

DIMORPHIC FUNGI

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Introduction

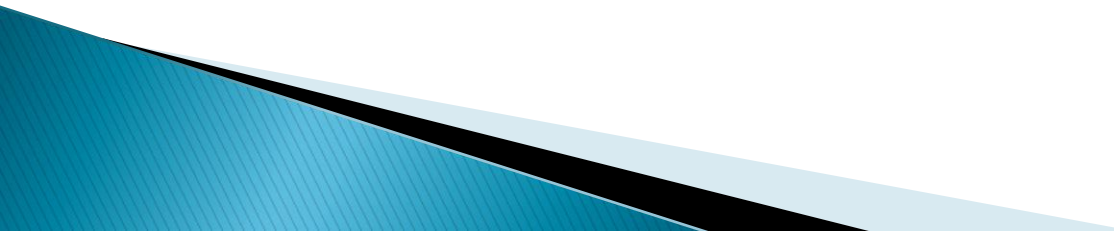
- ▶ Present two growth forms
 - ▶ mould when growing saprophytically in the environment or when on culture media at 25–30°C,
 - ▶ yeast or yeastlike form in animal tissues or when cultured on enriched media at 37°C.
 - ▶ Mould /mycelial phase- more
 - ▶ cause deep or systemic mycoses
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Table 41.1 Diseases and distribution of dimorphic fungi associated with disease in animals

Fungus	Disease(s)	Geographical distribution	Main host(s)	Usual habitat	Site of lesions
<i>Sporothrix schenckii</i>	Sporotrichosis (lymphangitis of limbs in horse)	Worldwide, more common in subtropical and tropical regions	Horses, dogs, cats, humans	Old wooden posts, rose thorns, dead vegetation, soil, moss	Subcutaneous nodules, lymphatics
<i>Blastomyces dermatitidis</i> (teleomorph: <i>Ajellomyces dermatitidis</i>)	Blastomycosis	Eastern regions of North America, sporadic cases in Europe, India, the Middle East	Dogs, cats, humans	Acidic soil rich in organic material	Primary lesions in lungs with metastases to skin and other organs
<i>Coccidioides immitis</i> and <i>Coccidioides posadasii</i>	Coccidioidomycosis	Semi-arid regions in southwestern USA, Central and South America	Dogs, horses, cats, humans	Soil of low-elevation deserts	Primary lesions in lungs with secondary lesions in bones
<i>Histoplasma capsulatum</i> var. <i>capsulatum</i>	Histoplasmosis	Mississippi and Ohio river valleys, sporadic cases in many other countries worldwide	Dogs, cats, humans	Nitrogenous soils enriched with bird or bat faeces	Primary lesions in lungs with dissemination to intestines and other organs
<i>Histoplasma capsulatum</i> var. <i>farcinosum</i>	Epizootic lymphangitis (African farcy)	Africa, Middle East, Asia	Horses, mules, donkeys	Soil	Skin, lymphatics, lymph nodes

Laboratory Diagnosis

- ▶ Direct microscopy:
 - ▶ – wet mounts of exudates and tissues
 - ▶ – histopathology on tissue sections

- ▶ • Culture and demonstration of the mould phase at 25–30°C and the yeast phase on enriched medium at 37°C
- ▶ • Microscopic appearance of cultures: fruiting structures and spores (colonies at 25–30°C) and yeast-forms (colonies at 37°C)

- ▶ • Nucleic acid probes (available commercially)
- ▶ • Exoantigen tests
- ▶ • Immunological & serological tests
- ▶ • Mouse inoculation

Table 37.2 Morphological features of pathogenic fungi in diagnostic specimens

Fungus	Techniques	Summary of diagnostic features
<i>Aspergillus fumigatus</i>	KOH, calcofluor white, periodic acid-Schiff (PAS) or silver impregnation stains	Septate hyphae, dichotomous branching at a 45° angle. Hyphae 3–6 µm and rarely up to 12 µm in diameter. Tissue reaction is granulomatous or necrotizing, but may not occur in an immunosuppressed host. May see distorted fruiting heads if fungus spreads into an air space in the body
Zygomycetes: <i>Rhizopus</i> , <i>Mucor</i> , <i>Rhizomucor</i> , <i>Absidia</i> and <i>Mortierella</i> spp.	KOH, calcofluor white, PAS or silver impregnation stains	Large, bulging, non-septate hyphae that can be twisted and fragmented. About 10–20 µm in diameter (range 3–25 µm) with irregular branching. The invading hyphae of <i>Mortierella wolfii</i> tend to be finer (2–12 µm diameter) than the other zygomycetes
<i>Candida albicans</i>	Gram stain, KOH, PAS or silver impregnation stains	Budding cells, oval or round, 3–4 µm diameter. Pseudohyphae may be present in tissue; these have regular points of constriction between individual elongated yeast cells. They must be distinguished from moulds with septate hyphae
<i>Malassezia pachydermatis</i>	Gram stain, methylene blue, KOH or calcofluor white	Bottle-shaped, small yeast (1–2 × 2–4 µm). Unipolar budding and reproduction is by bud-fission in which the bud detaches from the mother cell by a septum
<i>Cryptococcus neoformans</i>	India ink, KOH, PAS or Mayer's mucicarmine stain	Spherical budding yeast cells, 2–15 µm diameter, usually surrounded by a large capsule. Produces pinched-off buds, sometimes multiple. Cells vary greatly in size in a single preparation. Encapsulated pseudohyphae are very occasionally seen
<i>Blastomyces dermatitidis</i>	KOH, calcofluor white, FA technique, PAS or silver-impregnation stains	Large, budding yeast 8–15 µm (range 2–30 µm) in diameter with very thick walls. Buds are connected by a broad base. Intracytoplasmic contents are usually evident

<i>Histoplasma capsulatum</i>	Wright, Giemsa, PAS or silver impregnation stains	Small, budding yeast, spherical to oval, 2–5 µm, intracellular in monocytic cells. A clear halo can be seen around the darker staining cell. Buds are single with narrow bases. The fungus is difficult to detect in unstained preparations
<i>Coccidioides immitis</i>	PAS and silver-impregnation stains, KOH + calcofluor white	Large spherules present in tissue. When mature, up to 200 µm in diameter and contain numerous non-budding endospores (2–5 µm). Immature spherules vary in size and do not contain endospores
<i>Sporothrix schenckii</i>	Gram stain or KOH on exudates. PAS or silver-impregnation stains on biopsies	Small, cigar-shaped yeasts, 2–6 µm. May exhibit multiple budding. Only a small number are usually present in exudates and they may be hard to see
Dermatophytes: <i>Microsporum</i> and <i>Trichophyton</i> spp.	KOH, KOH + calcofluor white, DMSO + KOH, blue-black ink + KOH	Septate hyphae (2–3 µm diameter) surround affected hairs and fragment into arthrospores. Some hyphae may still be present but more usually a sheath of refractile round arthrospores (2–8 µm diameter) is present. These arthrospores must not be confused with fat globules or hair-pigment granules (melanosomes)
Fungi in mycetomas	KOH, calcofluor white, PAS and silver-impregnation stains	Irregular granules, 0.5–3.0 mm and variously coloured, are present in biopsies or scrapings. Within crushed granules are intertwined hyphae (2–5 µm) with swollen cells (15 µm or more) at the periphery
Fungi in chromoblastomycoses	KOH, calcofluor white, PAS and silver-impregnation stains	Single-celled or clustered, spherical (4–12 µm), thick-walled bodies and darkly pigmented (sclerotic) bodies. Hyphae may be present (2–6 µm) and are seen in skin scrapings and aspirates
<i>Pneumocystis carinii</i>	Giemsa stain, immunocytochemistry and methenamine silver stain	Trophic, cystic and spore forms may be found in lung tissue and bronchoalveolar lavage fluid of affected animals

Dimorphic fungus

Animal tissue (37°C)

Culture (37°C)

Brain-heart + 5% blood agar

Culture or environment (25°C)

Sabouraud dextrose agar

Sporothrix schenckii



Cigar-shaped, budding yeast cells, that may occur within neutrophils. Usually very few present. (2–4 μm in diameter) Asteroid bodies may occur

Single or multiple-budding yeast cells, 2–4 μm in diameter

Fine branching hyphae with 2–3 μm pyriform conidia in flowerettes from short conidiophores. Conidia connected by thread-like process

Blastomyces dermatitidis



Large (8–10 μm) round or oval, thick-walled yeast cells. Buds on a broad base, single buds. Cytoplasmic granulation is often obvious

Large (8–10 μm), round or oval thick-walled yeast cells budding on a broad base

Small (2–3 μm) oval or pear-shaped conidia borne on tips of short conidiophores on septate hyphae

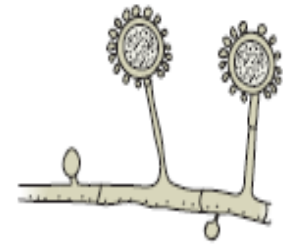
Histoplasma capsulatum



Small (2–5 μm) budding yeast cells intracellular in phagocytic cells. The yeast cells are usually surrounded by a halo

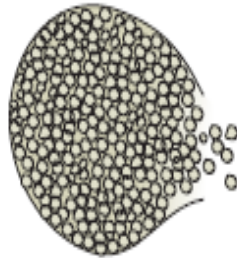


Oval budding yeast cells (3–4 μm diameter) with a narrow neck between mother and daughter cells



Two types of conidia; large (8–14 μm) tuberculate macroconidia that are sunflower-like and small tear-drop like microconidia

Coccidioides immitis

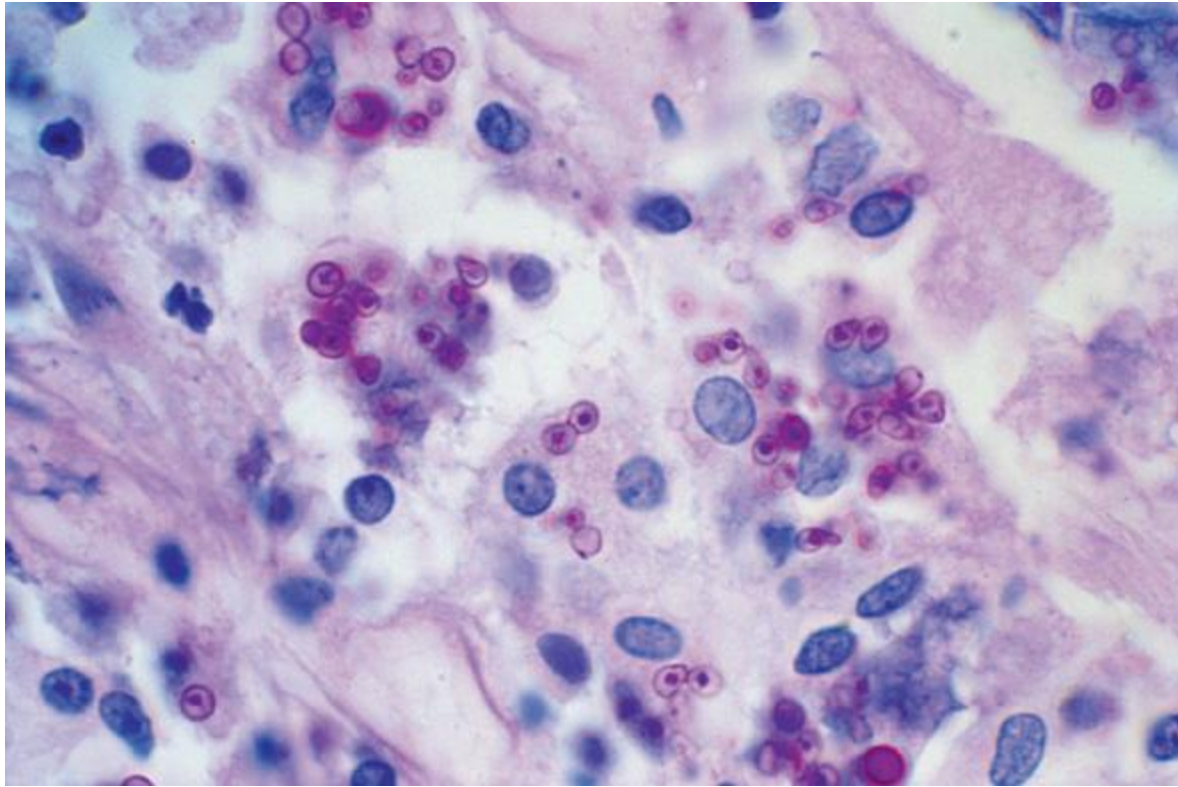


Spherules (15–60 μm), the mature forms filled with endospores. No endospores in immature spherules

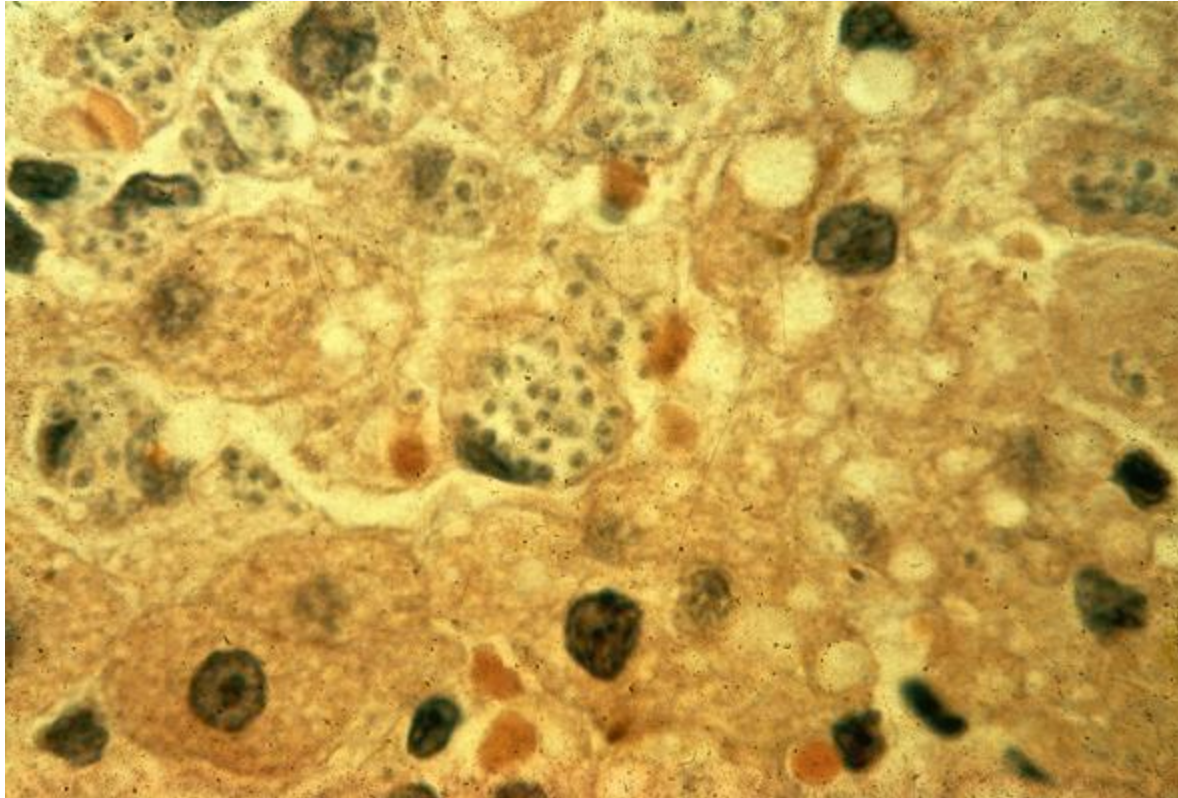


Septate hyphae branching at right angles. With age the hyphae dissociate into barrel-shaped arthrospores. These are separated by clear, non-viable cells. Arthrospores are wider than the hyphae. Cannot be converted easily to spherule form in vitro

Figure 41.1 Microscopic morphology of the dimorphic fungi.



Blastomyces dermatitidis yeast form in tissue.
(PAS-haematoxylin stain, $\times 1000$)

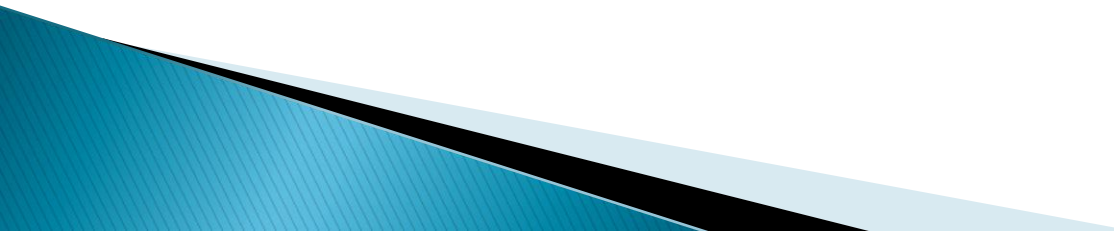


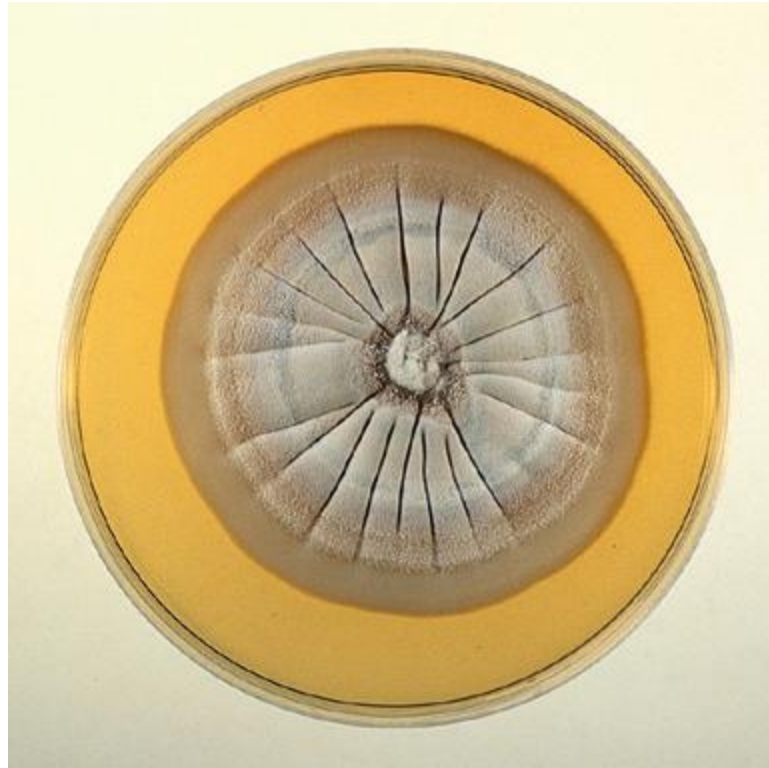
Histoplasma capsulatum yeast form in Kupffer's cells (dog's liver). (Silver stain, $\times 1000$)

Yeast conversion of the dimorphic fungi

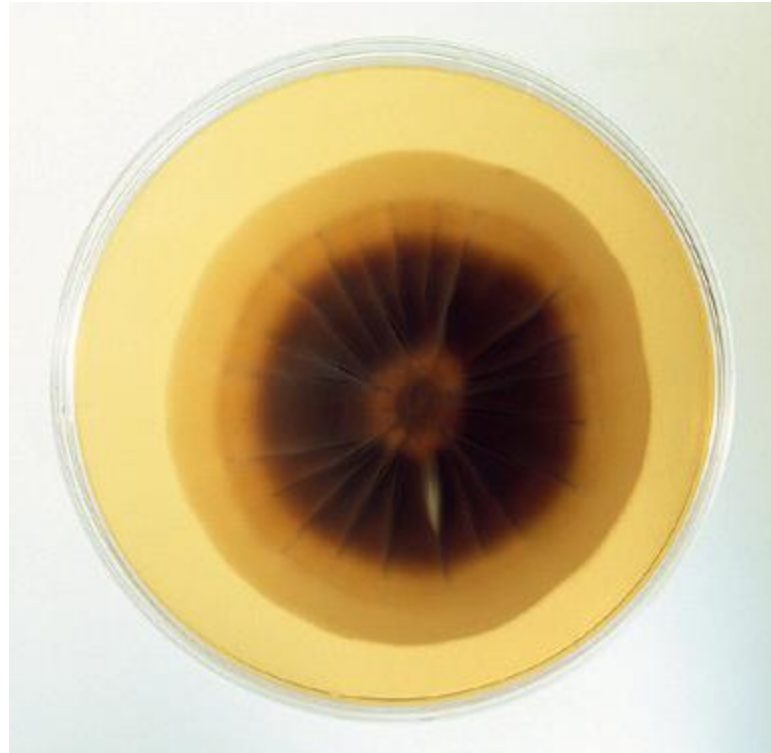
- ▶ For full identification of these fungi, an attempt to convert them to the yeast phase should be made on enriched media at 37°C.

Colonial morphology

- ▶ Sporothrix schenckii
 - ▶ At 25°C- growth is visible in three to five days. Colonies- white to cream at first, becoming wrinkled with delicate aerial hyphae and then later turning dark and leathery
 - ▶ At 37°C colonies are yeast-like, smooth, soft and cream to tan in colour. Growth occurs in about three to five days.
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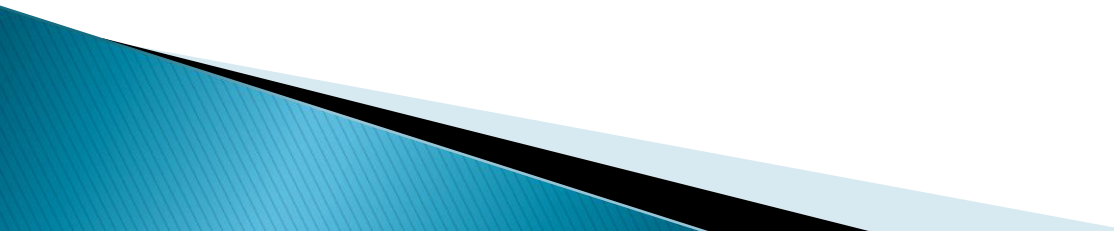


***Sporothrix schenckii* on Sabouraud agar at
25°C, 13 days.**



Sporothrix schenckii on Sabouraud agar,
13 days. Reverse.

Blastomyces dermatitidis

- ▶ At 25°C growth occurs in about two to four weeks.
 - ▶ The colonies are small and produce white, cottony aerial hyphae, becoming greyish or dark brown with age.
 - ▶ They vary from a flat, dull colony to a heaped fungal mass with hyphal tufts
 - ▶ At 37°C the waxy, yeast-like colonies are wrinkled and cream to tan in colour. They can have radiating 'prickles' from the surface.
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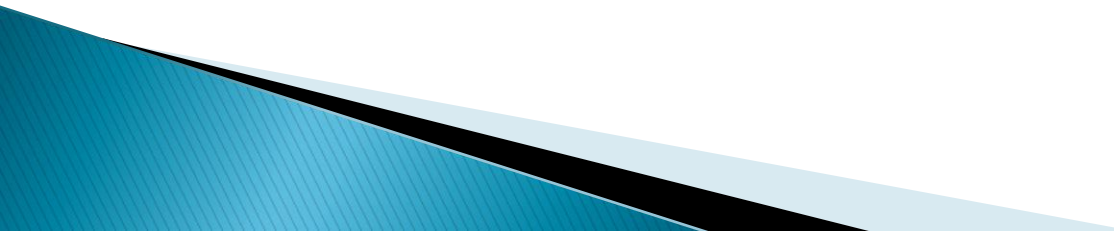


***Blastomyces dermatitidis* on Sabouraud agar.**
(25°C, 16 days)

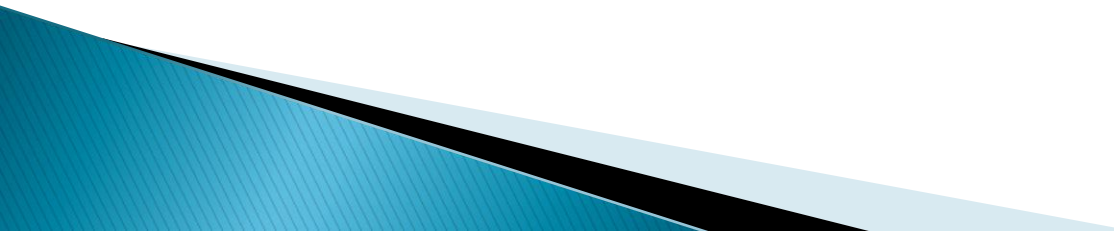
Histoplasma capsulatum var. *capsulatum*

- ▶ At 25°C white to cream colonies with cottony aerial hyphae are seen.
- ▶ They turn grey to brown with age and require two to four weeks' incubation. The colonies are similar to those of *B. dermatitidis*.
- ▶ At 37°C the colonies are smooth, yeast-like and cream to tan in colour.

Coccidioides immitis

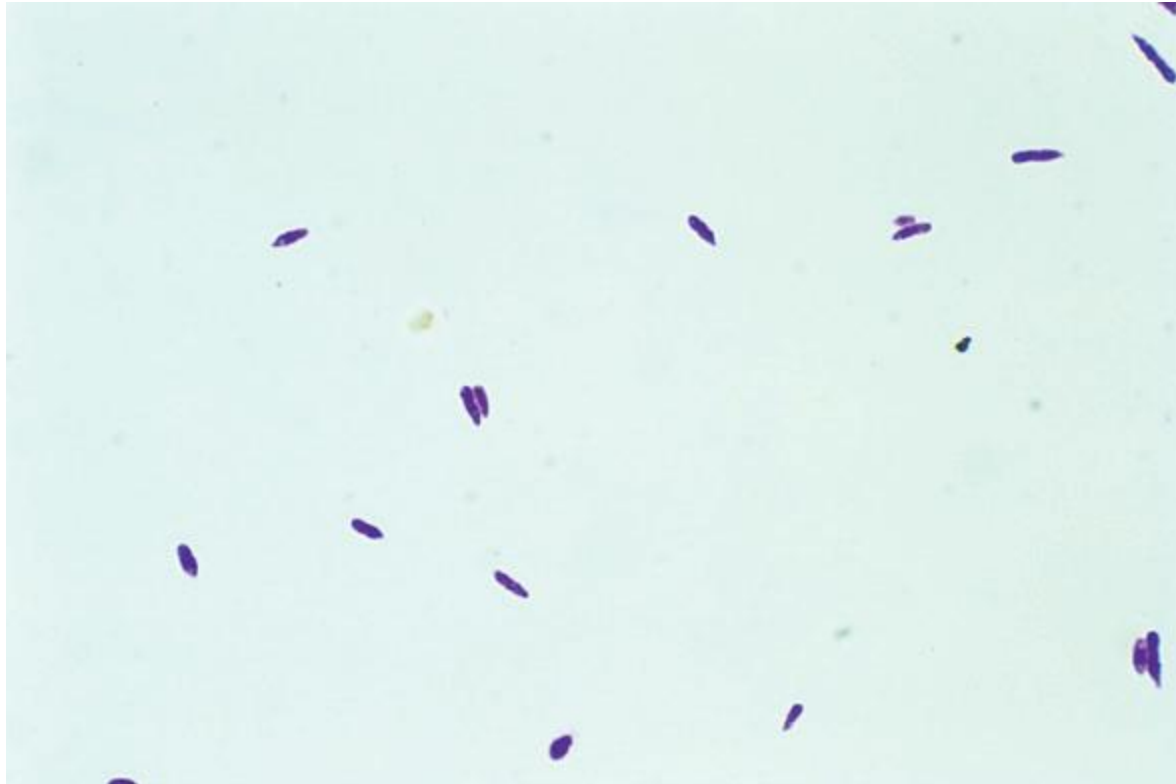
- ▶ At 25°C and 37°C delicate cobweb growth in three to 21 days occurs.
 - ▶ It causes a greenish discolouration on blood agar.
 - ▶ Colonies have fluffy areas alternating with areas adherent to the agar surface.
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Microscopic appearance

- ▶ Sporothrix schenckii
 - ▶ Large numbers of yeast cells may be seen in methyleneblue- stained smears
 - ▶ Yeast cells- histopathological sections stained by PAS or methenamine silver techniques.
 - ▶ Immunohistochemical staining may be used to specifically identify the yeast cells.
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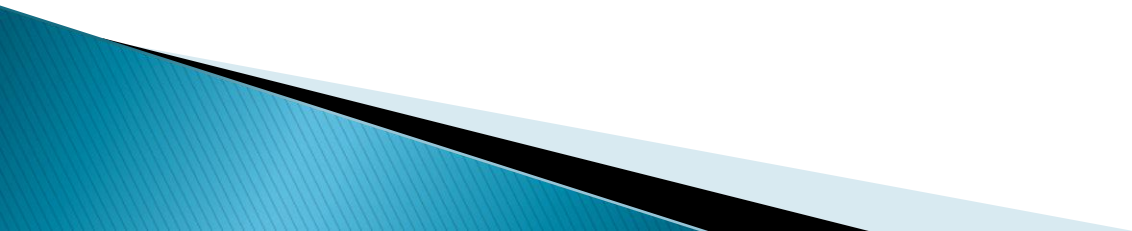


Sporothrix schenckii conidiophore and conidia.
Culture incubated at 25°C. (LPCB, ×400)

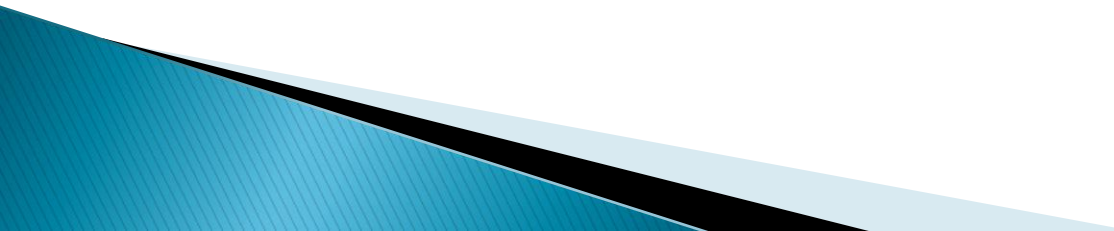


***Sporothrix schenckii* yeast cells. Culture**
incubated at 37°C. (LPCB, $\times 1000$)

Blastomyces dermatitidis

- ▶ Yeast cells may be demonstrated in cytological or histopathological preparations from lesions.
 - ▶ Methylene blue or Giemsa stains are suitable for smears from exudates or aspirates.
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Histoplasma capsulatum var. *capsulatum*

- ▶ Yeast cells in macrophages may be visible in Giemsa stained smears of exudates or aspirates.
 - ▶ Histopathological sections of affected tissues may demonstrate pyogranulomatous foci which contain yeast cells.
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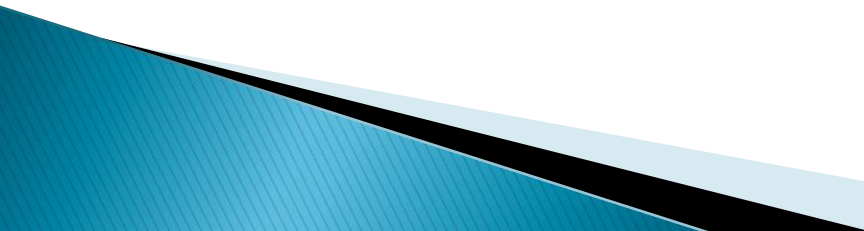
Coccidioides immitis

- ▶ Exudates and aspirates cleared with 10% KOH or stained tissue sections are suitable for the demonstration of the characteristic spherules.

Molecular techniques

- ▶ DNA probes
- ▶ *DNA* amplification techniques
- ▶ Nested PCR

Exoantigen test

- ▶ This test is used in some laboratories for *B. dermatitidis*, *H. capsulatum* and *C. immitis*.
 - ▶ *It is a relatively simple and rapid method for identification and if positive it obviates the necessity to convert the fungus to the yeast phase.*
 - ▶ The method is an immunodiffusion test that, using reference antisera, detects cell-free antigens (exoantigens) extracted and concentrated from a mycelial colony.
 - ▶ Kaufman and Standard (1987) have described the technique.
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Immunological tests

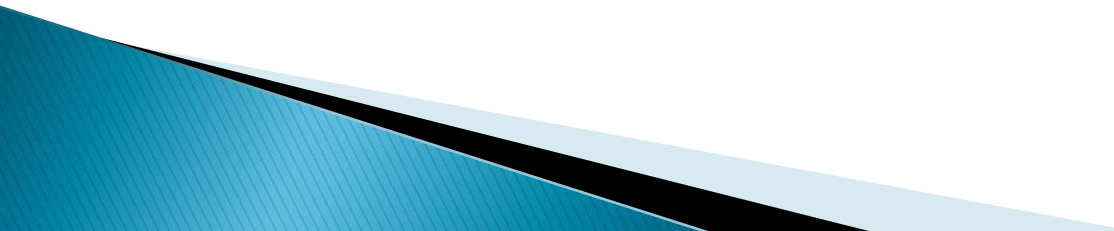
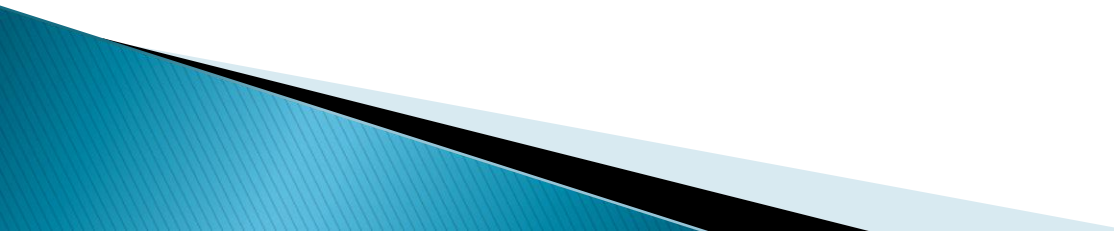
- ▶ *Coccidioides immitis* infection gives a strong immunological response and the serological tests are more reliable than for the other mycoses.
 - ▶ Immunodiffusion kits
 - ▶ Enzyme immunoassay
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Table 41.2 Immunological tests for dimorphic fungi

Fungus	Test	Comments
<i>Sporothrix schenckii</i>	Immunodiffusion, complement fixation test, latex agglutination	Antibodies are demonstrable only in the rare cases where systemic spread has occurred. Limited application in animals to date
	Immunofluorescence	For identification of yeast cells in exudates and tissues
<i>Blastomyces dermatitidis</i>	Skin test	Lacks sensitivity and specificity
	Immunodiffusion, complement fixation, ELISA, counter-immunoelectrophoresis	AGID and CFT are not considered to be sufficiently sensitive or specific
	Immunofluorescence	For identification of yeast cells in exudates and tissues
<i>Coccidioides immitis</i>	Skin test (coccidioidin)	Positive test may revert to negative as infection becomes disseminated and advanced, poor prognosis
	Immunodiffusion	Multiple bands tend to be associated with active infection whereas a single band is associated with chronic infection
	Complement fixation test	Antibody titre rises in disseminated disease and tends to remain high
	Latex agglutination test	Antibodies detected early in disease (IgM)
<i>Histoplasma capsulatum</i> var. <i>capsulatum</i>	Skin test (histoplasmin)	Positive reaction merely indicates exposure. Lack of reaction may be due to anergy
	Immunodiffusion	Erratic results obtained with animal sera. Of questionable usefulness
	Complement fixation test, latex agglutination	Useful in humans, reliability less certain with animal sera
	Immunofluorescence	For identification of yeast cells in exudates and tissues

Mouse inoculation tests

- ▶ All the dimorphic fungi cause lesions in mice. There is little need for animal inoculation now because of the availability of other specific confirmatory tests.
 - ▶ However, mouse inoculation might be the only method of recovery of the fungi from very contaminated specimens.
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Sporothrix schenckii

- ▶ *Mice are inoculated intratesticularly.*
- ▶ Orchitis develops in two to four weeks.

Blastomyces dermatitidis

- ▶ : *mice or guinea pigs are* inoculated intraperitoneally.
- ▶ Lesions may be found in liver, spleen, lungs and lymph nodes in three weeks.

Histoplasma capsulatum var. *capsulatum*

- ▶ *∴ mice are* inoculated intraperitoneally and the yeast-form can be recovered from the liver and spleen in two to four weeks.

Coccidioides immitis

- ▶ *: intraperitoneal inoculation into* mice.
- ▶ The mice are euthanized seven to 10 days postinoculation and nodules are found in the peritoneum, lungs and spleen.
- ▶ These nodules are examined for the characteristic spherules produced by the fungus.

HISTOPLASMA CAPSULATUM

VAR. FARCIMINOSUM

- ▶ About 90% of the cases of epizootic lymphangitis (African farcy) have been reported in horses and the remainder in mules and donkeys.
- ▶ The legs and neck are most commonly involved, displaying nodular, granulomatous and ulcerative lesions of skin, subcutaneous tissue and lymphatic vessels.
- ▶ The disease can become disseminated.
- ▶ The natural habitat, other than infected animals, remains unknown.
- ▶ Transmission is thought to occur mainly through breaks in the skin or via biting insects.

Direct Microscopy

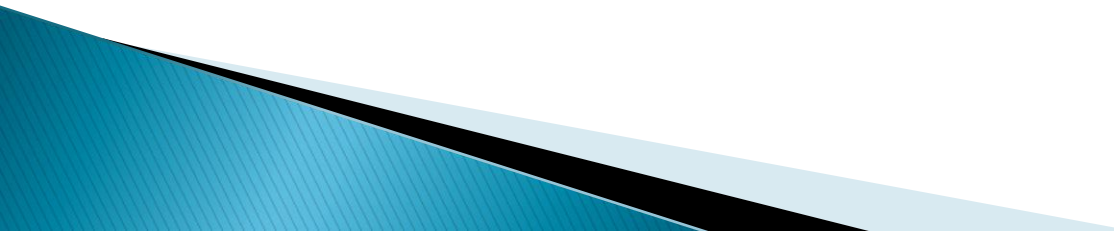
- ▶ Wet mounts of pus or exudates or biopsies can be examined for the intracellular, pear-shaped, double-contoured yeast cells (2–4 μm).
- ▶ They are usually present inside macrophages or neutrophils.

Culture

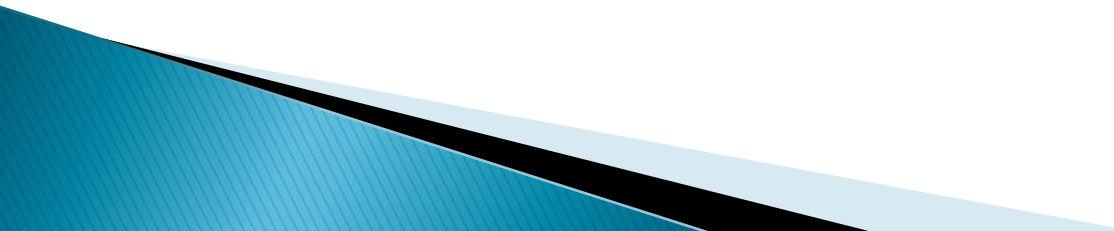
- ▶ Sabouraud dextrose agar, with and without antimicrobial agents, is inoculated with material taken aseptically from unruptured nodules and incubated at 25–30°C for two to eight weeks.
- ▶ For the conversion to the yeast phase, Hartley digest agar with 10% horse serum is inoculated and incubated at 37°C, under 20% CO₂ for two to eight weeks.

Identification

Colonial and microscopic appearance

- ▶ At 25°C the colonies appear as minute grey flakes later becoming dry and very wrinkled.
 - ▶ The colonies are often composed of sterile hyphae although very rarely chlamydospores (5–10 µm), arthrospores and blastospores are present.
 - ▶ At 37°C the small, grey, flaky colonies are composed of yeast cells and some hyphae.
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Immunology

- ▶ immunodiffusion test on mycelial extracts detects the **h and m genus-specific exoantigens.**
 - ▶ Skin sensitivity develops after exposure to the fungus
 - ▶ An ELISA and an indirect fluorescent antibody test
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Mouse inoculation

- ▶ Mice - intraperitoneally
- ▶ Impression smears from the liver and spleen, two to four weeks post inoculation, should reveal the yeast-form of the fungus.