Medical mycology : is the study of fungi that produce disease in humans and other animals, and deals with those infections in humans, and animals resulting from pathogenic fungi .

Course Objectives:

The purpose of the medical mycology laboratory section is to help students learn skills to work safely with fungi in practical applications.

Laboratory studies will include :

1. Collection of specimens and selection of samples like Hair, skin scrapings, sputum, blood, urine, corneal scratching, pus, and contaminated nails.
2. Clinical specimen microscopic analysis
3. Ultraviolet exam for fluorescent hair (Wood's Light)
4. Fungi-cultivation
5. Identification of specific dermatophyte isolates
6. Dermatophyte isolation from soil-hair bait technique
7. Cultivation of Yeasts
8. Germ tube test and chlamydospore development
9. Fermentation carbohydrate test for yeast identification
10. Fungi keratinolytic activity

Speciment collection and transport:

- Specimen should be collected from active lesion
- 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 , Oral rinse

Guidelines for fungal sampling:

- To avoid bacterial overgrowth, all specimens must be transported to the laboratory without any delay.

- Specimens other than skin and blood specimens can be refrigerated for a limited period of time in case of delay

- Using forceps, contaminated hair can be plucked. Hairs can be gathered in paper envelopes that are sterilized.

- Until sample collection, the surface of the skin must be sterilized with alcohol. Using a forceps, the tip of the injury is scraped and collected in sterilized paper envelopes.

Microbiologic Examination tests:

1- KOH test:

Potassium hydroxide (KOH) solution, being alkaline, has the ability to dissolve keratin that is scraped from the outer layer of skin, which allows the microscopic identification of organisms such as dermatophytes or scabies, helping to establish the correct diagnosis and facilitating effective treatment.

Procedure:

1- Place the specimen on a clean glass slide.

2- Add 1 drop of 20% KOH

3- Place the cover glass on top of the slide and gently press to get rid of any air bubbles

4- Place slide on the microscope stage and start with a low-power (10 ×) examination

5- Examine for fungal structures such as hyphae or yeast

The KOH preparation test used to find out if you have a fungal infection. This kind of infection can happen in various parts of the body, such as the skin, nails, mouth, or vagina.

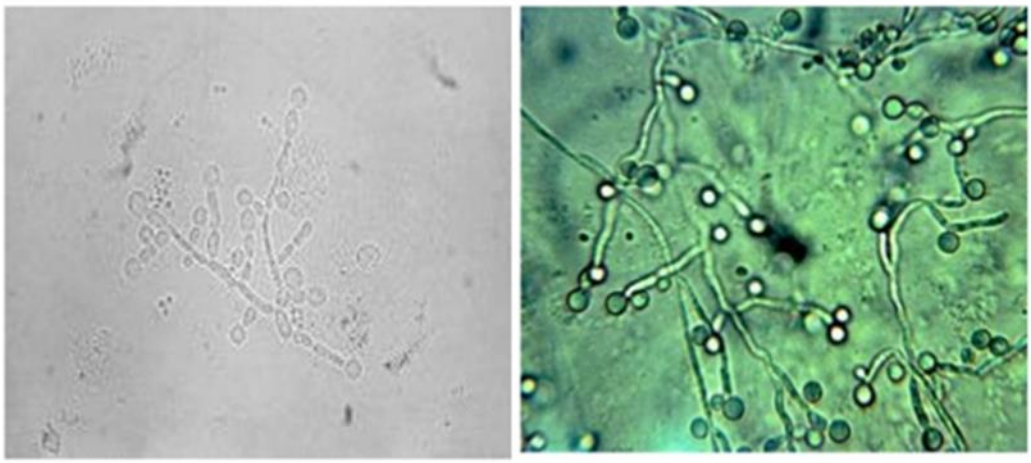


Figure1:KOH test of *candida sp.*

2-Calcofluor white stain test:

Calcofluor white is a chemifluorescent blue dye. It functions by being able to bind to 1-3 beta and 1-4 beta polysaccharides on chitin and cellulose that is present in cell walls on fungi, plants, and algae. Due to the speed in examining the cells, the stain was replaced by Potassium Hydroxide (KOH), which is quicker used to stain fungal and parasitic elements and to observe the presence of fungal and parasitic elements under a fluorescent microscope

Procedure:

1- Carefully put the specimen on a clean glass slide

2- Add a drop of Calcofluor white stain to produce an intense fluorescence

3-Add one drop of 10% Potassium Hydroxide

4- Cover the specimen with a coverslip and leave it to absorb the stain for 1 minute

5- Remove excess dye with a dry paper towel by gently pressing on the stain

6- Observe the stain under ultraViolet rays at x100-x400 magnification.

Fungi, Pneumocystis cysts, and parasites appear brilliant apple-green under UV fluorescent microscope, Violet and Blue light

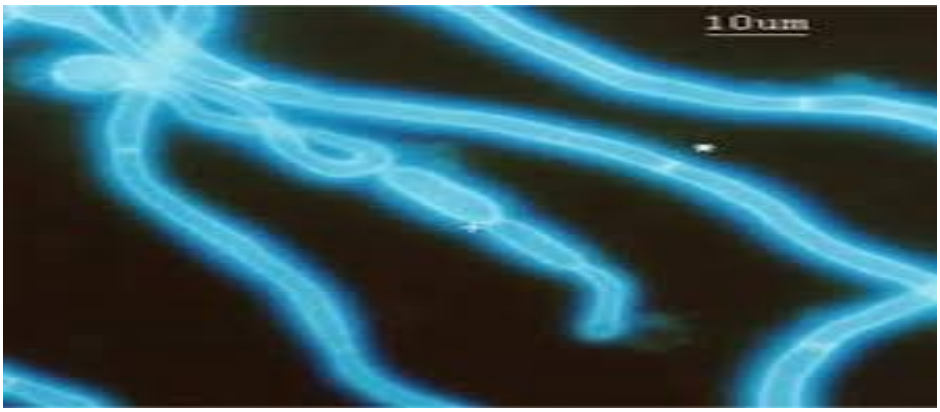


Figure2: Calcofluor white stained: The fluorescent staining of *candida albican* with calcofluor white. It shows a vivid blue color for the cell walls.

3-India ink test:

A diagnostic test used to detect the cryptococcal organism such as*Cryptococcus neoformans*. A dye, called India ink, is added to a sample like Cerebrospinal fluid (CSF), and if the fungi is present, they will become visible as the dye binds to the capsule surrounding the fungus

Procedure:

1-place a drop of India ink on to a clean glass slide

2-Add 1 drop of the specimen or liquid culture or rub a speck of material on the slide surface just beside the ink before mixing it into the ink. Sputum or pus can be cleared with KOH and heat and then mixed with India ink

Note: If preparation is too dark, it may be diluted with a small drop of water

3-Place a cover slip over the smear avoiding air bubbles, press it down gently through a sheet of blotting paper so that the film becomes very thin and pale in colour

4- Examine with a high-power lens (phase-contrast microscope) for the presence of encapsulated cells.

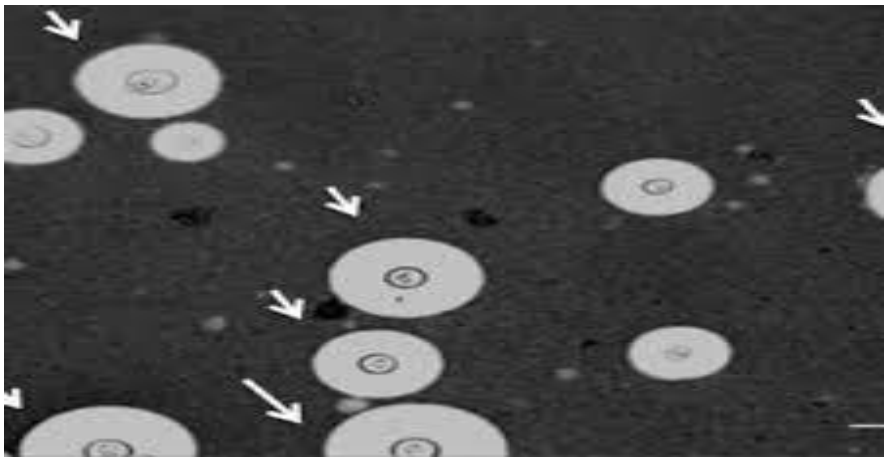


Figure3:Microscopy of CSF(India ink)

4-Culture test:

1- Culture the fungus On Sabouraud's agar medium

2- Incubated at room temperature For 21 days: Fast growers e.g, *candida sp.*, 2-3 days Slow growers e.g, *Trichophyton violaceum*, Macroscopic examination of colonies that grow on culture media and microscopic examination of slide mounts from culture often allow the identification of fungus

Culture for optimal recovery of fungal pathogen, the followings are added (Cycloheximide) is added to inhibit the growth of rapidly growing contaminating molds. An antibacterial agent (Chloramphenicol) is commonly added to control bacterial contamination.

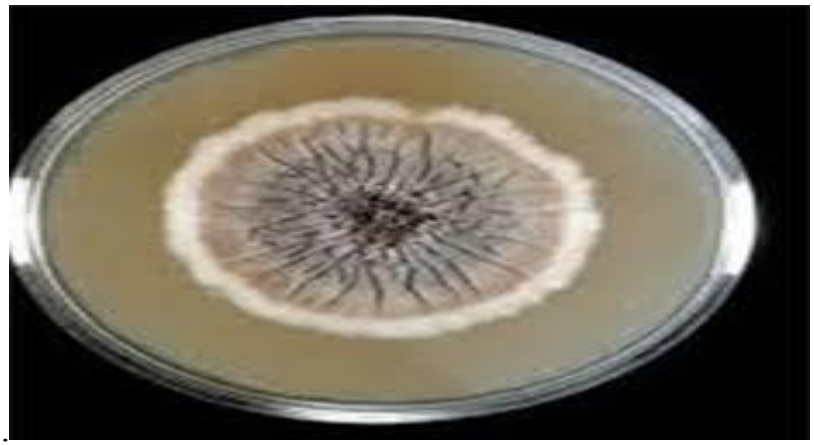


Figure 4*:T.violaceum* on Sabouraud's agar medium

5- GMS test:

Grocott-Gomori's Methenamine Silver stain is a histological stain that is used majorly for the identification of carbohydrates in fungal microorganisms. GMS tissue staining is often used in combination with microbiologic culture for diagnosis of fungal infections in people and animals .One advantage of GMS is that it produces better

staining contrast in tissues sections, and detects even degenerated and dead fungi The fungal species will stain black due to the reduction process of the silver nitrate solution. Silver nitrate solution after reduction forms silver ions which are black in color, thus producing a black stain for fungal cells.

Procedure:

1-Hydrate sections with distilled water

2- Oxidize the section with 4% aqueous chromic acid at room temperature and leave for 1 hr

3-Wash in water for a few seconds

4-Treat the sections with 1% sodium metabisulphite for 1 min

5-Wash in smoothly running tap water for 3 mins

6-Rinse thoroughly in distilled water Put the slides in pre-heated working silver solution in a water bath at 60°C for 15 to 20 mins until the section turns yellowish-brown Check microscopically to see fungi turned dark brown. Rinse well in distilled water

7-Add to the sections 0.2% gold chloride and leave for 2 mins Rinse well in distilled water Treat the sections with 2% sodium thiosulphate for 2 mins Wash with smoothly running tap water for 5 mins

8-Add the counterstain into the light green for 15 sec rinse the excess light green solution off the slide with alcohol dehydrate, clear and mount.

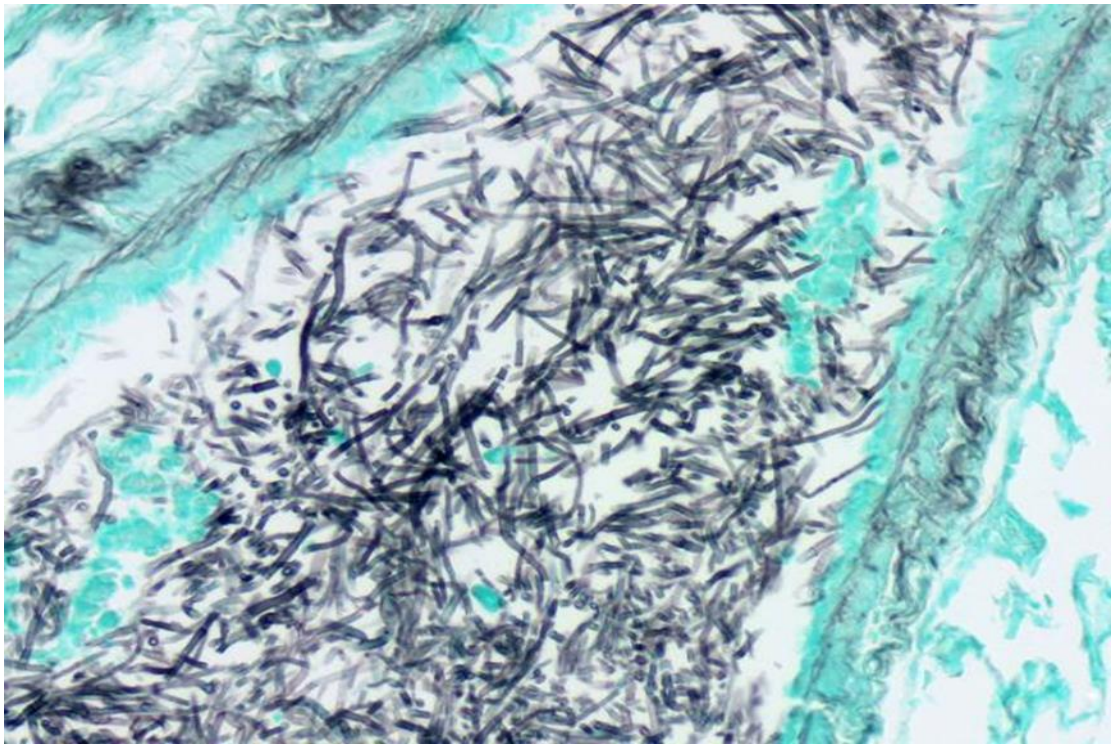


Figure5: GMS test of *Aspergillus sp*

6-Giemsa stain test:

Giemsa stain is used to obtain differential white blood cell counts It is also used to differentiate nuclear and cytoplasmic morphology of the various blood cells like platelets, RBCs, WBCs. Giemsa stain is used for staining *Histoplasma capsulatum*, *Penicillium* used in cytogenetics and for the histopathological diagnosis of malaria and other parasites.

Procedure:

1- Prepare a thin blood smear on a clean and dry microscopic glass slide and air dry it.
2- Dipping the blood smear quickly (two dips) in absolute methanol.
3- Dilution of Giemsa stain add 2 ml of stock solution of Giemsa stain to 40 ml of phosphate buffer solution You can also use the Distilled water instead of buffer

4-The Methanol fixed, Blood smear with diluted Giemsa stain (1:20, v/v) for 20 min. For this, put the slide in the Coplin jar containing the diluted Giemsa stain

5-Wash out the stained slides by Distilled water dried and observed by direct microscopy

Another techniques like :

- **Antibody detection • Antigen detection** typically involves measuring the level or titer of antibody. Serological tests ELISA,
- **Molecular techniques** may be used to detect the genetic material of the fungus causing the like such as DNA hybridization, PCR.

Culture media:

Culture media are specific mixtures of nutrients and other substances that support the growth of microorganisms , providing an environment securing of different kinds of microorganism and survival further Continuous propagation . It might contain nutrients carbon, nitrogen and vitamins, Glucose (dextrose), Fructose and mannose, growth factors , salts ,minerals and sources of energy like sugar.

Typical sequence of testing of fungal specimens:

1- Collection of specimen
2- preparation culture media
3- Fungi isolation
4- Incubation at optimal temperature
5- Check for growth
- Negative (No growth)
- Positive (Colonies on media)

6- Continue with identification procedures.

Specialized culture media:

1- Dermatophyte test media :

The use of dermatophyte test medium (DTM) is reliable, simple, inexpensive, and more definitive. Samples from hair, skin, or nails are obtained by scraping with a scalpel carefully, (especially for tinea capitis), and these are inoculated directly onto the test medium. After about 1 to 2 weeks, a color change from yellow to red in the agar surrounding the dermatophyte colony indicates positivity.

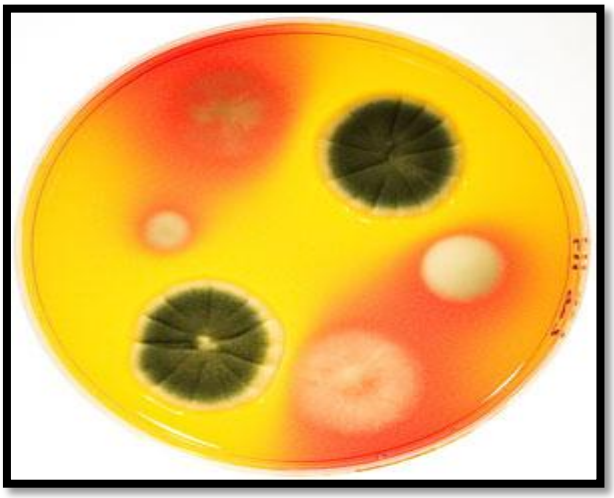


Figure (1) Dermatophyte test media

2- Sabourauds Dextrose Agar (SDA):

Is a standard in mycology laboratories and also in many dermatologists. It consist of dextrose energy source ,peptone protein source and agar for firm surface this media is good for yeasts and moulds like *candida albicans* .

3-Bird Seed Agar

For selective isolation of *Cryptococcus neoformans* and *C. gattii*. Most infections occur in the lungs which causes the human diseases (cryptococcosis) pulmonary infection.

Figure (2) Bird Seed Agar

4-Czapek Dox Agar:

This media used to cultivation of fungi, especially *Aspergillus*, *Penicillium*.

5- Modified Dixon's Agar:

For primary isolation and cultivation of Malassezia species which naturally found on the skin surfaces of many animals, and humans.

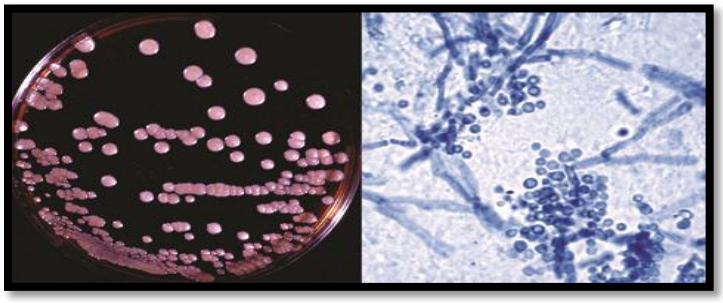


Figure (3) Malassezia in Modified Dixon's Agar

6- Hair Perforation Test for Dermatophytes

To distinguish between isolates of dermatophytes, particularly *Trichophyton mentagrophytes* and its variants.

Method :

- Place hair in water in vial.
- Inoculate with small fragments of the test fungus.
- Incubate at room temperature.
- Individual hairs are removed at intervals up to 4 weeks and examined microscopically in lactophenol cotton blue. Isolates of *Trichophyton mentagrophytes* produce marked localized areas of pitting and marked erosion whereas those of *Trichophyton rubrum* do not.

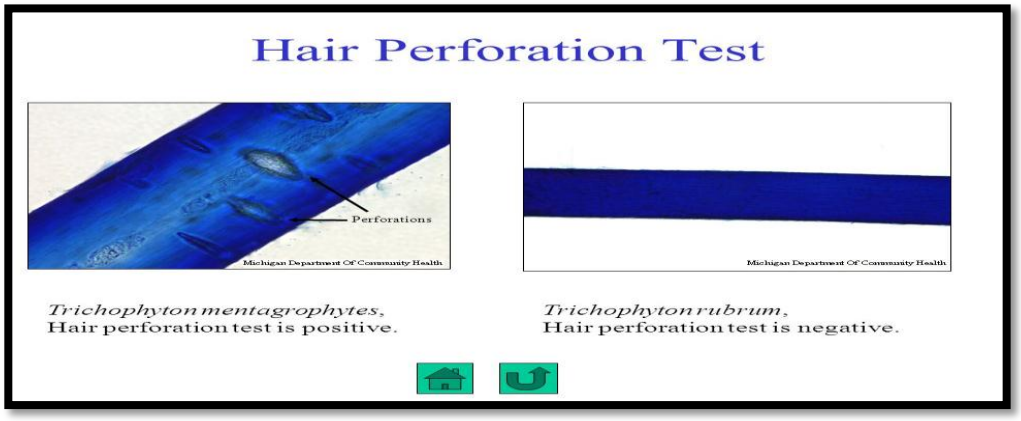


Figure (4) Hair Perforation Test

7- Potato Dextrose Agar:

For cultivation and identification of fungi.

8- Rice Grain Slopes:

For differentiation of *Microsporum audouinii* type of dermatophyte that colonizes keratinized tissues (hair) causing infection and *Microsporum canis* which infects the upper, dead layers of skin on domestic cats, occasionally dogs and humans.

Baits: Other kinds of baits might be pieces of wood, insects, carrot chunks, plastics, hair, or anything. Sometimes baits used to attract certain types of fungi, where a growth appear clear on these baits then transferred to the culture media.

Note: Antibacterial agents (Chloramphenicol) are used to kill the contaminating bacterial species.

Mycosis :

is a fungal infection of animals, including humans. Mycoses are common and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may initiate persistent infections; therefore, mycoses often start in the lungs or on the skin

Fungal infections of the skin was the 4th most common disease in 2010 affecting 984 million people .An estimation of 1.6 million people die each year of fungal infections.

Classification:

Mycoses are classified according to the tissue levels initially colonized.

A- Superficial mycoses

These are superficial fungal infections of the skin, hair or nails. No living tissue is invaded, however a variety of pathological changes occur in the host because of the presence of the infectious agent and its metabolic products.

Types of Superficial mycoses :

1- **Malassezia** species are basidiomycetes yeasts and form part of the normal skin flora of humans and animals. The genus now includes 14 species of which 13 are lipid dependent.

M. sympodialis ، *M. globosa*، *M. slooffiae* ، *M. restricta* and *M.* furfur are the most frequently found species responsible for colonization of humans .

Malassezia species may cause different skin manifestations including pityriasis versicolor, seborrhoeic dermatitis, dandruff, atopic eczema and folliculitis.

Note:

With the exception of *M. pachydermatis*, the primary isolation and culture of Malassezia species is challenging because in vitro growth must be stimulated by natural oils or other fatty substances. The most common method used is to overlay Sabouraud's dextrose agar (SDA) containing cycloheximide with olive oil or alternatively to use a more specialised media like modified Leeming and Notham agar or modified Dixon's agar .

Laboratory Diagnosis:

a- Direct Microscopy:

Skin scrapings taken from patients with Pityriasis versicolor stain rapidly when mounted in 10% KOH, glycerol and Parker ink solution and show characteristic clusters of thick-walled round, budding yeast-like cells and short angular hyphal forms up to 8um in diameter . These microscopic features are diagnostic for Malassezia furfur and culture preparations are usually not necessary.

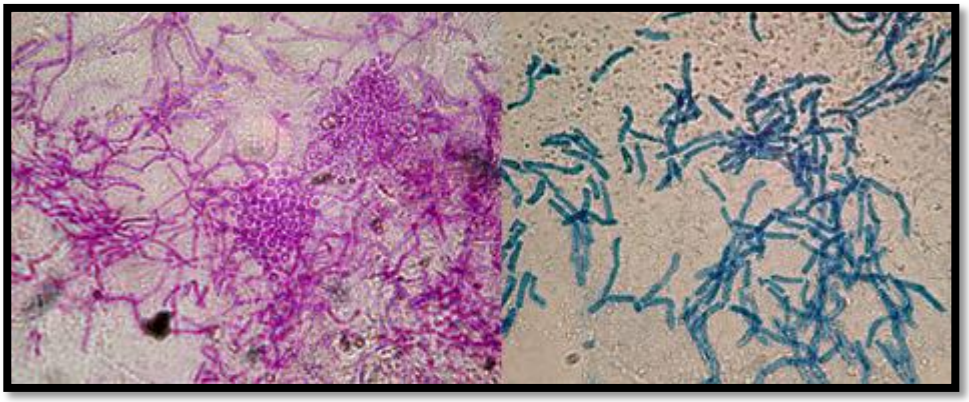


Figure (1) 10% KOH with Parker ink mount showing characteristic spherical yeast cells and short pseudohyphal elements typical of the fungus

b- Culture:

Culture is only necessary in cases of suspected . M. furfur is a lipophilic yeast, therefore in vitro growth must be stimulated by natural oils or other fatty substances. The most common method used is to overlay Sabouraud's dextrose agar containing cycloheximide with olive oil or alternatively to use a more specialized media like Dixon's agar which contains glycerol .

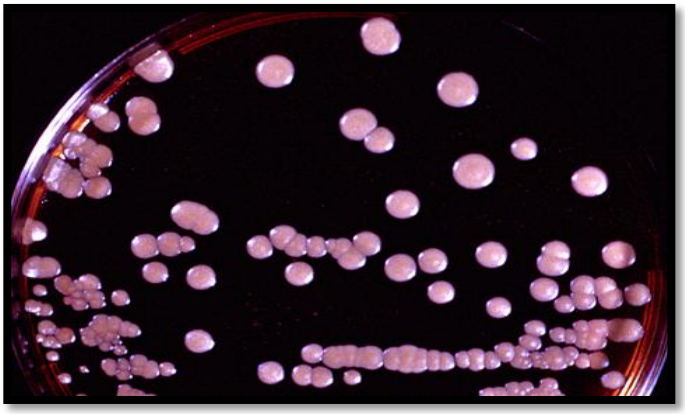
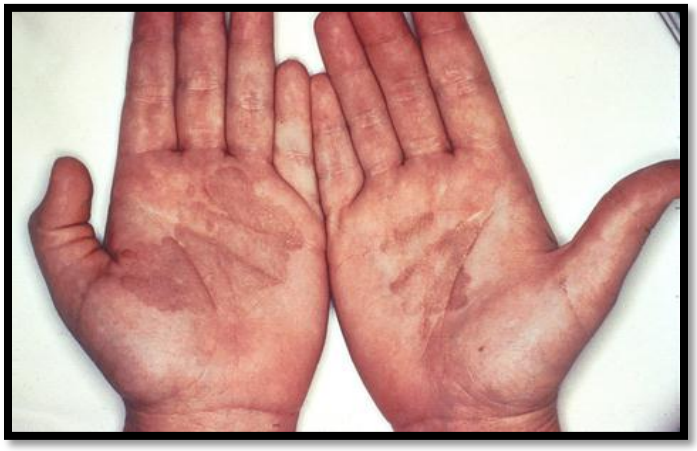


Figure (2) Culture of *M. furfur* on Dixon′s agar

2- *Tinea nigra*

A superficial fungal infection of skin characterised by brown to black which usually occur on the palmar aspects of hands and occasionally the plantar and other surfaces of the skin , Lesions are non-inflammatory . Familial spread of infection has also been reported. World-wide distribution, but more common in tropical regions of Central and South America, Africa, South-East Asia and Australia. The aetiological agent is *Hortaea werneckii* a common saprophytic fungus believed to occur in soil, compost, humus and on wood in humid tropical and sub-tropical regions.



Figure(3) Typical brown to black, on the palmar aspect of the hands. **Note:** there is no inflammatory reaction.

Laboratory Diagnosis:

a- Direct Microscopy:

Skin scrapings should be examined using 10% KOH and Parker ink .

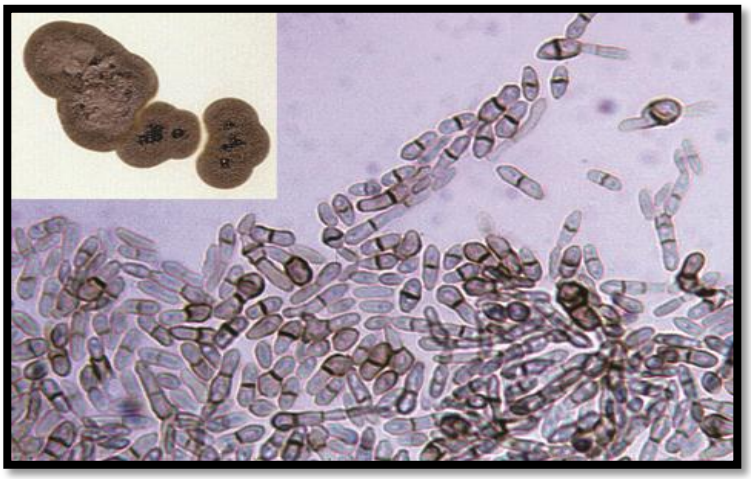


Figure (4) Colony and conidia of *Hortaea werneckii*

b- Culture:

Clinical specimens should be inoculated onto primary isolation media, like Sabouraud's dextrose agar.

3- **Black piedra**

Black piedra is a superficial fungal infection of the hair shaft caused by *Piedra hortae*, an ascomycetous fungus forming hard black nodules on the shafts of the scalp, beard, moustache and pubic hair. It is common in Central and South America and South-East Asia.

Laboratory Diagnosis:

a- Direct Microscopy:

Hairs should be examined using 10% KOH and Parker ink or calcofluor white. Look for darkly pigmented nodules that may partially or completely surround the hair shaft. Nodules are made up of a mass of pigmented with a stroma-like centre containing asci.

b- Culture:

Hair fragments should be implanted onto primary isolation media, like Sabouraud's dextrose agar. Colonies of *Piedra hortae* are dark, brown-black and take about 2-3 weeks to appear .

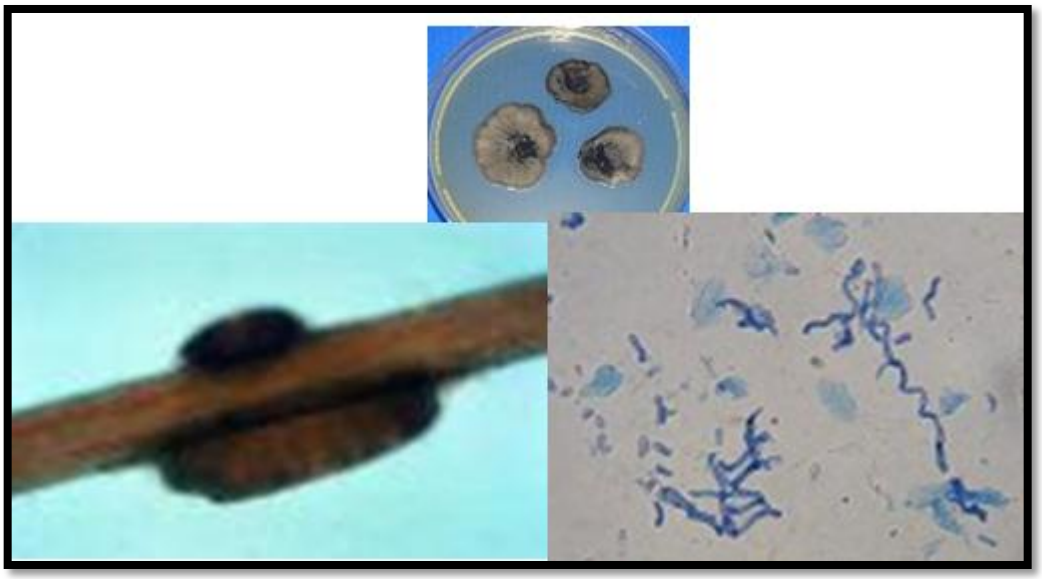


Figure (5) *Piedra hortae*

2- Cutaneous mycoses

Are infections that extend deeper into the epidermis. It invades hair and nails and causes diseases. The diseases are referred to as ringworm or tinea, also called (dermatophytes) Dermatophytes are keratinophilic - "keratin loving". Digest the keratin Which is found in the skin, nails and hair by their keratinase enzyme.

❖ Tinea Pedis

Also known as Athlete's foot is a dermatophyte infection of the skin of the foot. Symptoms include: itching or burning on the skin of the foot, peeling or flaking skin, dry skin, blisters or thick patches of dry red skin. Three species of fungi, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum* are together responsible for the vast majority of cases of tinea pedis throughout the world.

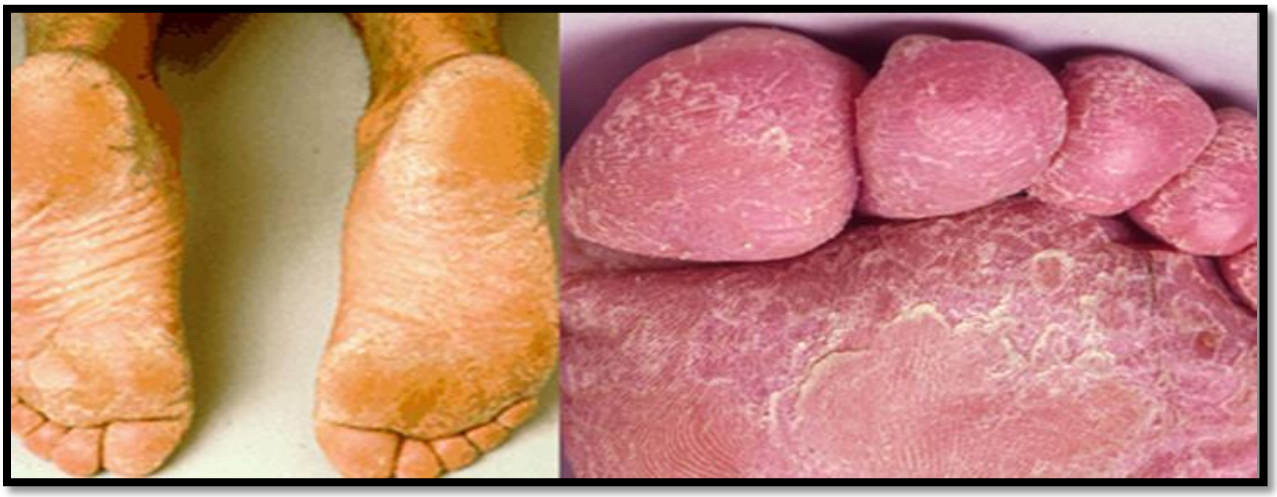


Figure (1) Tinea pedis caused by *Epidermophyton floccosum*

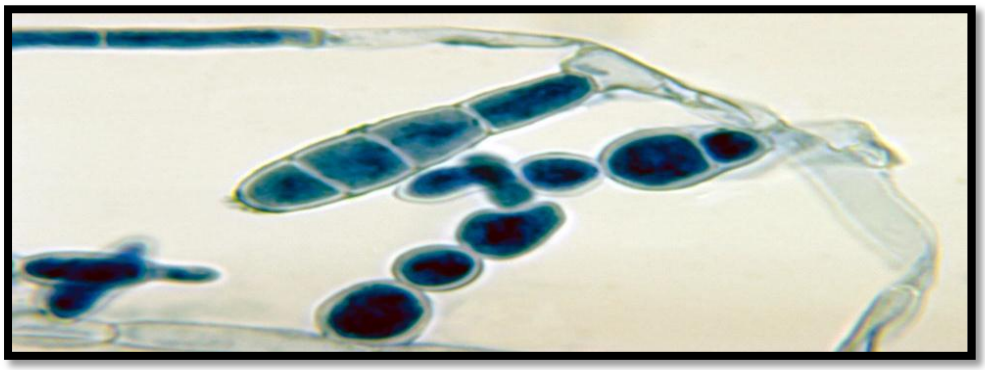


Figure (2) *Epidermophyton floccosum*: Thin-walled macroconidia growing directly from the hyphae and numerous clamidoconidia

❖ Tinea corporis

Dermatophytosis of the body (tinea corporis) affects the glabrous skin and is commonly known as ringworm. Clinically, the lesions present as annular plaques with central clearing, leading scale, and a ring-shape appearance .They occur mainly on the trunk, limbs, and may be single or multiple, of varying sizes, and may coalesce *Microsporum canis* is a frequent cause of human infection,

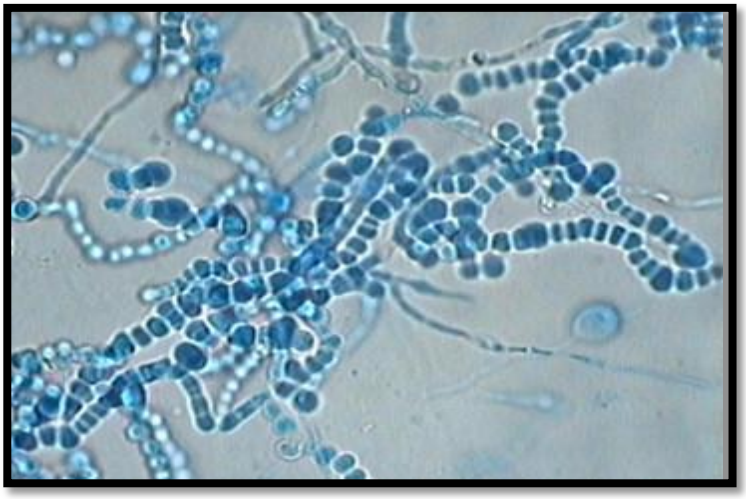


Figure (3) *Microsporum canis* :Micromorphology: Spindle-shaped, thick-walled macroconidia with more than 6 cells, and few pyriform to claviform microconidia

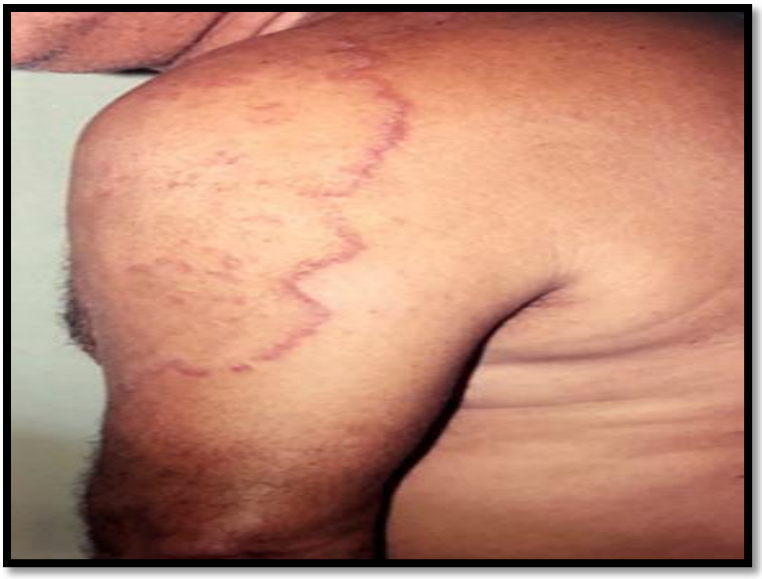


Figure (4) Tinea corporis: The lesions present as annular plaques and a ring-shape appearance

❖ Tinea cruris

Tinea ruris is infection of the groin with a dermatophyte fungus. It is most often seen in adult men. Tinea cruris is commonly known as jock itch. *Trichophyton rubrum* and *Epidermophyton floccosum* are the most common causes. Symptoms of jock itch are mainly seen in the groin region, inner and upper thighs and on the male genitalia. Some of the symptoms of jock itch infection are: Red or pink ring-shaped rash, Dry and roughened skin, Small blisters (formation).

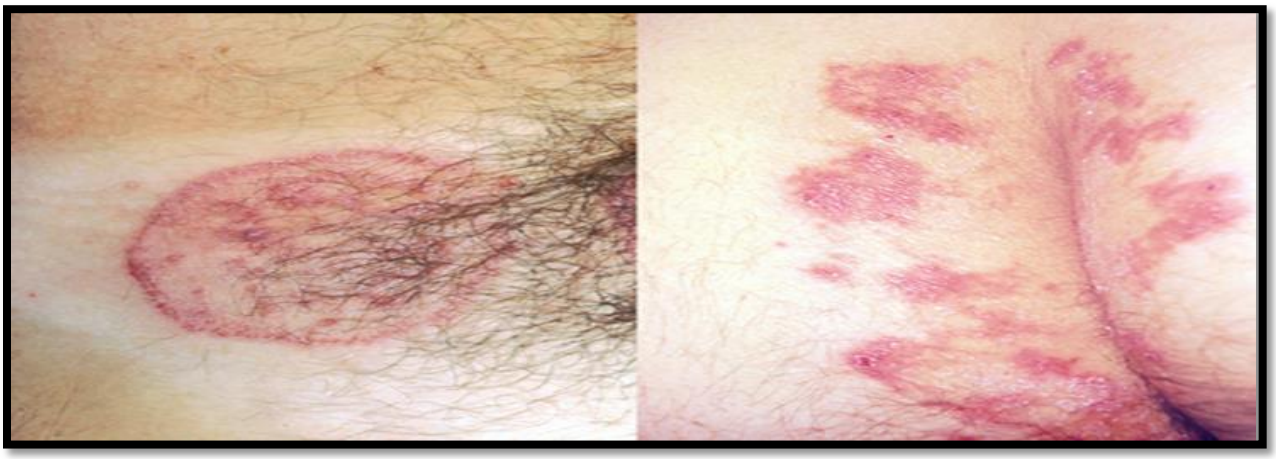


Figure (5) Tinea of the groin and buttocks caused by *T. rubrum*

❖ Tinea unguium

onychomycosis a fungal infection of the nail, usually caused by a dermatophyte *Trichophyton rubrum* and *T. interdigitale*

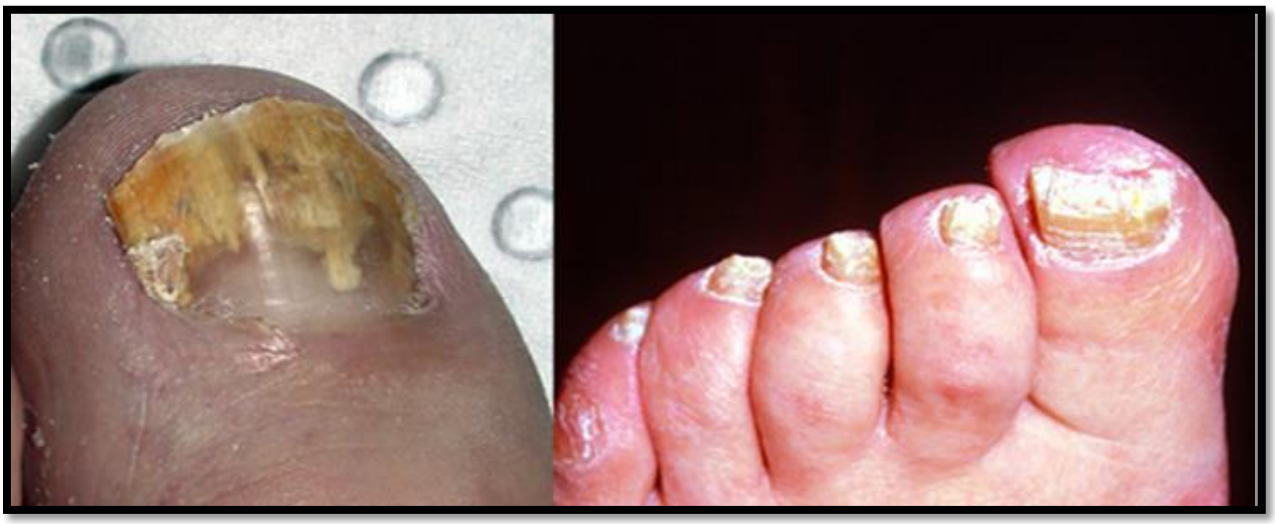


Figure (6) Tinea of the toe nails caused by *T. rubrum*

❖ Tinea capitis

is a fungal infection of the scalp, involving both the skin and hair. It is also known as scalp ringworm. Symptoms of tinea capitis include hair loss, dry scaly areas, redness, and itch .Tinea capitis is caused by dermatophytic fungi capable of invading keratinised tissue, such as the hair and nails. *Trichophyton tonsurans* is an anthropophilic dermatophyte that is the most common cause of tinea capitis in the United States. Examples of other anthropophilic fungi that cause tinea capitis include: *T. soudanese, T. schoenleinii, M. audouinii*.

The infection occur Following invasion of the keratinised stratum corneum of the scalp ,the fungus grows downwards into the hair follicle and the hair shaft. It penetrates the hair cuticle and typically invades the hair shaft in one of three ways:

- Ectothrix infection: the dermatophyte grows within the hair follicle and covers the surface of the hair. Fungal spores are evident on the outside of the hair shaft and the cuticle is destroyed. M. canis is an ectothrix dermatophyte.

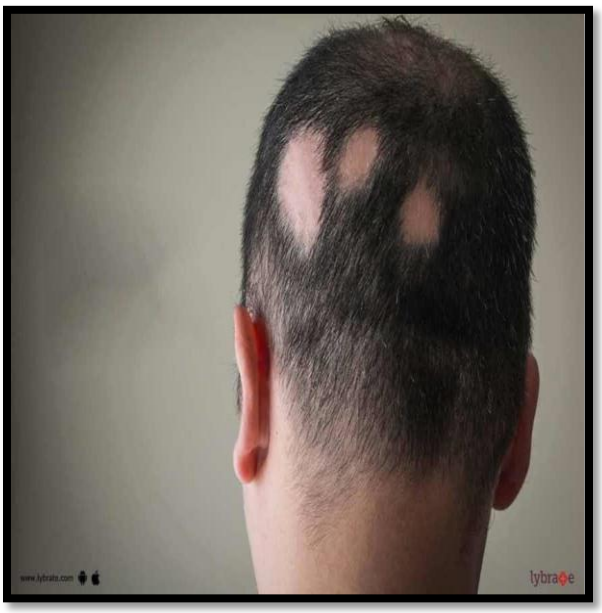


Figure (7) Ectothrix Tinea capitis showing Places hair loss caused by *M. canis*

- Endothrix infection: the dermatophyte invades the hair shaft and grows within it. Fungal spores are retained inside the hair shaft, and the cuticle is not destroyed. T. tonsurans is an endothrix dermatophyte.

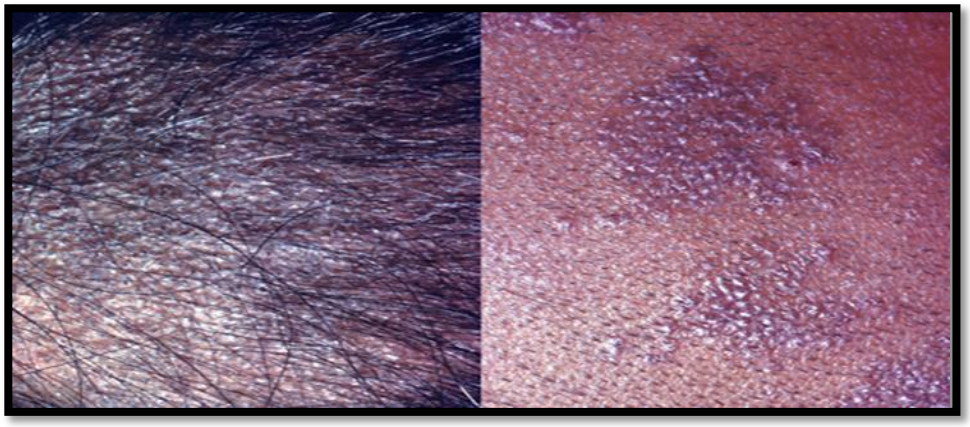


Figure (8) Endothrix tinea capitis caused by *T. tonsurans* and black dot tinea capitis caused by *T. violaceum*

- Favus infection: a chronic dermatophyte infection caused by T. schoenleinii and characterised by clusters of hyphae at the base of the hairs, with air spaces in the hair shafts. Clinically there is yellow crusting around the hair shaft.

Figure (9) Favus of the scalp showing extensive hair loss and numerous small scuttle caused by *T. schoenleinii*

Laboratory Diagnosis:

Direct Microscopy:

Skin Scrapings, nail scrapings and epilated hairs should be examined using 10% KOH and Parker ink or calcofluor white mounts.

Culture:

Specimens should be inoculated onto primary isolation media, like Sabouraud's dextrose agar containing cycloheximide and incubated at 26-28˚C for 4 weeks. The growth of any dermatophyte is significant.

Wood lamp examination

Wood lamp is diagnostic when hair fluorescence is seen, Bright green fluorescence of infected hairs is observed in tinea capitis caused by Microsporum species (*M. ferruginium, audouinii, canis,* and *distorum*). Identifying affected hairs in this way may help with obtaining an appropriate specimen for microscopy and culture. Procedure that uses transillumination (light) to detect bacterial or fungal skin infections.

A Wood lamp examination is a test that uses ultraviolet (UV) light to look at the skin closely. A Wood's lamp is a light that uses long wave ultraviolet light. When an area of scalp that is infected with tinea (a type of ringworm fungus) is viewed under a Wood's light, the fungus may glow.

Figure (10) Wood lamp fluorescence device

Figure (11) Tinea capitis: Wood lamp fluorescence

3- Subcutaneous Mycoses

Mycoses that penetrate the epidermis and the dermis to infect deeper tissues are called subcutaneous mycoses.

a- Sporotrichosis :

Sporotrichosis also known as rose gardener's disease is a chronic mycotic infection of the cutaneous or subcutaneous tissues and adjacent lymphatic's characterized by nodular lesions which may suppurate and ulcerate. Infections are caused by the traumatic implantation of the fungus into the skin, or very rarely, by inhalation into the lungs.

Clinical manifestations:

1- Fixed cutaneous sporotrichosis:

Primary lesions develop at the site of implantation of the fungus, usually at more exposed sites mainly the limbs, hands and fingers. Lesions often start out as a painless nodule which soon become palpable and ulcerate often discharging a serous or purulent fluid. Importantly, lesions remain localised around the initial site of implantation Isolates from these lesions usually grow well at 35˚C, but not at 37˚C .

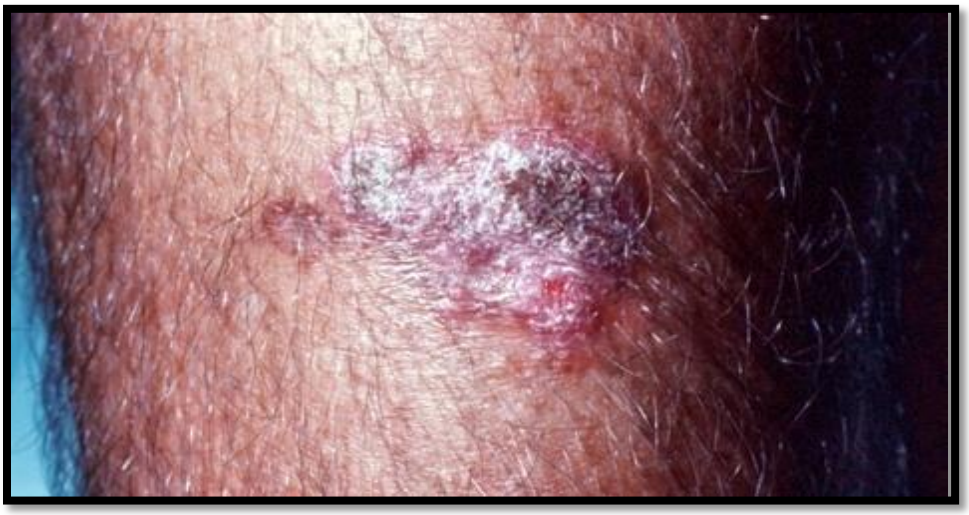
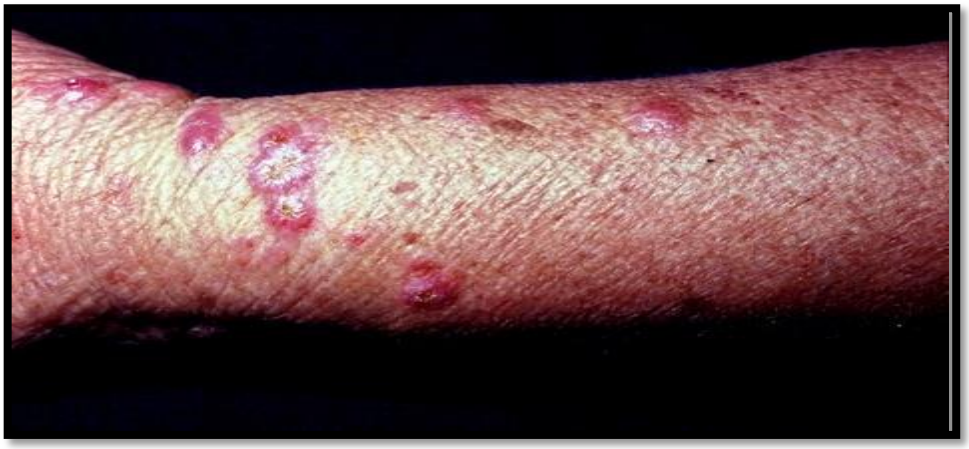


Figure (1) Fixed cutaneius sporotrichosis showing an ulcerating lesion on the leg

2- Lymphocutaneous sporotrichosis:

The primary lesion start out as painless nodules which soon become palpable and ulcerate. No systemic symptoms are present. Isolates from these lesions usually grow well at both 35˚C and 37˚C.



Figure(2) Lymphocutaneous sporotrichosis showing typical elevated subcutaneous nodules

3- Pulmonary sporotrichosis:

This is a rare entity usually caused by the inhalation of conidia . Symptoms are nonspecific and include cough, sputum production, fever, weight loss and upper-lobe lesion. Haemoptysis may occur and it can be massive and fatal. The natural course of the lung lesion is gradual progression to death .

4- Osteoarticular sporotrichosis:

Most patients also have cutaneous lesions and present with stiffness and pain in a large joint, usually the knee, elbow, ankle or wrist. Osteomyelitis seldom occurs without arthritis; the lesions usually confined to the long bones near affected joints .

Laboratory diagnosis:

A tissue biopsy is the best specimen.

1- Direct Microscopy:

Tissue sections should be stained using PAS digest, Grocott's methenamine silver (GMS) or Gram stain.

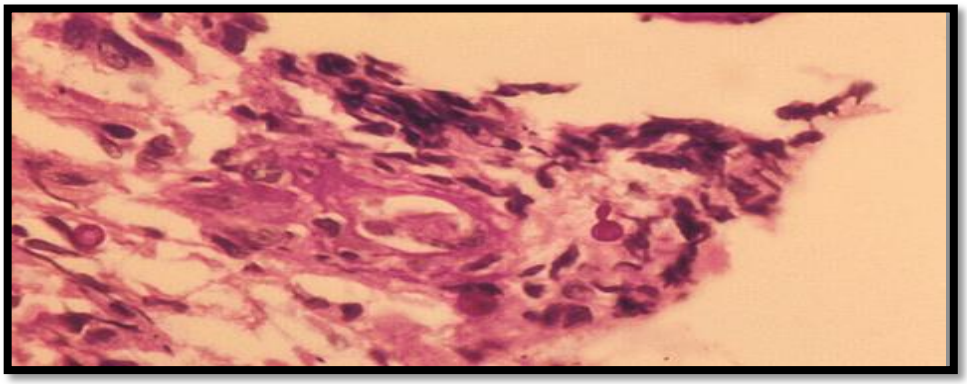


Figure (3) Section from a fixed cutaneous lesion showing budding yeast-like cells. Sporothrix

2- Culture:

Clinical specimens should be inoculated onto primary isolation media, like Sabouraud's dextrose agar and Brain heart infusion agar supplemented with 5% sheep blood .

b- Chromoblastomycosis

A mycotic infection of the cutaneous and subcutaneous tissues characterised by the development in tissue (brown-pigmented), . Infections are caused by the traumatic implantation of fungal elements into the skin and are chronic, slowly progressive and localised. Tissue proliferation usually occurs around the area of inoculation producing crusted, wart-like lesions. The species Fonsecaea pedrosoi and Cladophialophora carrionii are prevalent in regions where the disease is endemic .

Clinical Manifestations:

Lesions of chromoblastomycosis are most often found on exposed parts of the body and usually start a small scaly papules or nodules which are painless but may be itchy. the disease develops rash-like areas enlarge and become raised irregular plaques that are often scaly , lesions may become tumorous and even cauliflower-like in appearance. Other prominent features include epithelial hyperplasia, fibrosis and microabscess formation in the epidermis.

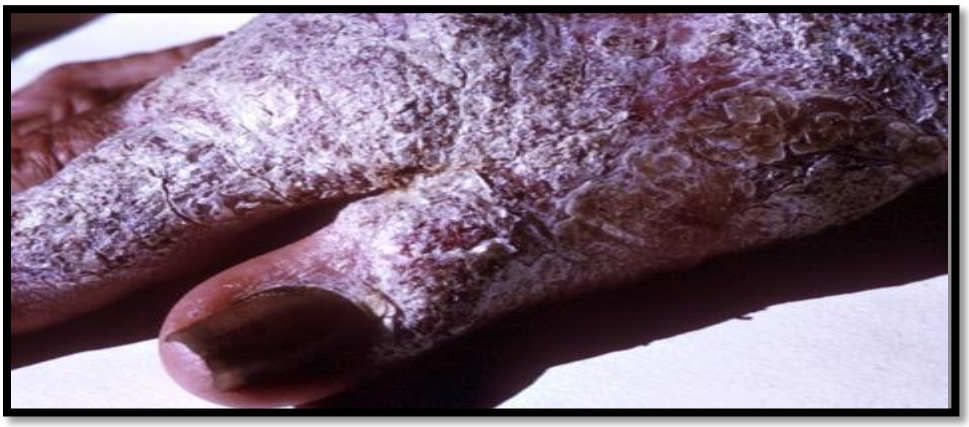


Figure (4) Chronic chromoblastomycosis of the hand due Cladophialophora carrionii

Laboratory Diagnosis:

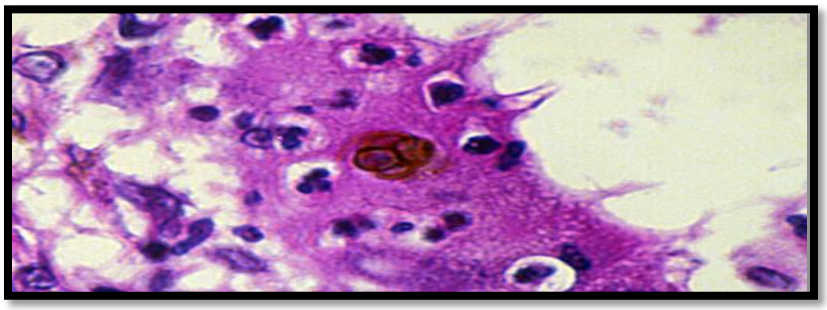
By Skin scrapings and/or biopsy.

1- Direct Microscopy:

(a) Skin scrapings should be examined using 10% KOH and Parker ink or calcofluor white mounts, to see round Sclerotium bodies.

Figure (5) Skin scrapings from a patient with chromoblastomycosis mounted in 10% KOH, rounded Sclerotium bodies.

(b) Tissue sections should be stained using H&E, PAS digest, and Grocott's methenamine silver (GMS), to see round sclerotic bodies.



Figure(6) H&E stained section showing characteristic dark brown ,rounded Sclerotium bodies.

2- Culture

Clinical specimens should be inoculated onto primary isolation media, like Sabouraud's dextrose agar with Cycloheximide .

Figure (7) Chromoblastomycosis Sabouraud's dextrose agar

Candidiasis :

Candidiasis is a fungal infection which is caused by a type of fungus called candida. Candida is a type of yeast that lives in our mouth, throat, gut or vagina (in case of women), or on the skin. There are many species of the fungus candida causes of fungal infection.: *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis,* but *candida albicans* are the most that usually cause the infections.

Clinical manifestations:

1- Oropharyngeal candidiasis: including thrush, glossitis, stomatitis and angular cheilitis.

Oral candidiasis is the most common oral fungal infection. Oral candidiasis is considered an opportunistic infection, occurring more frequently in persons with impaired immunity.

In thrush, white or yellow patches appear on the tongue, gums, lips, inner cheeks, and roof of the mouth. There is also redness and soreness in the mouth and throat. If the infection spreads to the throat, then there may be pain in swallowing too.

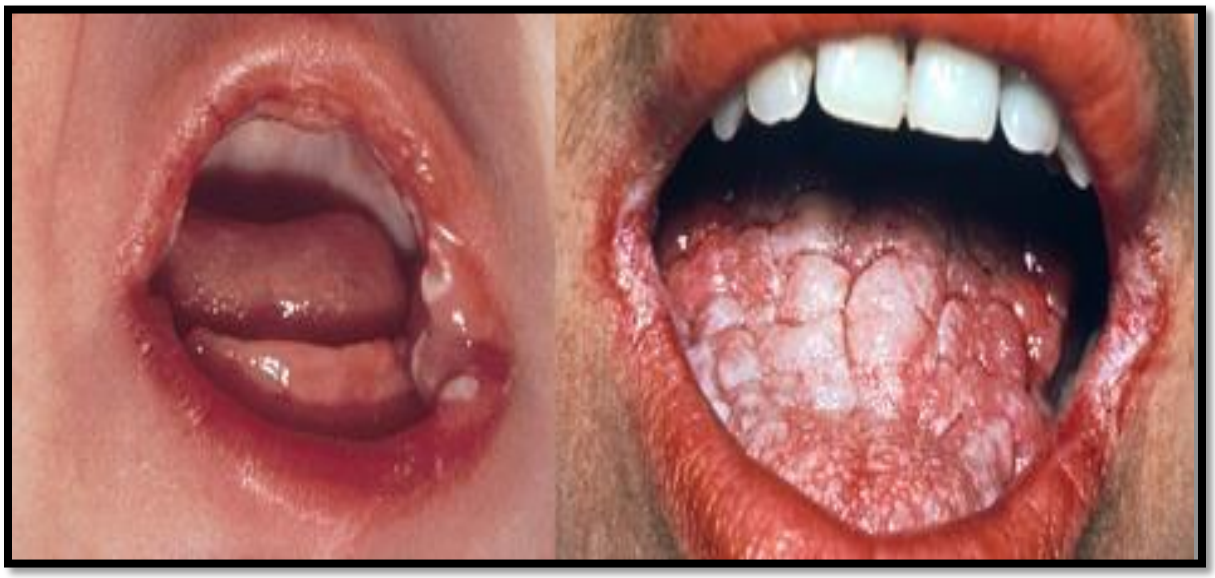


Figure (1) Oral candidiasis in a new born (left) and in an immunosuppressed patient (right).

2- Cutaneous candidiasis: including intertrigo, diaper candidiasis, paronychia , onychomycosis and Vulvovaginal .

Candidiasis skin infection. Hand of a patient affected by candidiasis, a fungal infection of the skin. Candidiasis is a common infection of the moist areas of the body, Candidiasis causes an itchy red rash Irritation Pain, Blisters

Cracked skin those who have a compromised immune system are at an increased risk of developing a severe infection. candidiasis is most commonly seen in the axillae, groin, inter- and sub-mammary folds, interdigital spaces, and umbilicus. Moisture, heat, friction and maceration of the skin are the principle predisposing factors in the normal patient.

Vulvovaginal candidiasis is a common condition in women, , low vaginal pH and diabetes mellitus. Sexual activity and oral contraception may also be contributing factors and infections may extend to include the perineum, the vulva and the entire inguinal area. Symptoms include acute vulval Itch, burning and erythema associated with a fluid white .

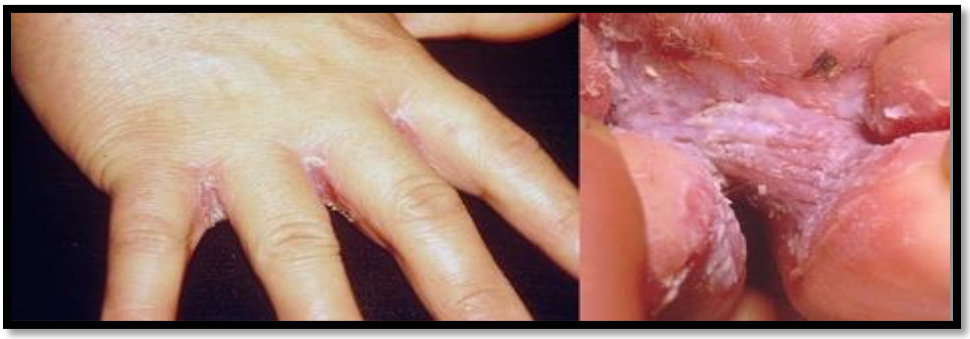


Figure (2)Interdigital candidiasis and candidiasis between the toes mimicking tinea.

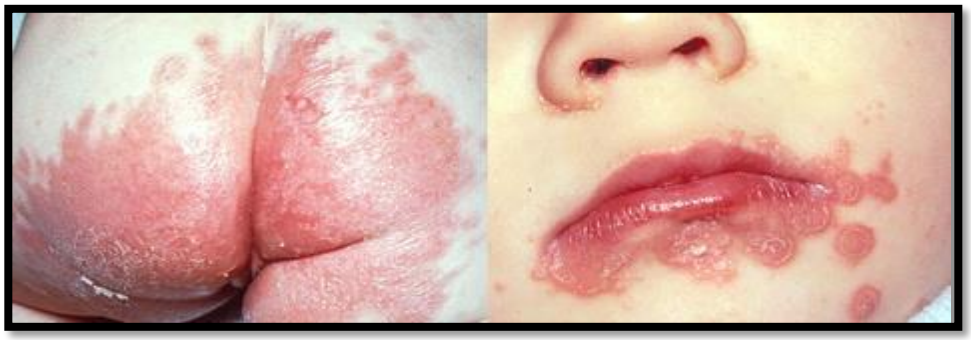


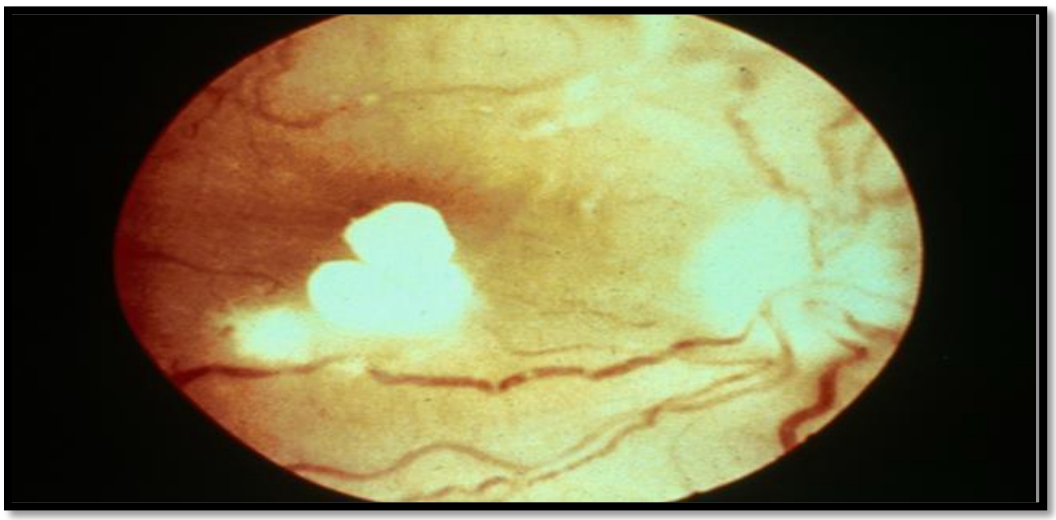
Figure (3) Nappy rash candidiasis in an infant which spread to the mouth area.

3- Candidemia (Candida septicemia)

Candidemia refers to the isolation of pathogenic species of Candida from a blood culture specimen. And the presence of species of Candida in the blood may then occur to one or more other organ systems Candida species have been reported to cause up to 15% of cases of septicemia seen in hospital patients.

4- Ocular candidiasis:

Candida endophthalmitis is often associated with candidemia, When this fungus infects the eye, the condition is known as ocular candidiasis. The infection is transmitted through the bloodstream, and affects the blood vessels of the retina. Lesions (yellow, centre and centre right) form and can cause blindness if left untreated.



Figure(4) Endophthalmitis due to *Candida*

5- Osteoarticular candidiasis:

Arthritis may be a late sequel of candidemia in neonates, Prosthetic or rheumatoid joints are also prone to infection by Candida . The knee is the main site involved with pain on weight bearing or on full extension. The diagnosis depends on the isolation of yeasts from joint fluid obtained by needle aspiration or from synovial biopsies .

Laboratory diagnosis:

1- Direct Microscopy:

(a) Skin and nails should be examined using 10% KOH and Parker ink or calcofluor white mounts **.**

(b) body fluids should be centrifuged and the sediment examined using either 10% KOH and Parker ink or calcofluor white mounts and/or gram stained smears

(c) Tissue sections should be stained using PAS digest, Grocott's methenamine silver (GMS) or Gram stain. Note Candida may be missed in H&E stained sections.

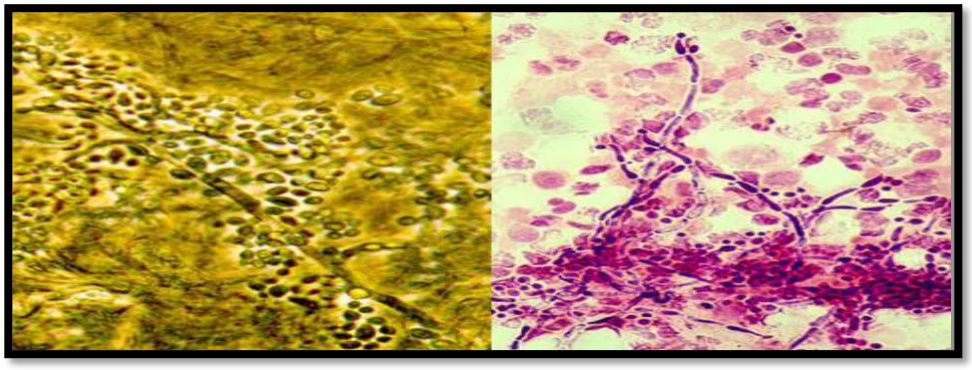


Figure (5)10% KOH mount showing the presence of budding yeast cells and pseudohyphae in a skin scraping

2-Culture:

Colonies are typically white to cream colored with a smooth, glabrous to waxy surface.

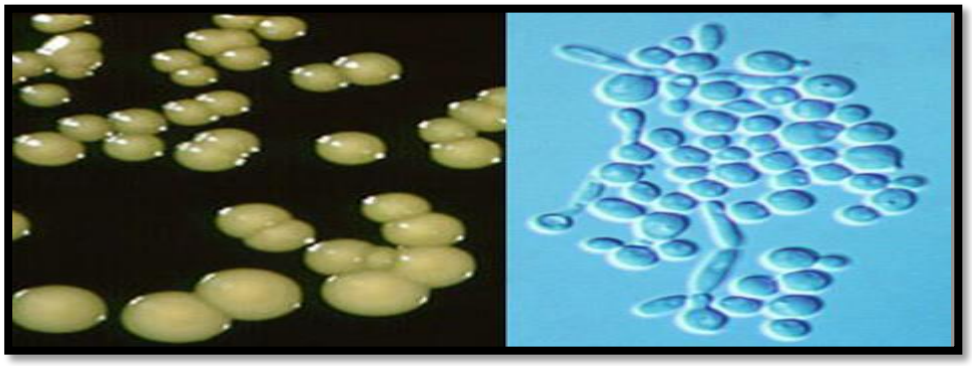


Figure (6) Typical moist colonies of Candida.