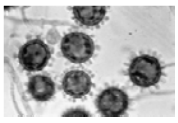

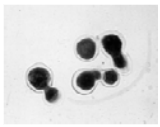

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Systemic Mycoses

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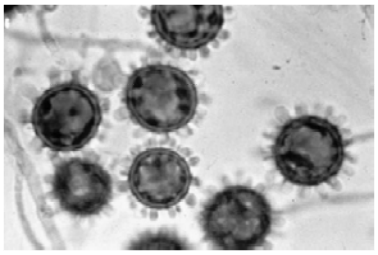
Systemic Mycoses

- ***Histoplasma capsulatum***
- ***Blastomyces dermatitidis***
- ***Coccidioides immitis***
- ***Coccidioides posadasii***
- *Paracoccidioides brasiliensis*
- *Penicillium marneffei*
- *Sporothrix schenckii*
- *Aspergillus species*
- *Emmonsia species*

Dimorphism

- **Majority systemic mycoses are caused by dimorphic fungi**
  - Molds in their normal soil environment, and on laboratory media when kept at 25-30°C.
  - Yeast when the temperature is raised to 37°C (as in the human host).
  - Nutritional factors such as certain amino acids can also enter into dimorphism, but are generally less important than temperature.

Histoplasmosa capsulatum var. capsulatum



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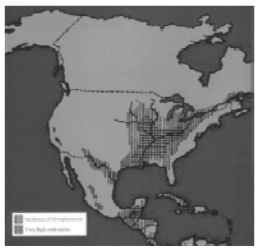
Histoplasmosis

- Occurs throughout the world
- Endemic areas
  - Mississippi and Ohio River Valleys in the US.
  - Mexico, Central and South America
- Once thought to be a highly lethal form of pneumonia with up to 90% mortality
- Now known to be a rather common infection in endemic areas.



Histoplasma habitat

- Soil saprobe
- Loves droppings from bats or birds.
  - Requires high levels of creatinine and nitrogen
- Birds not susceptible to infection with Histoplasma
  - Likely related to their high body temperature
    - 40-42C (104-108F)
- Occupational risk for people working with chickens.
- Clearing Starling roosts has been associated with large outbreaks of fatal infections.

Histoplasma capsulatum Disease

- Usually self limiting flu-like illness and does not require medical intervention.
  - 90% asymptomatic
  - 4:1 male predominance for clinical disease
  - Infants and young children more likely symptomatic
  - Chronic pulmonary disease
  - Disseminated disease in immunocompromised

Pathobiology

- In human infection---small yeast 2-5 µm in diameter.
  - predominantly in macrophages.
- Nonactivated macrophages do not effectively kill H. capsulatum and can actually spread the disease.
  - Can multiply intracellularly, kill the phagocyte, and infect additional cells

Pathobiology

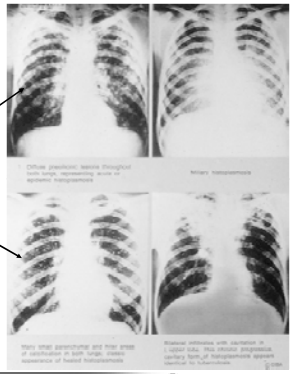
- Granulomatous lesions in the lungs
  - Very similar to tuberculosis lesions
  - Lymphocytes, macrophages, Langhans' giant cells
- Severity of infection directly proportional to the number of conidiospores inhaled.
  - Miliary lesions when large numbers of the spores are inhaled.
- In most infections the cure is spontaneous and lasting immunity occurs.
- Histoplasma may remain viable and recurrence possible with decrease in CMI

Histoplasmosis

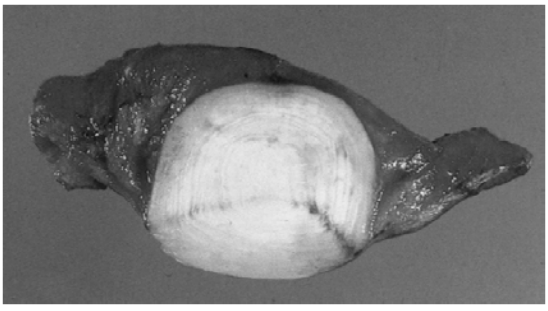
Diffuse pneumonic histoplasmosis

•Radiologic variations

Calcified miliary histoplasmosis



Calcification



Immunity and Treatment

- Immunity dependent on CMI.
  - Antibody is of little importance
- Healing of lesions leads to calcified granulomas similar to that seen in tuberculosis.
  - Old calcified nodules on chest x-ray not uncommon
- Treatment reserved for life-threatening infections
  - Amphotericin B
  - Itraconazole

Laboratory Diagnosis

- **Histology**
  - GMS stain
  - Wright stain of blood or bone marrow
- **Direct Microscopic Exam**
  - KOH, Calcofluor---2-5um yeast
- **Culture**
  - Enriched media (BHI with Blood, Yeast Extract Phosphate, Inhibitory Mold Agar)
  - 2-4 weeks at 30C
- **Antigen Detection-**-----Urine EIA (miravistalabs.com)
- **Serology**
  - Complement fixation
  - EIA
  - Immunodiffusion
- **Real-time PCR**

Specimens for Fungal Infections

- Respiratory secretions, tissues, blood, CSF, other body fluids
- Discourage swabs
- Transport at room temp
  - Specimens with endogenous flora, refrigerate if >2hr delay
- Blood--Lysis centrifugation, BACTEC MYCO/F Lytic, or BacT ALERT MB
- CSF—large volume (10-20ml)
  - Centrifuge 2000g, 10 minutes
  - Inoculate pellet

Specimens for Fungal Infections

- Urine and other body fluids
  - Centrifuge 2000g, 10 minutes
    - Plate pellet
- Mince tissue, do not grind
  - Place 3-4 pieces on plate and press into agar
  - Exception for Histoplasma—want to grind to release intracellular organisms

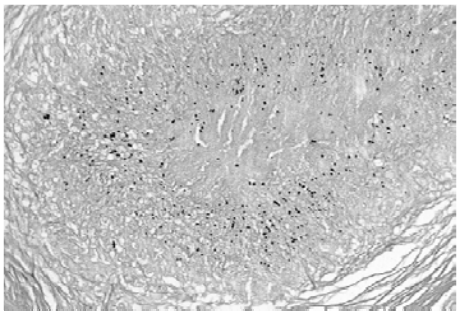
Media for Primary Isolation of Systemic Fungi

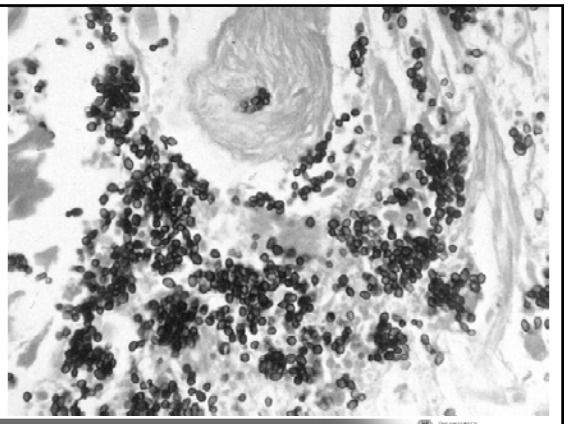
- **Non-inhibitory media**
  - Sabouraud's dextrose agar
  - Potato Flake Agar
  - Potato Dextrose Agar
- **Selective Media**
  - Mycobiotic or Mycosel agar--- cyclohexamide and chloramphenicol

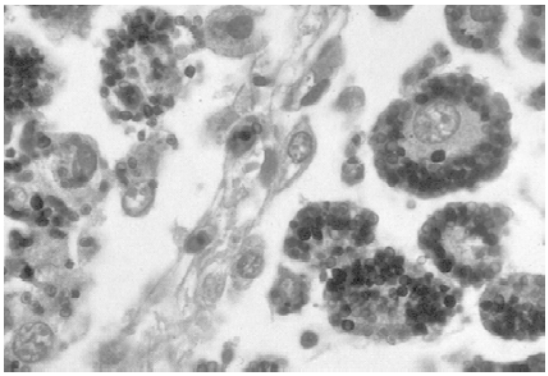
Media for Primary Isolation of Systemic Fungi (2)

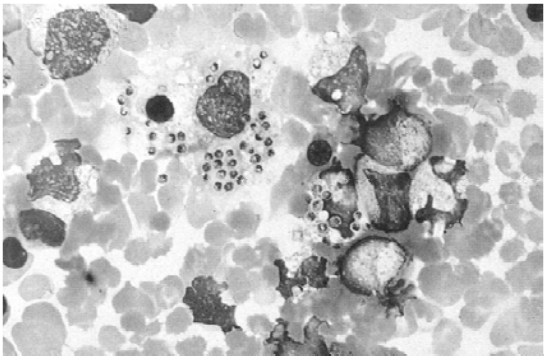
- **Enriched media w/ or w/o antibiotics**
  - Inhibitory mold agar---chloramphenicol and cyclohexamide
  - BHI with sheep blood w/wo antibiotics
  - Yeast extract phosphate agar with ammonia
- **Incubate plates or tubes at 30ºC or 25ºC**
  - Hold 4 weeks
- **For Blood Cultures**
  - Lysis Centrifugation
  - BACTEC MYCO/F or BacT ALERT MB

Silver Stain

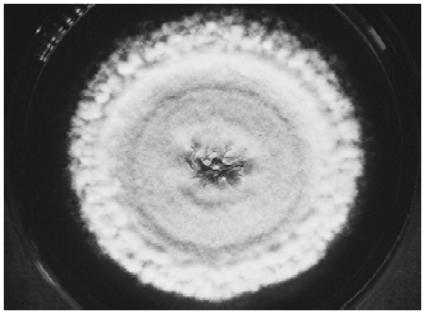






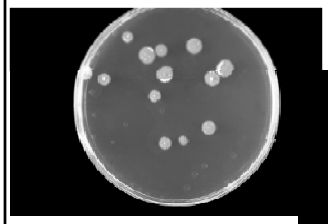


Culture



Histo at 3 weeks, 30C

Histoplasma capsulatum



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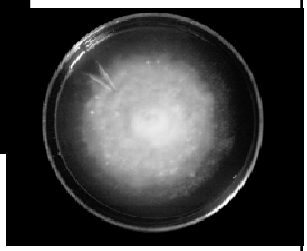

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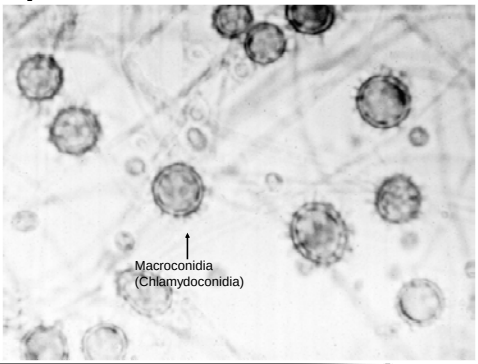
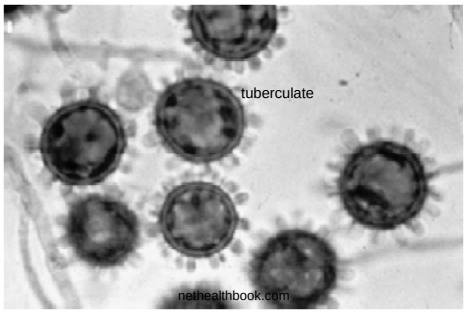
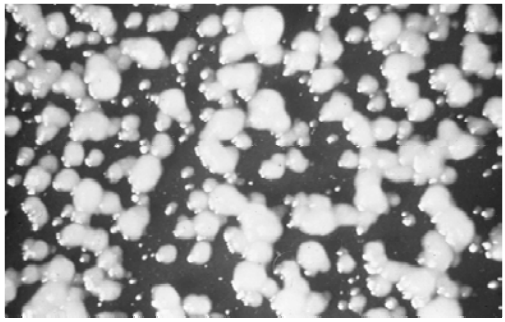
H. capsulatum conidia 30C Incubation



H. capsulatum



H. capsulatum—Yeast phase



Differentiation from other Fungi

- Must differentiate from *Sepedonium* and *Chrysosporium* species that produce tuberculate macroconidia
  - More rapid growing
  - Not dimorphic
  - Usually will not grow in the presence of cycloheximide
  - Distinguish using DNA probe

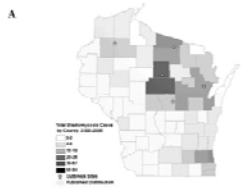
Nucleic Acid Probe Identification

- GenProbe® Assay
  - Rapid
  - Chemiluminescent assay using labeled probes specific for each agent
  - Labeled DNA probe hybridizes with rRNA of the fungus
  - Available for *H. capsulatum*, *Blastomyces dermatitidis*, and *C. immitis*

BLASTO!

Blastomycosis

- Blastomyces dermatitidis
- Agent of North American Blastomycosis,
- Geographical distribution is similar to H. capsulatum
- More common in Wisconsin than H. capsulatum.



Kurt Reed et. al *PLOSone* 3(4): e2034, 2008

Blastomycosis

- The epidemiology is poorly understood
  - Lack of a good skin test reagent
  - Ecologic niche not well established
- Difficult to recover from the soil in endemic areas.
- Eagle River, Wisconsin outbreak 1985
  - First time Blastomyces isolated from the environment at the site of an outbreak
  - Isolated from soil containing decayed vegetative matter and from decomposed wood.

Clinical Manifestations

- **Two clinical presentations**
  - A **primary cutaneous infection** which usually remains localized to one area of the body
    - May indicate systemic disease
  - **Primary pulmonary infection** with possible secondary dissemination.
    - 30-45 day incubation
    - Mimics flu progressing to cough, weight loss, chest pain, low grade fever
    - 75% with isolated pulmonary disease
    - Infection may involve any organ
    - Secondary cutaneous infection
  - **Asymptomatic in >50% of those infected**

Systemic Disease

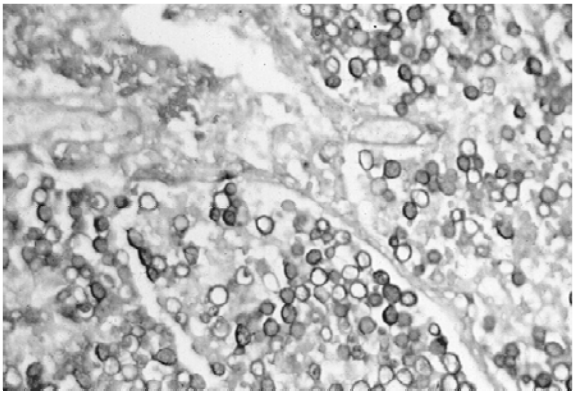
- **Common sites of infection in systemic disease**
  - Bones---long bones, ribs, vertebrae
  - Joints
  - Genitourinary tract----prostate, epididymis
  - CNS-----common in AIDS (40%), uncommon in immunocompetent (<5%)

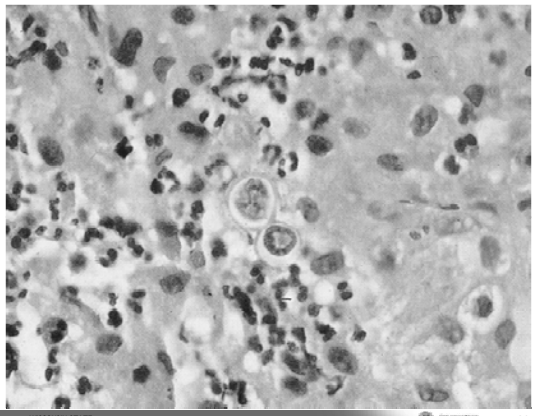
Cutaneous Form

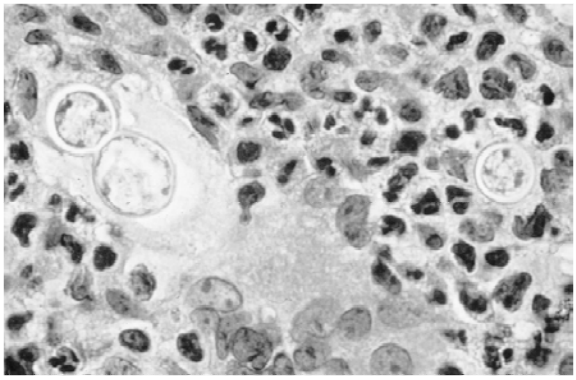
- A chronic suppurative granulomatous lesion.
- The presence of epithelial microabscesses and characteristic yeasts in the tissues is considered diagnostic.
- It is important to obtain urine and sputum samples from a patient with cutaneous blastomycosis since systemic spread may occur.

Histology

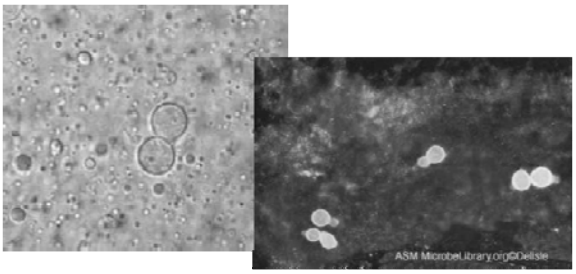
- **Body fluids or tissue specimens**
  - Look for the characteristic yeast form.
    - Large (8-15 µm) and thick walled.
    - The wall is prominent; "doubly refractile" on bright field microscopy.
    - A single daughter cell (bud) is present with a broad connection between the two cells **(<u>BROAD-BASED BUDDING</u>).**







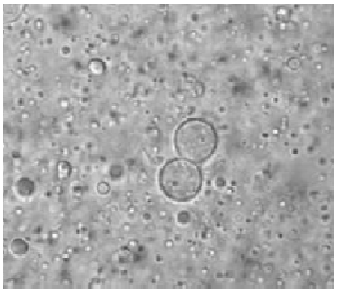
Direct Exam



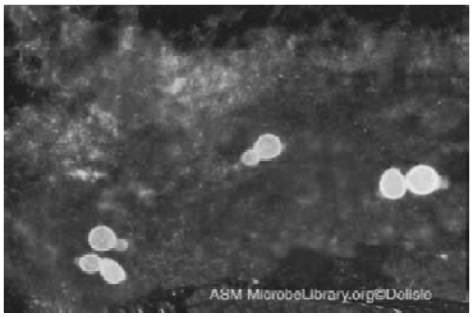
KOH Prep

Calcofluor White

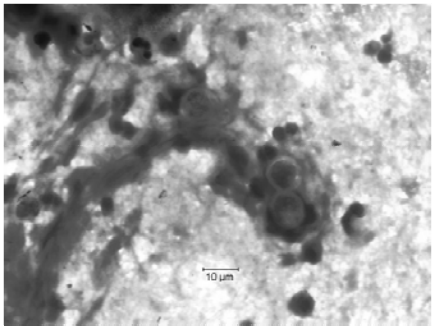
KOH Exam



Calcofluor White



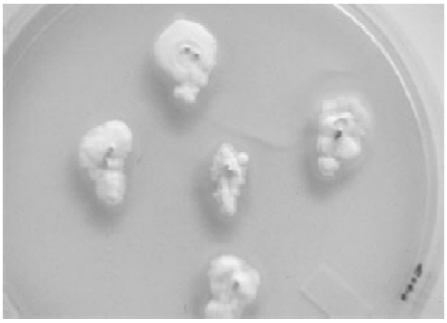
Gram Stain

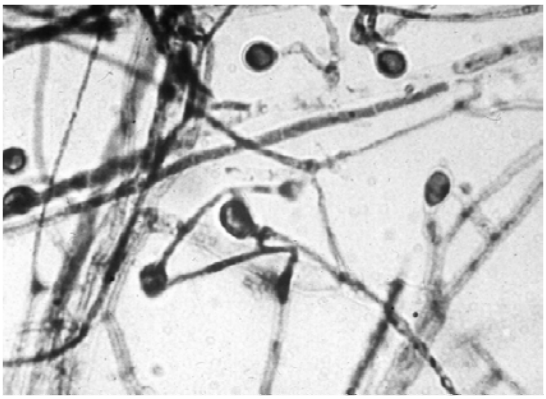


Culture Characteristics

- **On culture**:
  - Slow growing gray/white mold
  - Delicate, septate hyphae
  - Conidia usually absent on blood-containing media. May be sparse on PDA and SAB
  - "Lollypop" conidiation

Blastomyces dermatitidis

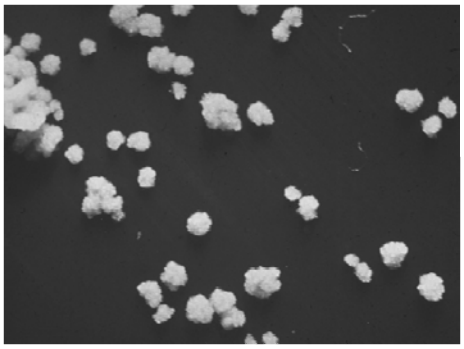




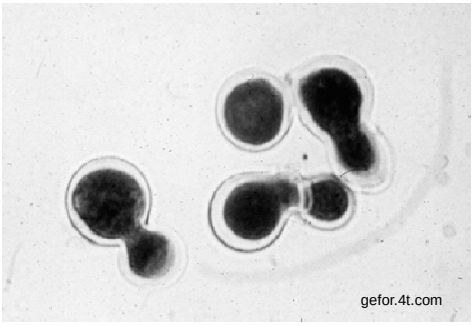
Blastomyces Mold Phase

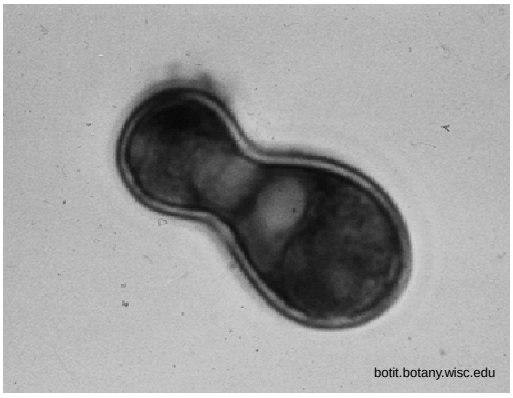
- This form of conidia is also found in such fungi as Chrysosporium sp., Pseudallescheria boydii (Scedosporium), and various Trichophyton sp.
- Differentiation from these other species can be made by the following characteristics:
  - Slower growth
  - Growth in the presence of cycloheximide
  - Dimorphism
  - Nucleic acid probes

Blastomyces Yeast Phase

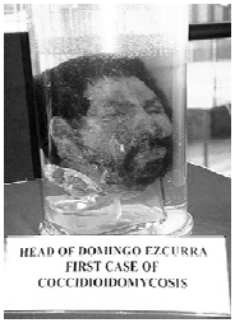


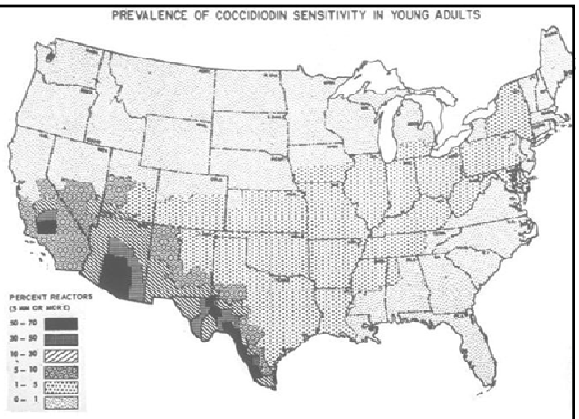
Blastomyces Conversion





Coccidioides immitis and C. posadasii





Coccidiodes immitis

- Coccidiomycosis is sometimes known as "**San Joaquin Valley fever**". Up to 95% of the residents of the endemic area are skin test positive (coccidioidin test positive)
- Lower Sonoran Life Zone
  - Arid climate, hot summers, few winter freezes, low altitude, alkaline soil, sparse flora
  - Drought followed by heavy rains---Increased infections
  - 100,000 infected annually in U.S.
- Variety of animals infected
  - Positive cultures around rodent burrows
- Archaeology students discover new "infected" sites

Clinical Manifestations

- The primary disease is pulmonary, secondary to inhalation of small numbers of arthrospores
- Usually resolves spontaneously as an influenza-like infection.
  - 60% asymptomatic
  - 40% influenza-like illness, LRI or systemic illness
    - Cough, sputum, chest pain, malaise, fever, chills, night sweats, arthralgias, anorexia

Clinical Manifestations

- In a minority of cases a more chronic pulmonary infection occurs
  - Granulomatous lesions of the lung
  - Can lead to cavitation
- In rare cases (0.5%) dissemination occurs which can lead to rapidly fatal results.
- Reactivation infection occurs

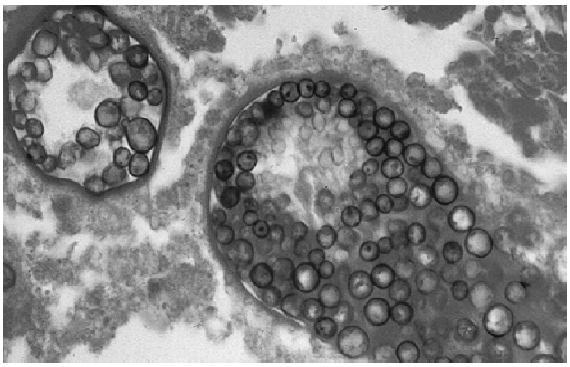
Coccidiomycosis

Lesions variable:

Papules
Pustules
Plaques
Nodules
Ulcers
Abscesses

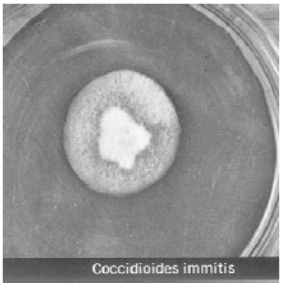
Histology

- Histological examination useful in confirming a diagnosis.
  - Spherules
  - 10-60 µm in diameter, but they may be as big as 200 µm.
- Immature spherules can be similar in size to the large yeast cells of *B. dermatitidis*
- The spherules contain **endospores** 2-5 µm in diameter
  - Similar in size to *Histoplasma capsulatum*
  - Will not see budding



Culture

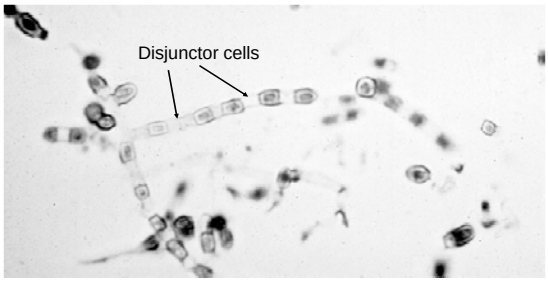
- **Culture**:
  - The organism grows fairly rapidly.
    - Visible growth on Sabouraud's agar within a few days.



Microscopic Features

- **Arthrospores:**
  - Formed by fragmentation of hyphae
  - Very thick walled. Provides them with resistance to drying.
  - "Barrel-Shaped"
    - As a culture ages on media the entire hyphal mass may fragment and form arthrospores.
  - **Spores are extremely infectious - Handle with extreme care.**
- **Remember**: Arthrospores can be made by other fungi.
  - Malbranchea sp., Gymnoascus uncinatus, Auxarthron sp.
  - Geotrichum and Trichosporon can also form arthrospores
- Confirm the identification nucleic acid probe test

Coccidioides---Arthrospores



Select Agent Regulations

- Report to CDC within 7 days of ID
  - Responsibility of lab confirming ID
  - Select Agent APHIS/CDC Form 4
- Secure against loss, theft, or release
- Destroy all subcultures and specimens
- Good News
  - Proposed to remove Coccidioides from SA list

WORK SAFELY!