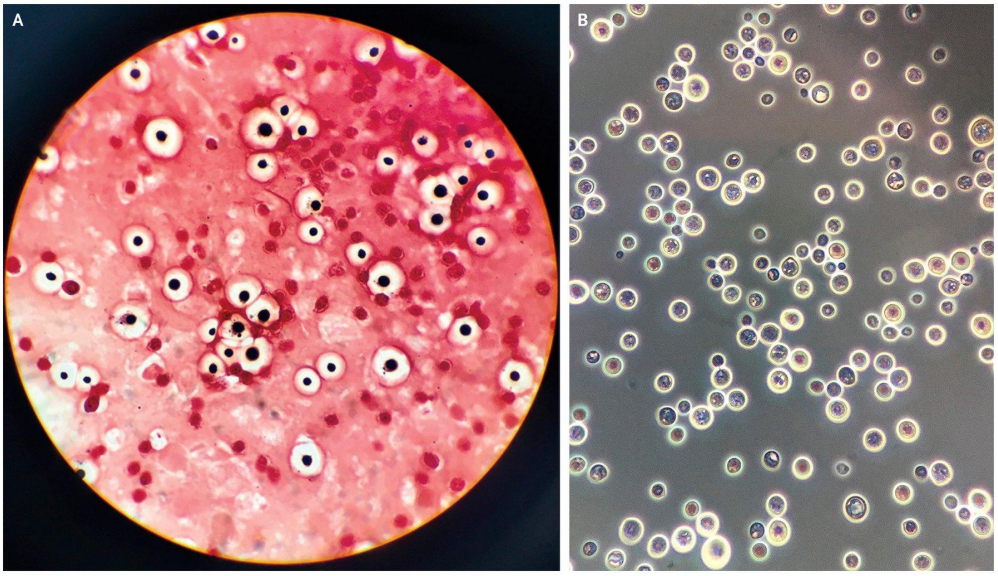


Figure 1: India ink preparation showing encapsulated budding yeast cells of *Cryptococcus neoformans*. (400X)









? DIAGRAMS / FLOWCHARTS

? Fungal Culture Workflow

Clinical sample
?
Direct microscopy (KOH)
?
Culture on SDA
?
Colony morphology
?
LPCB mount
?
Identification

? Slide Culture Technique

Agar block
?
Inoculation
?
Coverslip placement
?
Incubation
?

? EXAM PEARLS

- SDA ? **most important culture medium**
- KOH mount ? **bedside diagnosis**
- India ink ? **Cryptococcus hallmark**
- Germ tube test ? **Candida albicans identification**
- GMS ? **most sensitive tissue stain**

? VIRULENCE FACTORS & HOST IMMUNITY (VERY HIGH-YIELD)

? VIRULENCE FACTORS OF FUNGI

? Adhesins

- Surface molecules that **facilitate attachment** to host tissues
- Important in **Candida adherence to mucosa**

? Enzymes

- **Keratinase** ? invasion of keratinized tissues (dermatophytes)
- **Proteases** ? tissue destruction

- **Phospholipases** ? membrane damage
- Aid in **invasion and spread**

? Capsule

- Seen in **Cryptococcus neoformans**
- Polysaccharide capsule
- Functions:
    - Anti-phagocytic
    - Inhibits immune response

? Biofilm Formation (Candida) (VERY HIGH-YIELD)

- Structured microbial community attached to surfaces
- Seen on:
    - Catheters
    - Prosthetic devices
- Leads to:
    - **Drug resistance**
    - Persistent infections

? Phenotypic Switching

- Ability to change morphology (yeast ? hyphae)
- Helps in:
    - Immune evasion
    - Adaptation to host environment

? HOST IMMUNITY IN FUNGAL INFECTIONS

? Innate Immunity (VERY IMPORTANT)

- **Neutrophils**
    - Primary defense
    - Kill fungi via oxidative burst
- **Macrophages**
    - Phagocytosis
    - Present antigen to T cells
- Physical barriers:
    - Skin
    - Mucosa

? Adaptive Immunity

Cell-Mediated Immunity (Th1) (VERY HIGH-YIELD)

- Most important defense against fungi
- T-cell activation ? cytokine release
- Activates macrophages

Humoral Immunity

- Limited role
- Antibodies not highly protective

? IMMUNOCOMPROMISED STATES (CLINICALLY IMPORTANT)

? HIV/AIDS

- ? CD4 count
- Predisposes to:
  - Candidiasis
  - Cryptococcosis
  - Pneumocystis

? Diabetes Mellitus

- Predisposes to:
  - **Mucormycosis (VERY HIGH-YIELD)**
- Mechanism:

- Impaired neutrophil function
- High glucose environment

? Steroid Therapy / Immunosuppressants

- ? Cell-mediated immunity
- Increased risk of:
  - Aspergillosis
  - Candida infections

? TABLES (VERY HIGH-YIELD)

? Fungal Virulence Factors

FACTOR	FUNCTION	EXAMPLE
Adhesins	Attachment	Candida
Enzymes	Tissue invasion	Dermatophytes
Capsule	Anti-phagocytic	Cryptococcus
Biofilm	Drug resistance	Candida
Phenotypic switching	Adaptation	Candida

? Innate vs Adaptive Immunity in Fungi

FEATURE	INNATE IMMUNITY	ADAPTIVE IMMUNITY
Main cells	Neutrophils, macrophages	T cells
Response	Immediate	Delayed
Mechanism	Phagocytosis	Cytokine-mediated
Importance	First-line defense	Long-term protection
Key role	Neutrophils	Th1 cells

? DIAGRAMS / FLOWCHARTS

? Host Immune Response to Fungi

Fungal entry
?
Innate immunity
? Neutrophils (phagocytosis)
? Macrophages
?
Antigen presentation
?
T-cell activation
?
Cell-mediated immunity (Th1)
?
Fungal clearance

? Candida Biofilm Formation

Adhesion to surface
?
Microcolony formation
?
Maturation of biofilm
?
Extracellular matrix formation

?
Drug resistance + persistence

? EXAM PEARLS

- **Neutrophils = most important defense** against fungi
- **Th1 immunity = key protective mechanism**
- Capsule ? Cryptococcus virulence
- Biofilm ? antifungal resistance
- Diabetes ? mucormycosis risk
- HIV ? opportunistic fungal infections

? LABORATORY DIAGNOSIS OF MYCOSES (SYSTEMATIC)

? DIRECT MICROSCOPY (FIRST-LINE, VERY HIGH-YIELD)

? Specimens

- Skin scrapings
- Hair
- Nail clippings

- Sputum / BAL
- CSF
- Tissue biopsy

? Methods

- **KOH mount (10–20%)**
  - Dissolves keratin ? clears background
  - Demonstrates hyphae / yeast
- **Gram stain**
  - Yeasts ? Gram-positive
- **India ink**
  - Capsule of Cryptococcus ? clear halo

? CULTURE

? Media

- Sabouraud dextrose agar (SDA)
- SDA + antibiotics
- SDA + cycloheximide

? Features

- Incubation:
    - 25°C ? mould
    - 37°C ? yeast
- Colony morphology:
    - Texture ? cottony / velvety
    - Pigmentation (surface + reverse)

? Identification

- LPCB mount
- Slide culture

? HISTOPATHOLOGY

? Specimens

- Tissue biopsy
- Blood / body fluids

? Stains

- **PAS stain**
    - Fungi ? magenta
- **GMS stain**

- Fungi ? black (VERY HIGH-YIELD)

- H&E (less specific)

? Findings

- Hyphae
- Yeast forms
- Tissue invasion

? SEROLOGY

- Detection of antibodies
- Limited utility
- Useful in:
  - Histoplasmosis
  - Coccidioidomycosis

? ANTIGEN DETECTION (VERY HIGH-YIELD)

- **Cryptococcal antigen (CrAg)** ? CSF/serum
- **Galactomannan antigen** ? Aspergillus
- **?-D-glucan assay** ? broad fungal detection

? MOLECULAR METHODS (PCR)

- Detects fungal DNA
- Highly sensitive and specific
- Rapid diagnosis
- Useful in:
    - Invasive fungal infections
    - Non-culturable fungi

? TABLE (VERY HIGH-YIELD)

? Diagnostic Methods Comparison

METHOD	SAMPLE	ADVANTAGE	LIMITATION
KOH mount	Skin, nail	Rapid	No species ID
Culture (SDA)	All samples	Gold standard	Slow
Histopathology	Tissue	Shows invasion	Needs biopsy
Serology	Blood	Useful in systemic	Low specificity
Antigen detection	Blood/CSF	Early diagnosis	Limited organisms
PCR	Any	Highly sensitive	Expensive

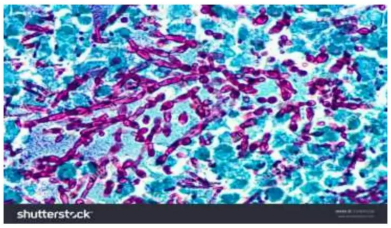
? SLIDES (EXAM FAVORITE)

? Histopathology (PAS / GMS)

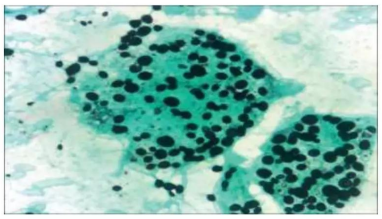
Direct Examination

- **HISTOPATHOLOGICAL STAINS –**
- **PERIODIC ACID SHIFF (PAS) STAIN**
- Recommended stain for detecting fungi.
- PAS positive fungi appear magenta/deep pink – nuclei – blue.

- **GOMORI METHENAMINE SILVER (GMS) –** used as an alternative to PAS stain for detecting fungi.
- It stains both live and dead fungi.
- As PAS can stain only live fungi.
- GMS stains the polysaccharide component of the cell wall.
- Fungi appears – Black
- Background tissue – Pale green color.



Budding Yeast, Pseudohyphae, *Candida spp.*



Pulmonary Histoplamosis mimicking lung carcinoma

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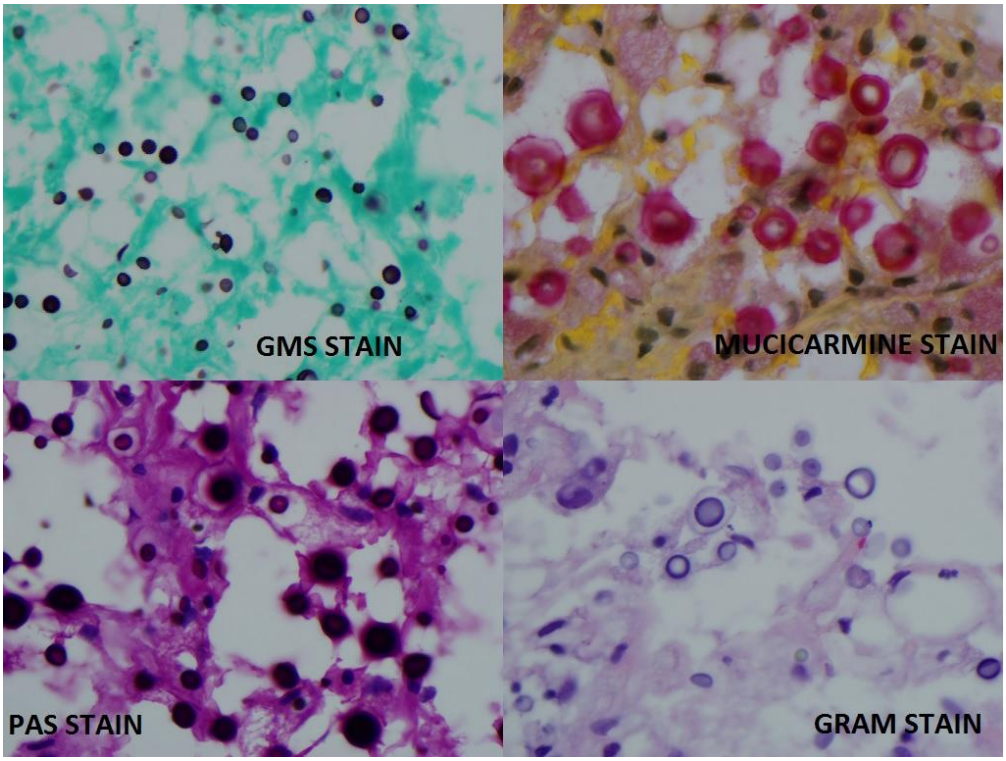
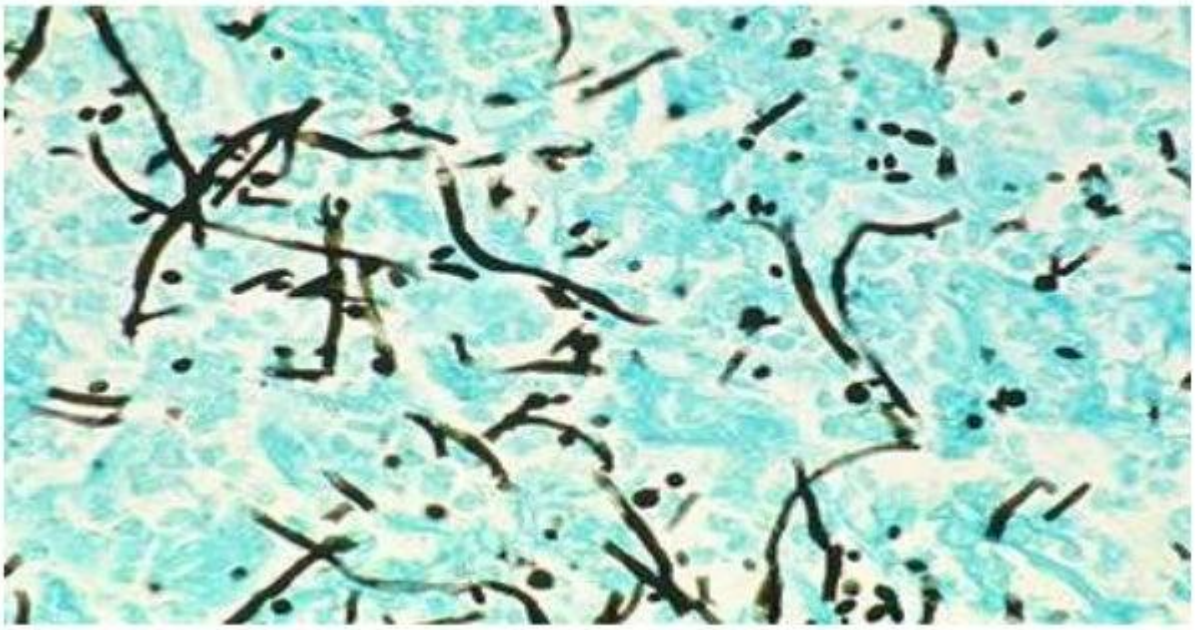
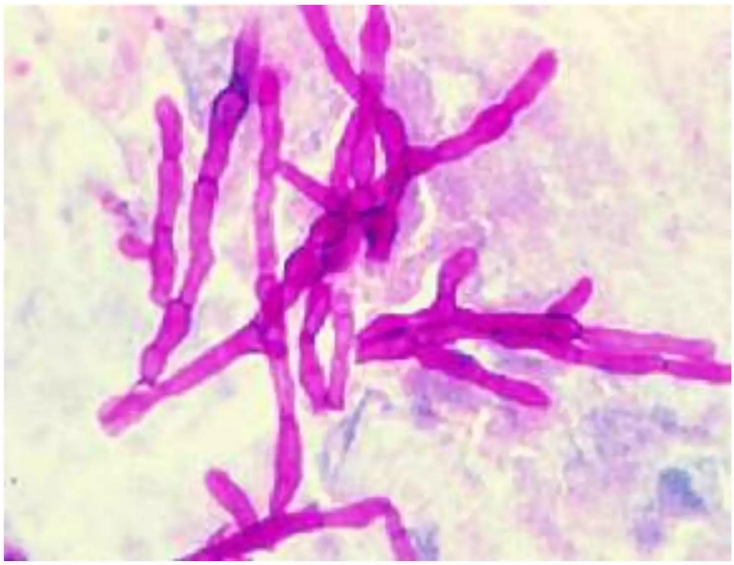
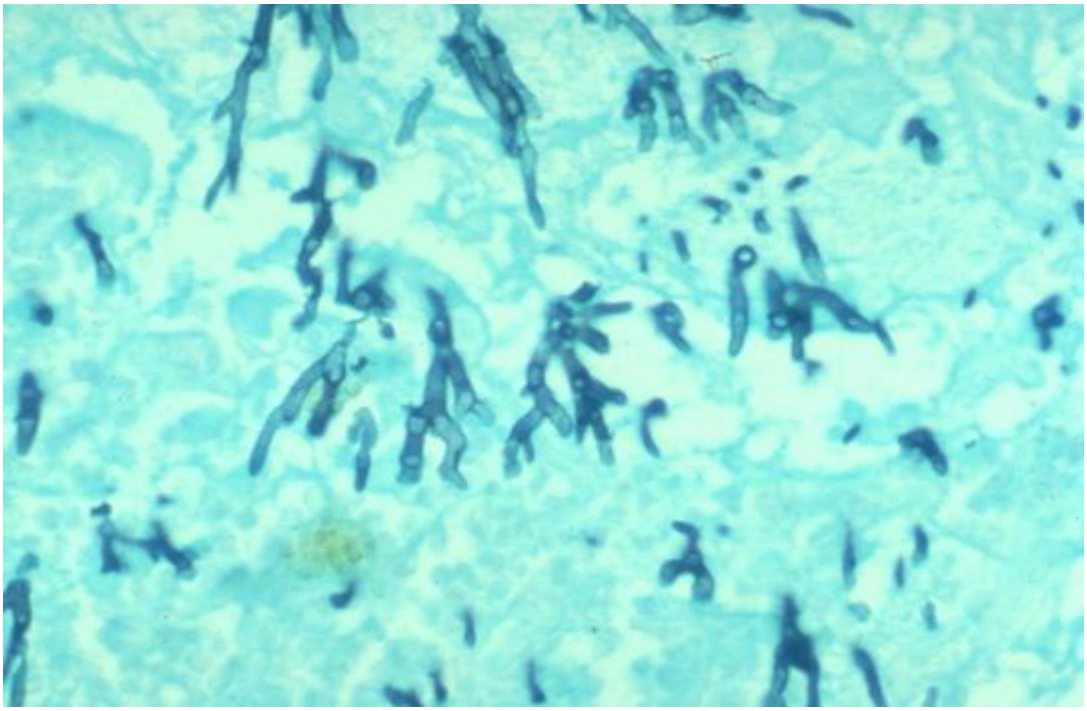


GMS stain shows characteristic pseudohyphae and buds of Candida



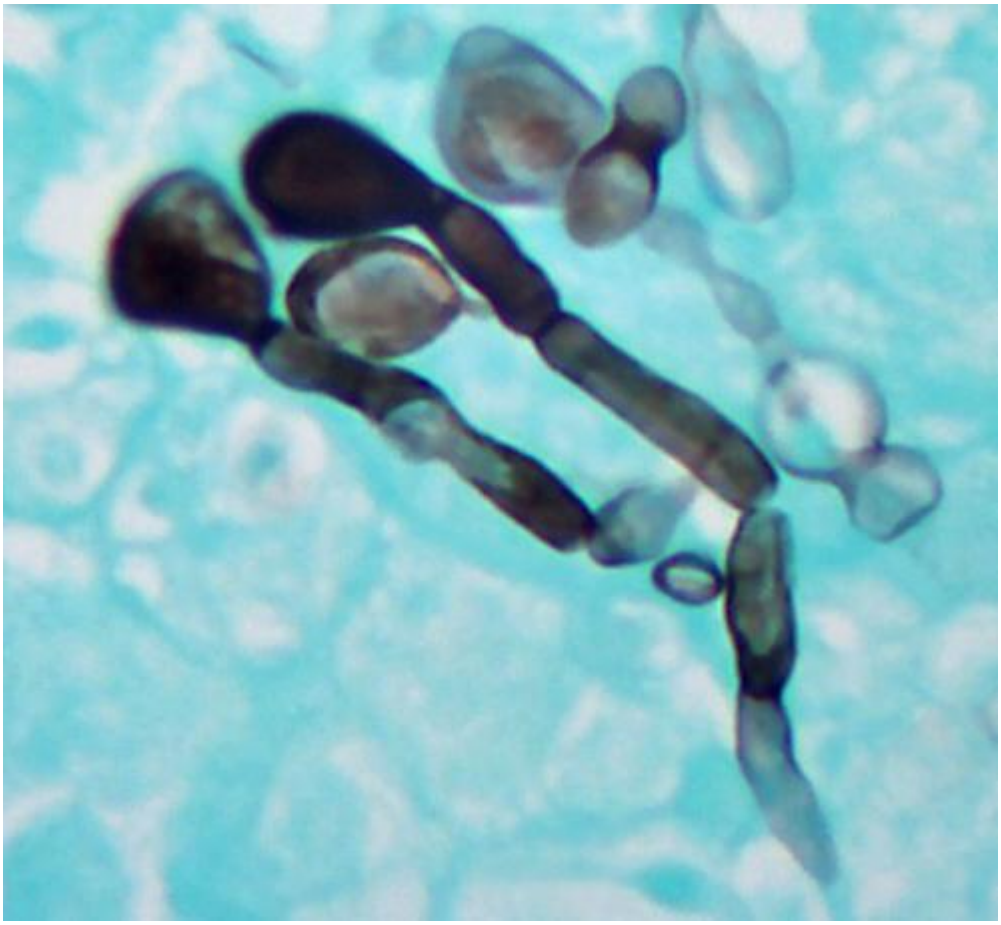


Candida seen on PAS stain

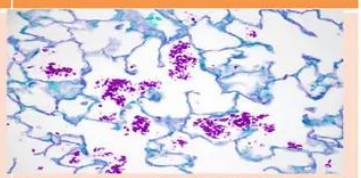
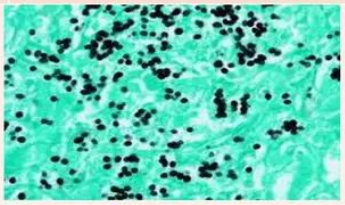
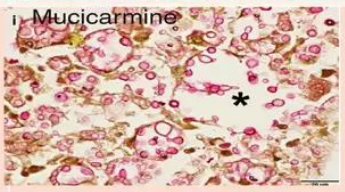


Fungal Stains

Stain	Comments
Mycology Lab	
Calcofluor white	fluorochrome that binds glucans and chitin in fungal cell wall;
Lactophenol Cotton Blue	cotton blue (aka analine blue) dye stains chitin and allows visualization of fungal elements following scraping or tape preparation from culture
Pathology	
Hematoxylin and esoin (H&E)	used to visualize host cell response to fungi but doesn't stain most fungi; exceptions are *Aspergillus* spp. and the Mucorales.
Grocott methenamine silver (GMS)	stains fungal cell wall black
Periodic acid-Schiff (PAS)	stains polysaccharide component of fungal cell wall magenta
Selected specific stains	
India ink	"negative" stain that outlines the polysaccharide capsule of *Cryptococcus* sp; not as sensitive as Ag detection for cryptococcal meningitis; some strains are reportedly acapsular.
Mucicarmine	can be used to stain mucopolysaccharide capsule of *Cryptococcus* sp.; also stains *Blastomyces* and *Rhinosporidium*
Fontana-Masson	silver stain used to detect melanin pigment in cell walls of *Cyptococcus*



HISTOPATHOLOGICAL STAINS

METHOD	USE	IMAGE
▪PAS stain (Periodic acid schiff) stain	•Recommended method for detecting fungi. •PAS positive fungi appear magenta/ deep pink, whereas nuclei stain blue.	
▪Gomori methenamine silver (GMS) stain:	•Stains both live and dead fungi. •Stains the polysaccharide component of cell wall. •Fungi appear black and the background tissue takes pale green colour.	
▪Mucicarmine stain	•Used for staining carminophilic cell wall of *Cryptococcus* and *Rhinosporidium.*	

? Fungal Culture Plates


Trichophyton rubrum
Aspergillus fumigatus
Candida albicans
Colony Morphology in SDA
Blastomyces dermatitidis
Aspergillus flavus
Aspergillus niger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Candida albicans
Sabouraud agar
HansN.

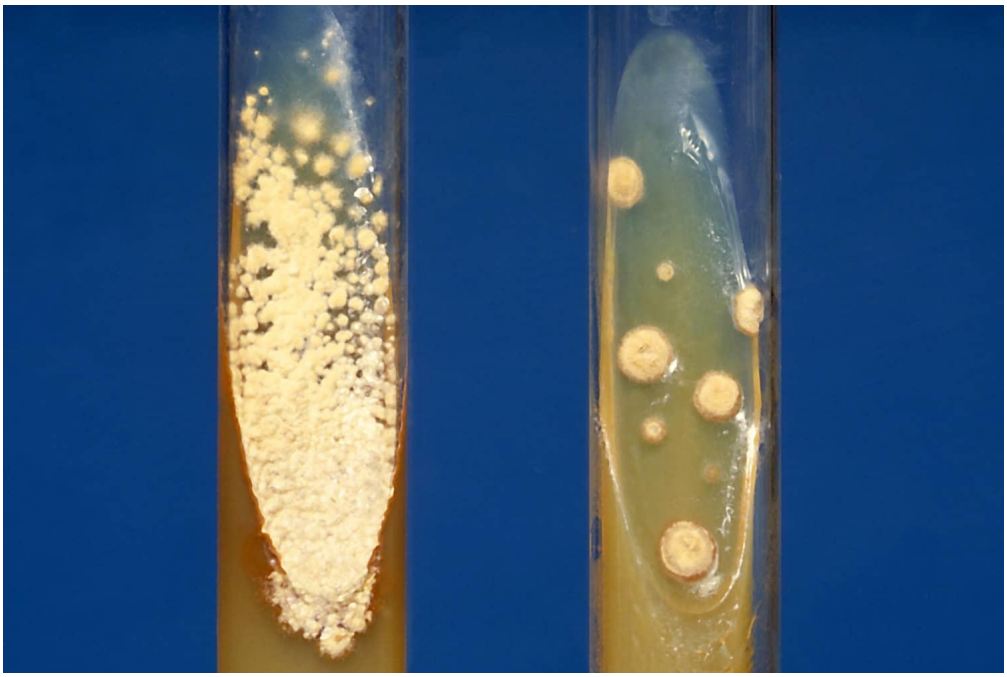
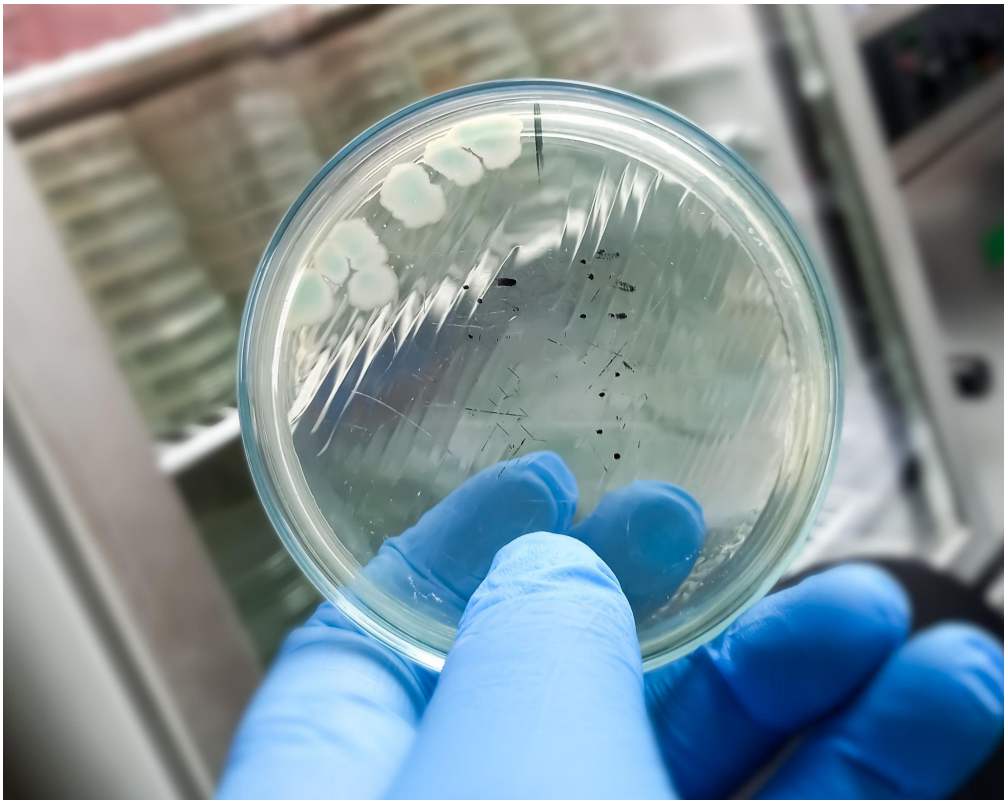


Figure 5.16: Fungal growth on Sabouraud Dextrose Agar media a) yeast growth b) mold growth



? DIAGRAM / FLOWCHART

? Diagnostic Algorithm of Fungal Infections

Clinical suspicion
?
Sample collection
?
Direct microscopy (KOH / Gram / India ink)
?
Culture (SDA)
?
Identification (LPCB / slide culture)
?
Advanced tests
? Histopathology
? Antigen detection
? PCR
?
Final diagnosis

? EXAM PEARLS

- KOH mount ? **fastest bedside test**
- Culture ? **gold standard but slow**
- GMS stain ? **most sensitive tissue stain**
- India ink ? **Cryptococcus diagnosis**
- Galactomannan ? **Aspergillus marker**
- ?-D-glucan ? **pan-fungal marker**