



Diagnosis of Coccidioidomycosis (Valley Fever): The Ill Wind Blows

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- Clinical Diagnosis of Coccidioidomycosis often difficult as presentation can be protean
- Most presentations are of a respiratory nature but often can't separate from other respiratory infections
- At times patient does not realize that he/she has more than a virus until progression occurs
- As we have heard it may take months before a true diagnosis is made
- Must have high degree of suspicion and must understand laboratory studies (pros/cons; shortcomings)

Case 1: Pulmonary Presentation

- A 52 y/o caucasian male presented moderately ill with pneumonia: chest X-ray showed a unilateral infiltrate
- Sputum Gram-stain showed many WBCs and light oropharyngeal contamination/without any PO associated with WBCs
- Sputum culture grew light growth of oropharyngeal flora
- The patient was treated with ceftriaxone and erythromycin for two days and sent home in stable condition on oral levofloxacin.

Case 1: Pulmonary Presentation

- After initial improvement the patient continued to have fevers and showed persistence of the infiltrate.
- He was seen by a pulmonologist for further workup and underwent a BAL.
- Routine, fungal and AFB cultures failed to determine the etiology after 4 weeks incubation.
- Coccy serologies were negative at 1, 2 and 3 weeks after initial presentation.
- After the 4th week the IMDF IgM turned positive and a week later both the IMDF IgG and the CF titer turned positive (at only 1:2).
- At 6 weeks the CF titer peaked at 1:4

Case 1: Pulmonary Presentation

- The patient began to defervesce without specific antifungal therapy and was seemingly normal after a total of ten weeks.
- The patient did well for a period of 4 years without any specific symptoms (but he did complain of tiredness and some night sweats; he had no fever and now had negative IMDF and CF serologies.
- Significantly, his SED rate continued to be elevated.
- After 4 years, the patient suddenly complained of pain in his ankle.

Case 1: Pulmonary Presentation

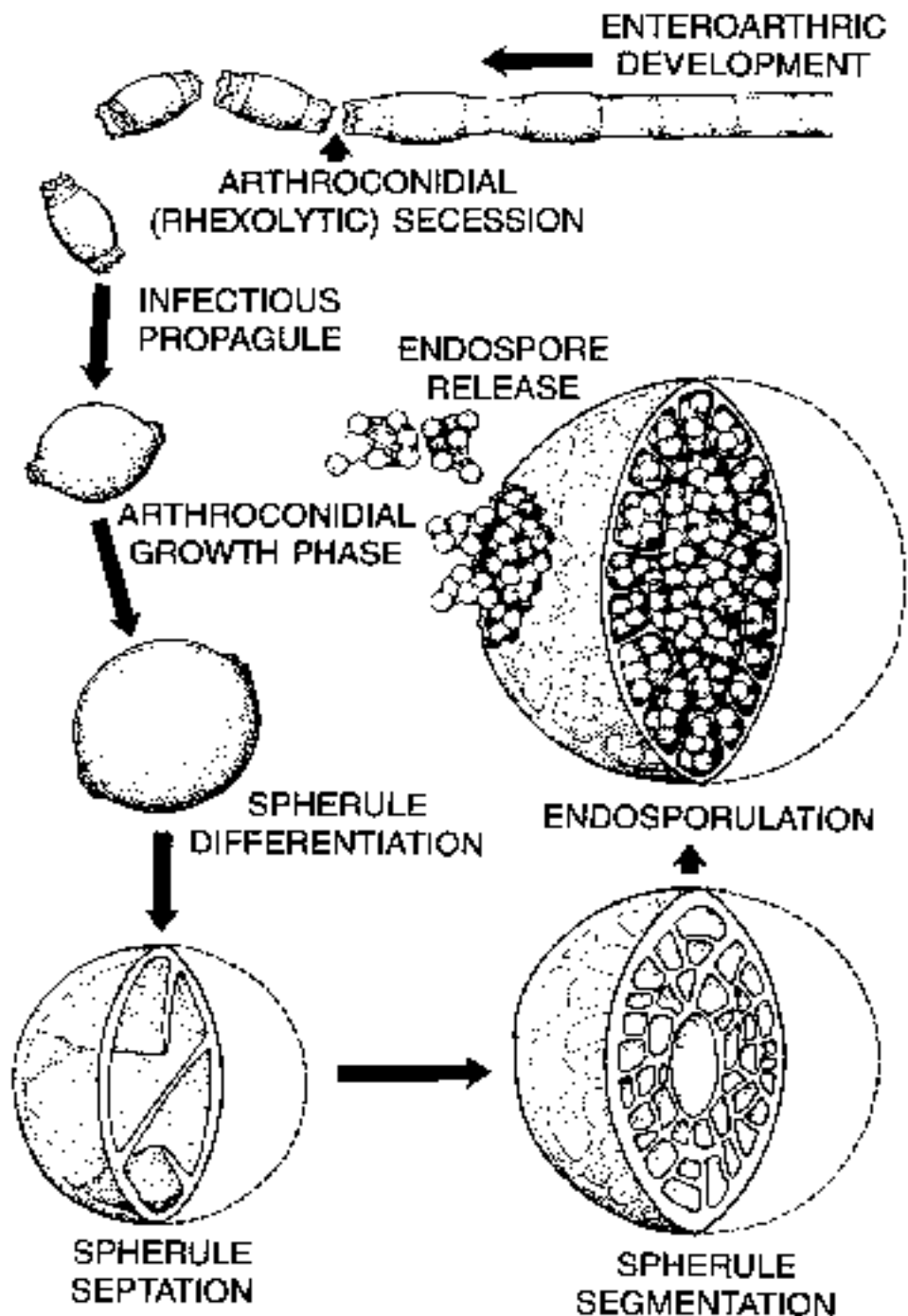
- He presented without any fever but with increased WBC count and some eosinophilia.
- A scan of his ankle revealed a localized osteomyelitis.
- His Coccy CF serologies were now at 1:2 and became 1:4 two weeks later.
- He had no new pulmonary infiltrates.
- Therapy with high dose fluconazole ameliorated the pain and reduced the CF titer to 1:2 three weeks later and negative at six weeks. He was kept on fluconazole for six months and showed no symptoms other than a continued elevated SED rate.

Case 1: Pulmonary Presentation

- His fluconazole was stopped after 8 months; within three weeks his ankle pain returned and his CF titer became elevated at 1:2 (Davis Lab).
- He was placed on fluconazole and remained on fluconazole until his death 10 years later from other unrelated causes.

Coccidioidomycosis

The Mycology

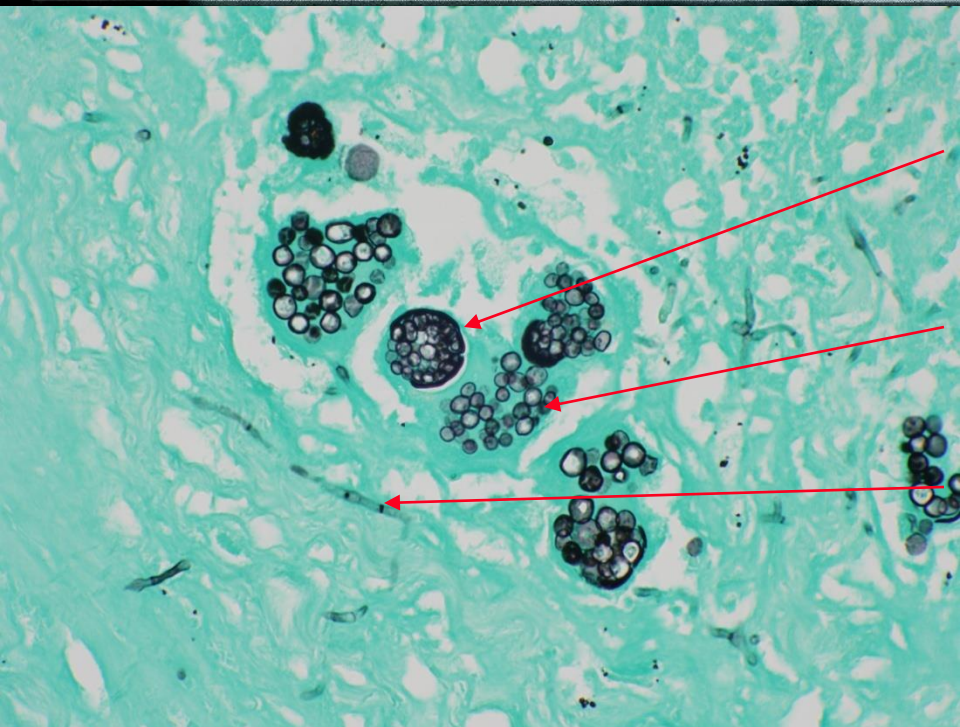
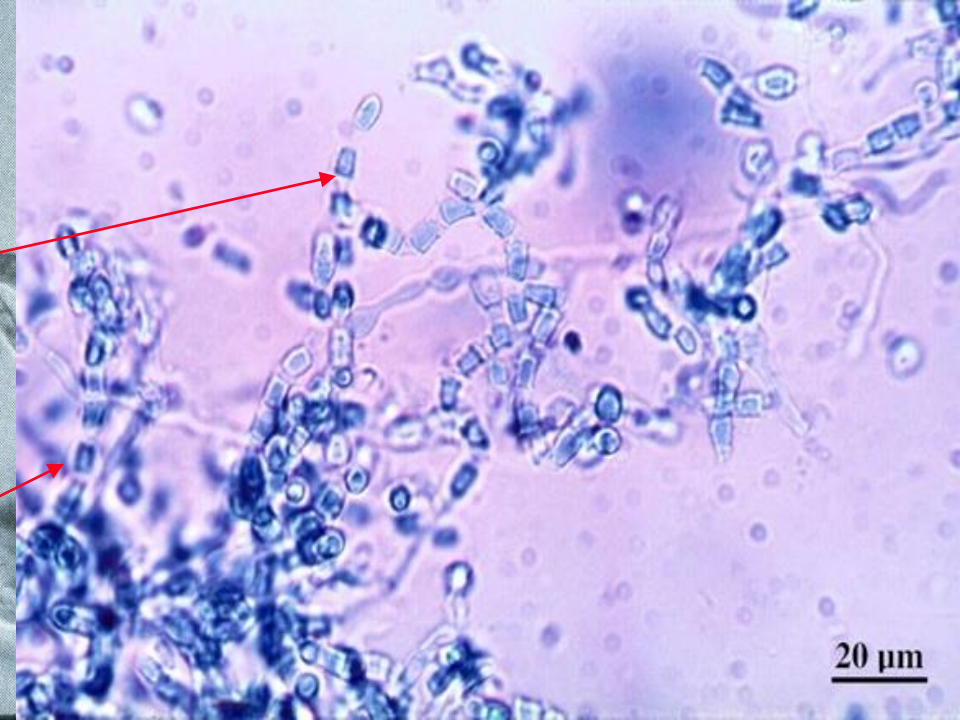
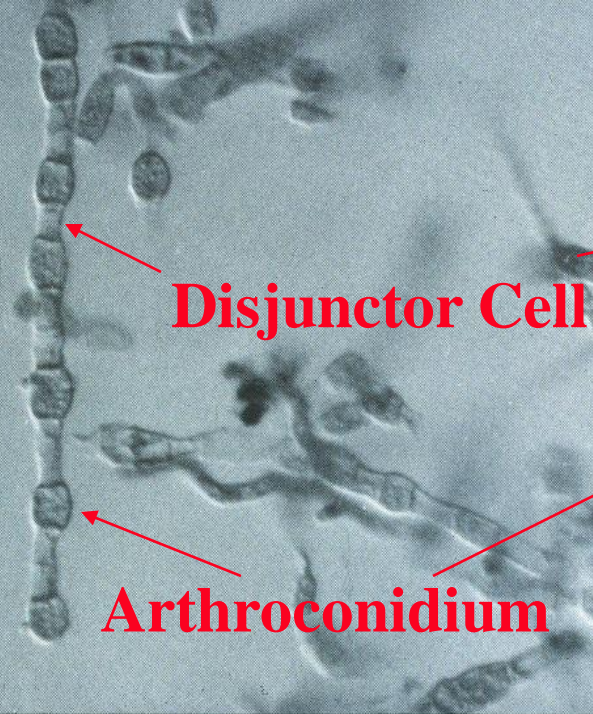


Dimorphic fungus found in sandy soil:

C. immitis (CA)

C. posadasii (other)

- Spherules: 10-80 μm
- Endospores: 2-5 μm
- Arthroconidia:
2-5 by 3-6 μm
- Temp-independent



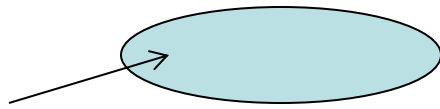
Spherule phase

Endospore phase

Mycelium phase

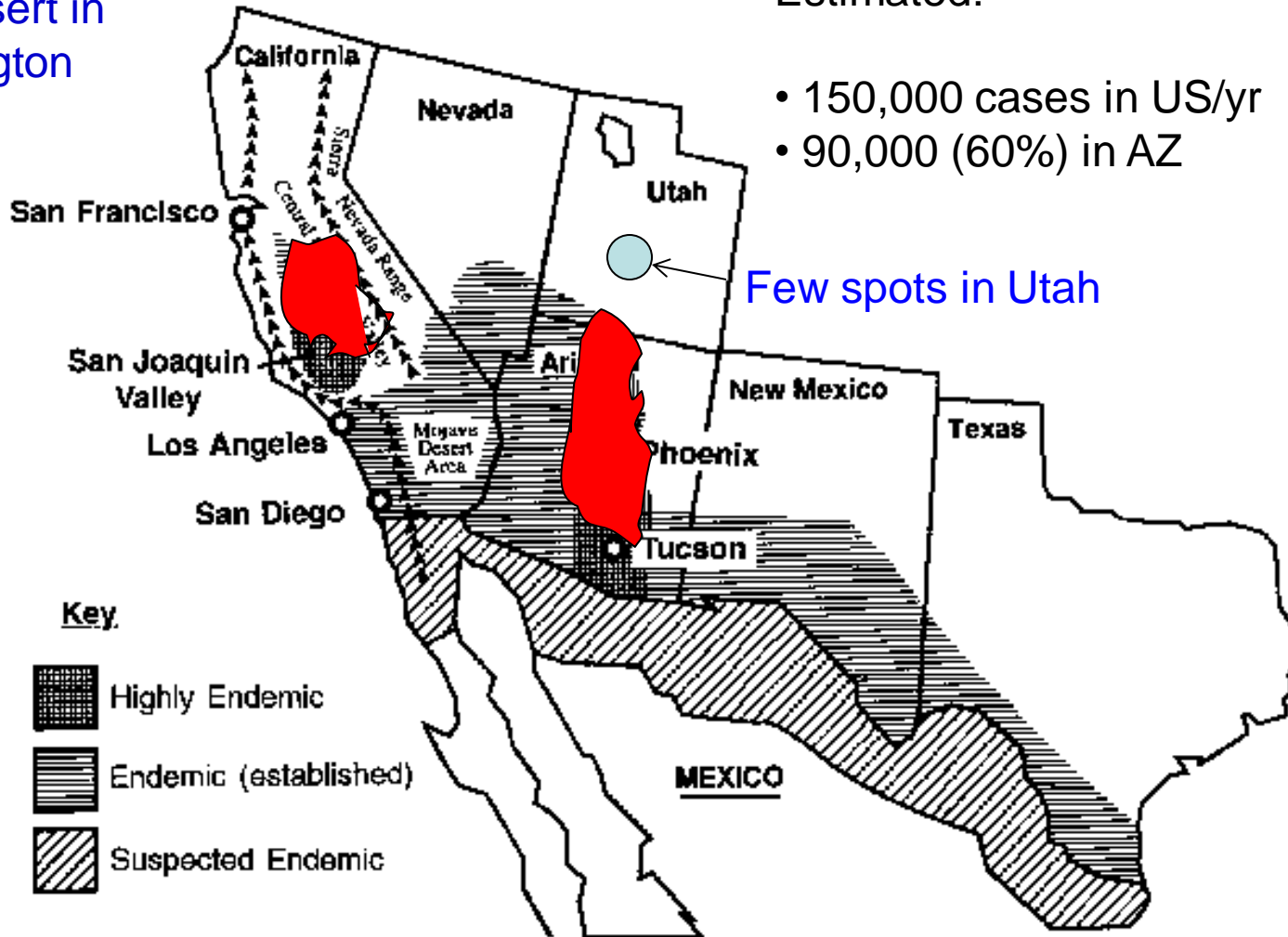
Grocott methenamine silver (GMS) stain of tissue from edge of cavitory lung lesion (x450)

Now reported in few cases from high desert in Washington



Estimated:

- 150,000 cases in US/yr
- 90,000 (60%) in AZ



Few spots in Utah

P. Q. Edwards and C. E. Palmer. Prevalence of sensitivity to coccidioidin, with special reference to specific and nonspecific reactions to coccidioidin and to histoplasmin. *Dis.Chest* 31:35-60, 1957

Disease Estimates



- **Estimated 150,000 cases in US annually**
- **90,000 (60%) in Arizona**
- **36,000 (40%) symptomatic AZ cases/year**
- **Arizona had less than 5,000 reported cases/yr in 07-08**
 - **54% male (81 cases/100,000)**
 - **46% female (68 cases/100,000)**

From Arizona Dept. Health Services (Epidemiology)

Issues with Coccidioidomycosis in Arizona

- Although, 40% are symptomatic
- < 1/3 are clinically evaluated,
- It is estimated that only 8-10% of total infections are serologically confirmed
- Only serologically confirmed are reported to public health

Advancing Arthroconidial Storm (Haboob)



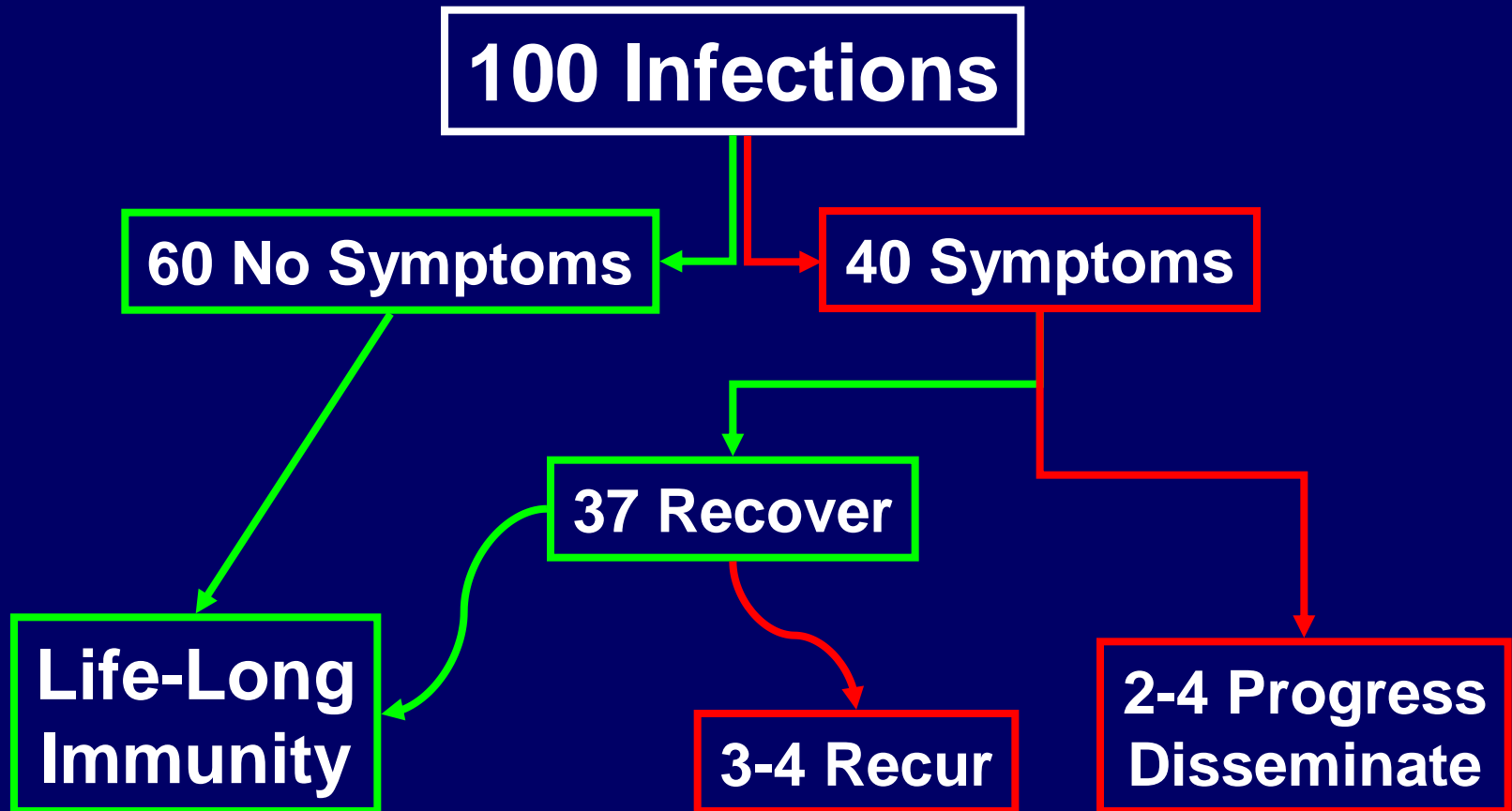
**New Homes of
Unsuspecting
Virgin Population**



Coccidioidomycosis

The Infection

Coccidioidomycosis Spectrum of Disease



Coccidioidomycosis

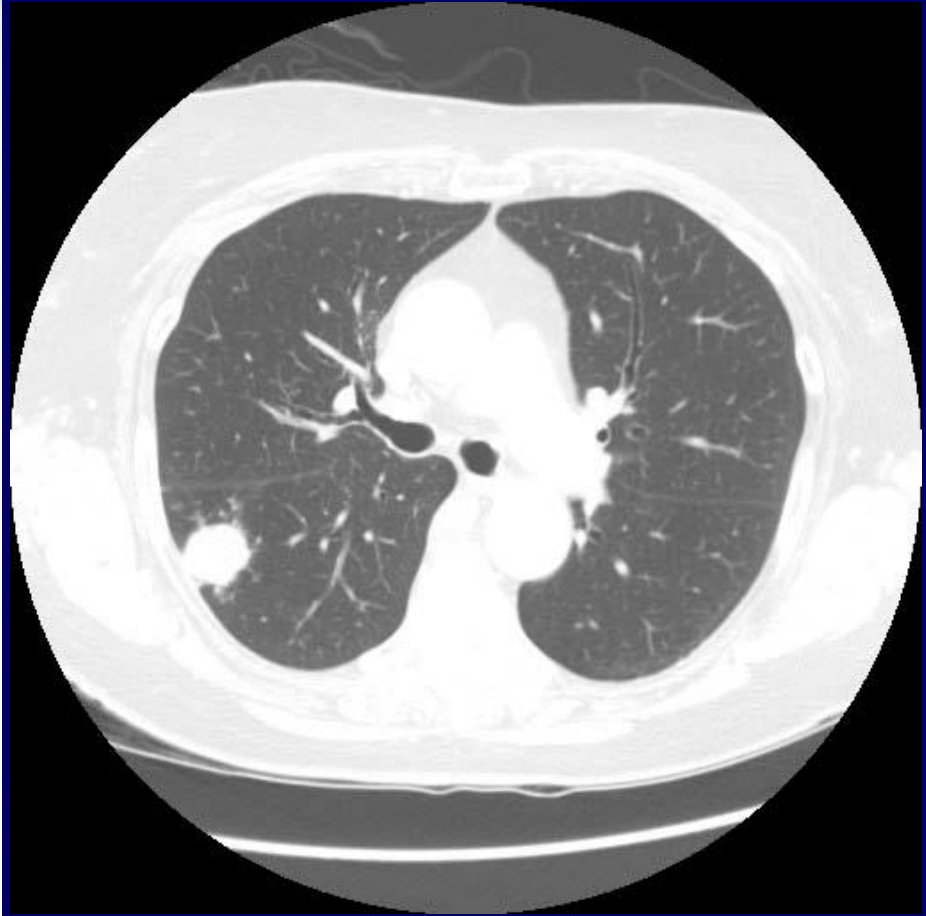
- Incubation 7-28 days
- Primary Pulmonary (asymptomatic to mild to severe; erythema multiforme or nodosum usually good prognostic signs)
- Disseminating
 - Respiratory: pulmonary or extrapulmonary (pleural, chest wall)
 - Extrapulmonary: lymphatic, cutaneous, subcutaneous, skeletal, CNS, cardiac, endocrine, ophthalmic, urogenital

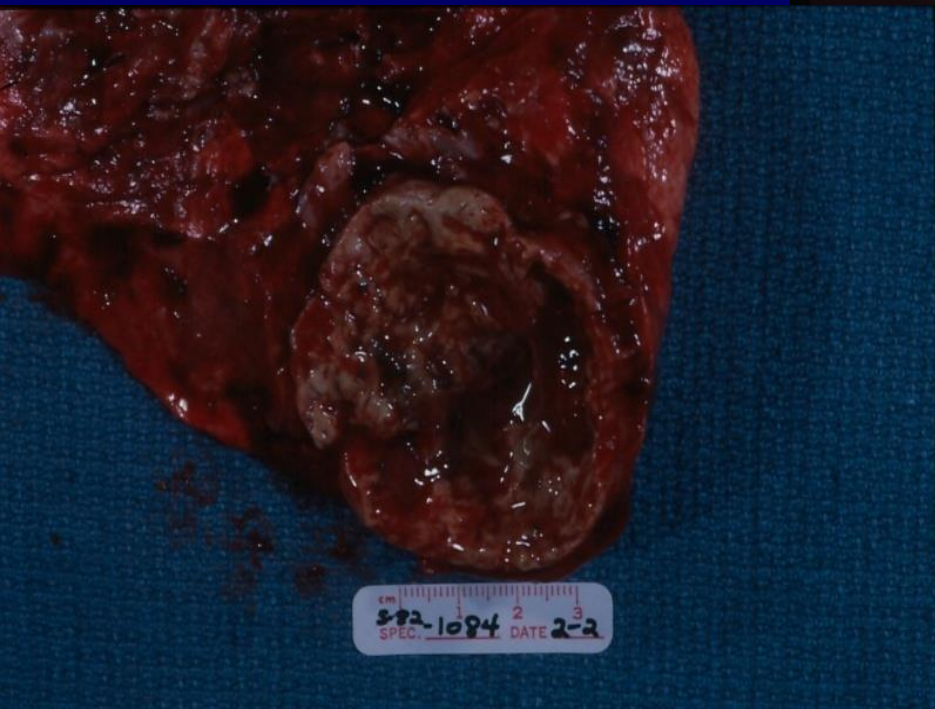
Coccidioidal Lesion



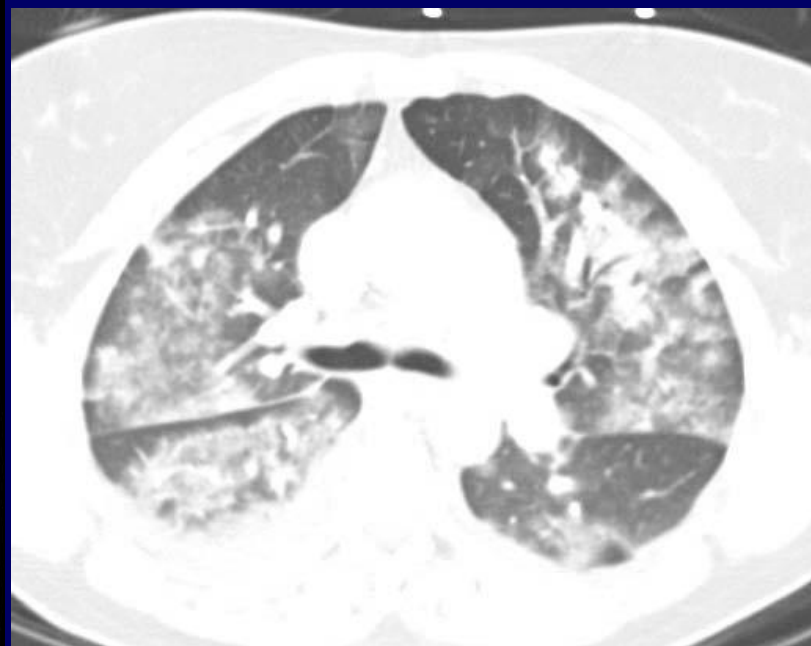
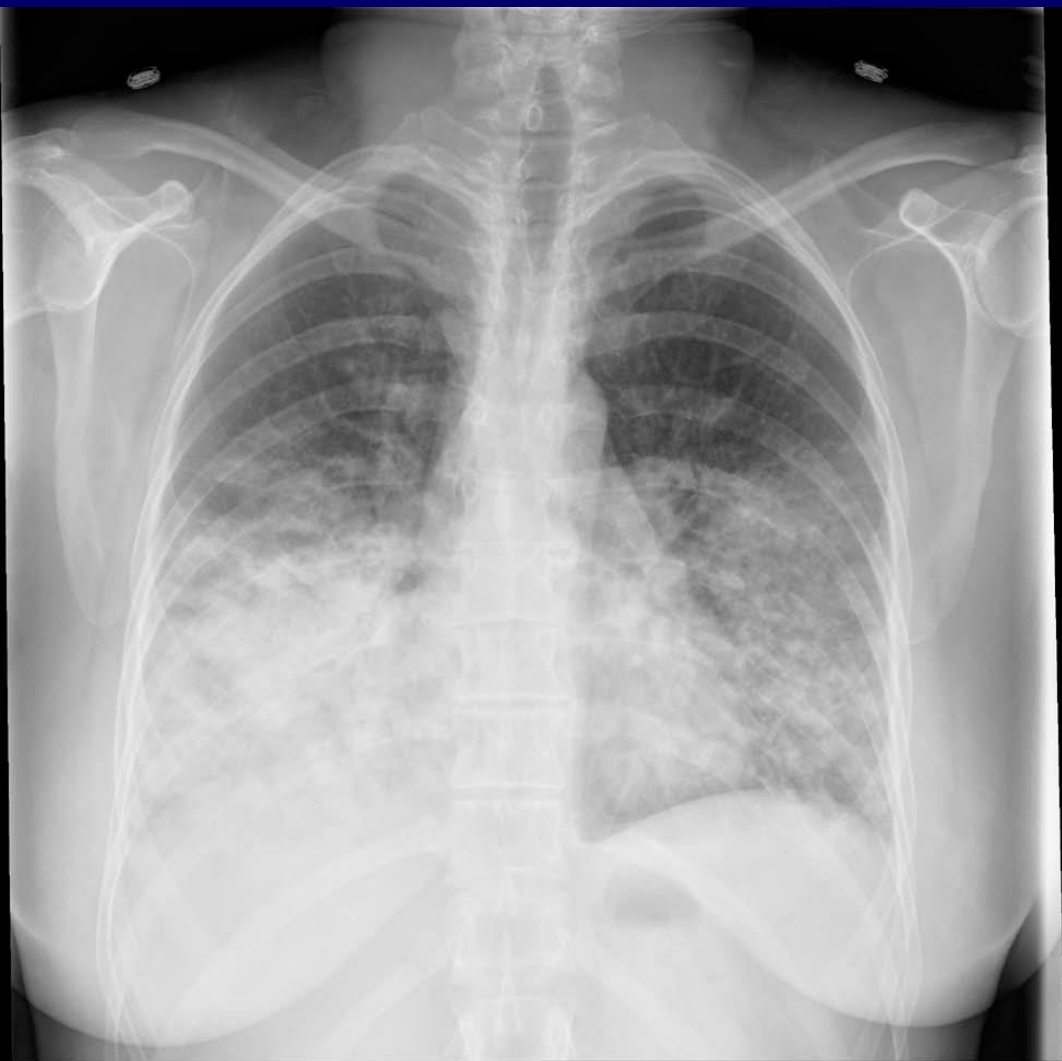
Big Hearted







cm
SPEC. 1084 DATE 2-2

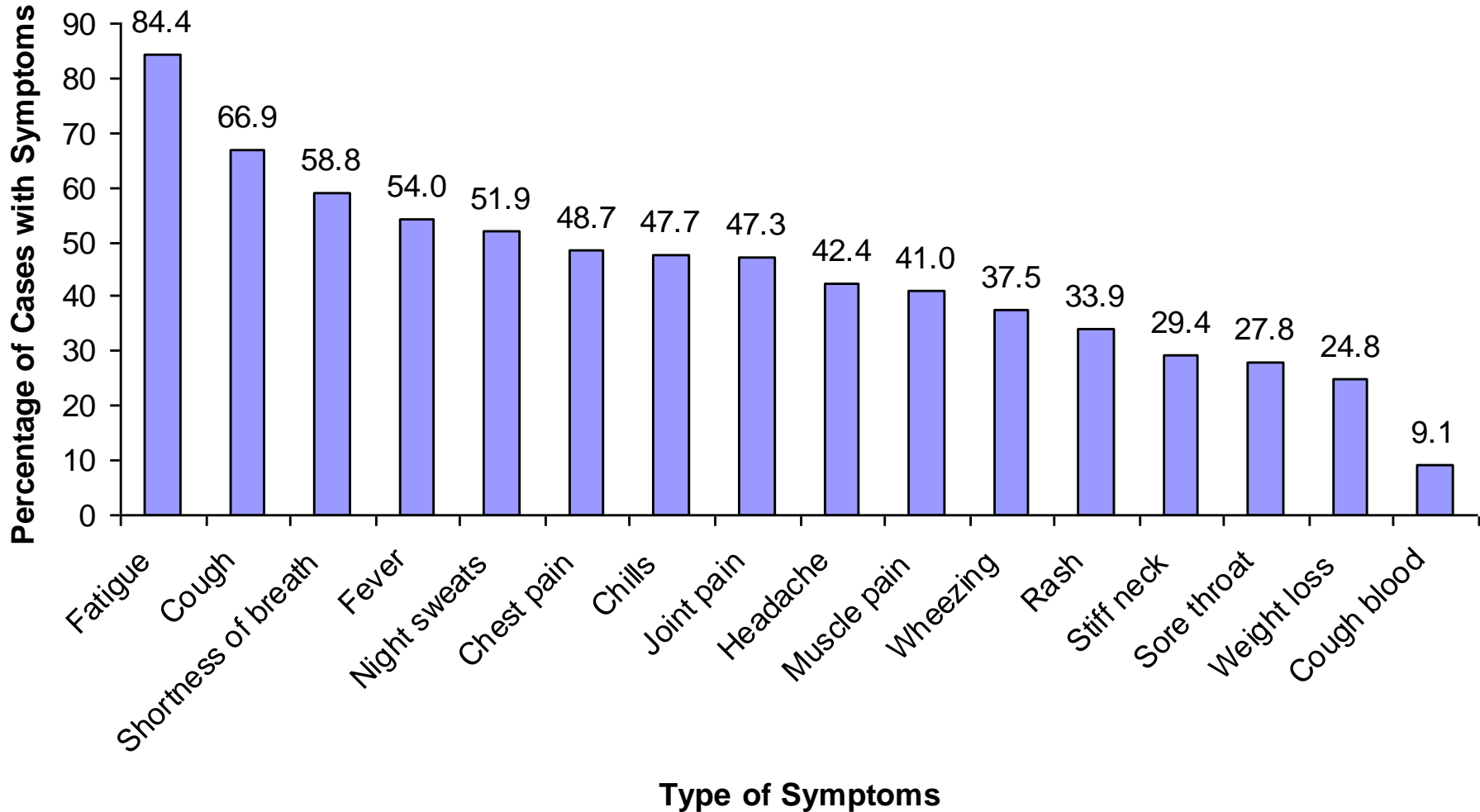


Lymph node





Prevalence of Symptoms seen in CAP caused by *Coccidioides* spp.



Coccidioidomycosis

Laboratory Diagnosis

Laboratory Diagnosis (especially in CAP)

- Most beneficial for sicker patients (may benefit most from Rx)
- Other benefits of Dx may include:
 - Avoidance of use of bacterial antimicrobics
 - Avoidance of use of corticosteroids
 - Earlier identification of complications
 - Decreased need for added expensive Dx studies
 - Reduction in patient anxiety

Coccidioidomycosis: General Laboratory Diagnosis

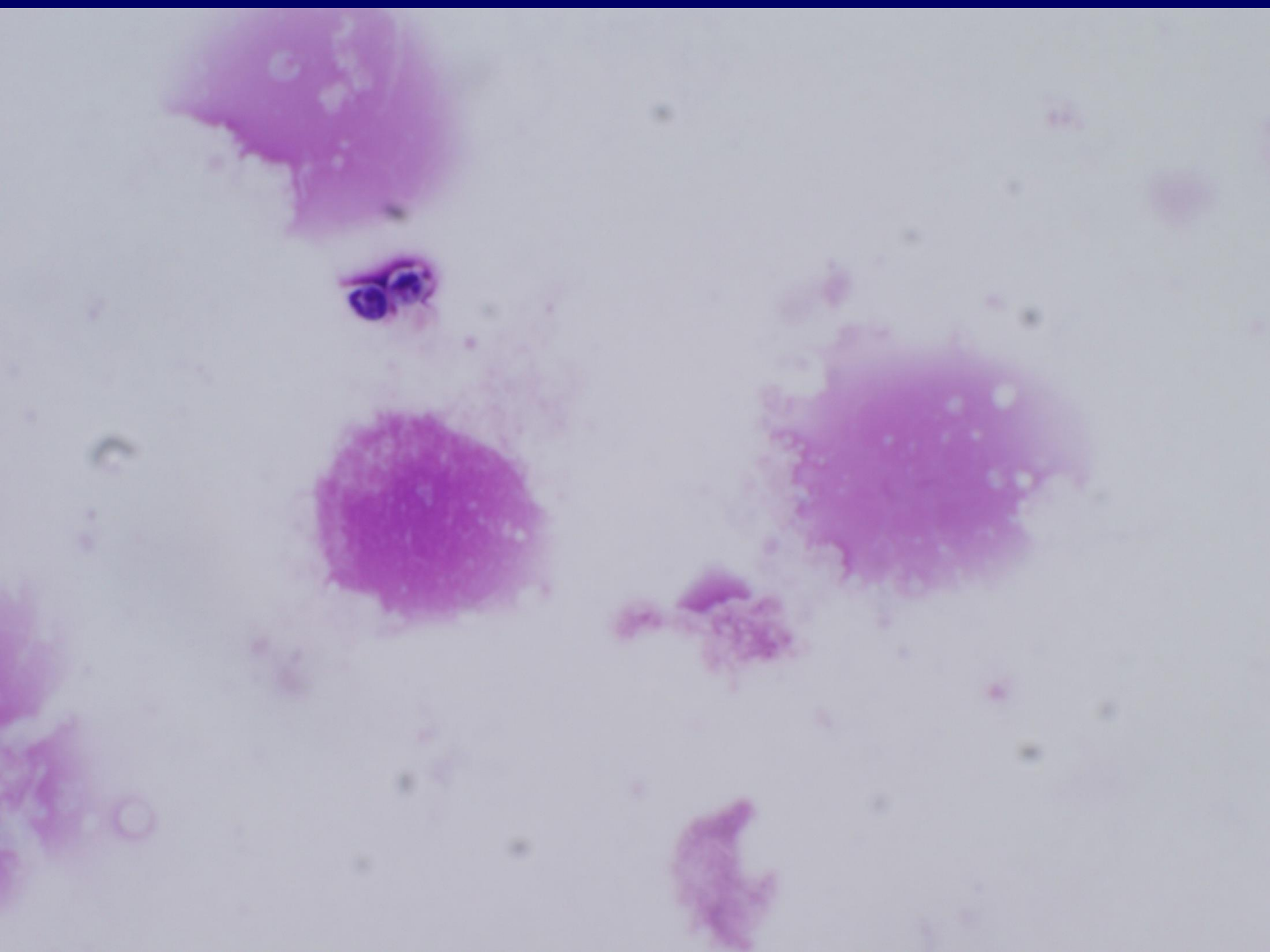
- Hematologic
 - elevated erythrocyte sedimentation rate
 - eosinophilia
- Meningitis (CSF)
 - variable overall increased cell count
 - predominance of lymphocytes over PMNs
 - low to moderate elevation of protein
 - moderate decline in glucose

Clinical Example: Case 2

- 67 y/o caucasian male presents to ED with deterioration of mental status over past 4-5 days
- Skin lesion on forearm
- History of:
 - Nursing home / COPD
 - Cerebrovascular accident
 - Ventriculoperitoneal shunt inserted 2 months previously for normal pressure hydrocephalus

Clinical Case 2 (continued)

- Temperature within normal limits
- MRI of brain consistent with meningitis
- Peripheral WBC count: $8,800/\text{mm}^3$:
- CSF: $88/\text{mm}^3$
 - 18% segs,
 - 27% lymphs,
 - 54% “other” cells noted as being “plasmocytoid”



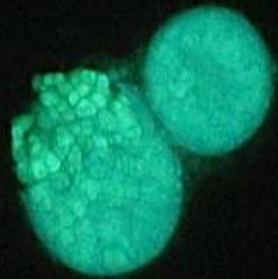
Clinical Case (continued)

- CSF grew *Coccidioides species* (*C. immitis* / *posadasii*) within 3 days
- CSF serologies showed a CF titer of 1:8
- Serum serologies showed a CF titer of 1:128

Coccidioidomycosis: Laboratory Diagnosis

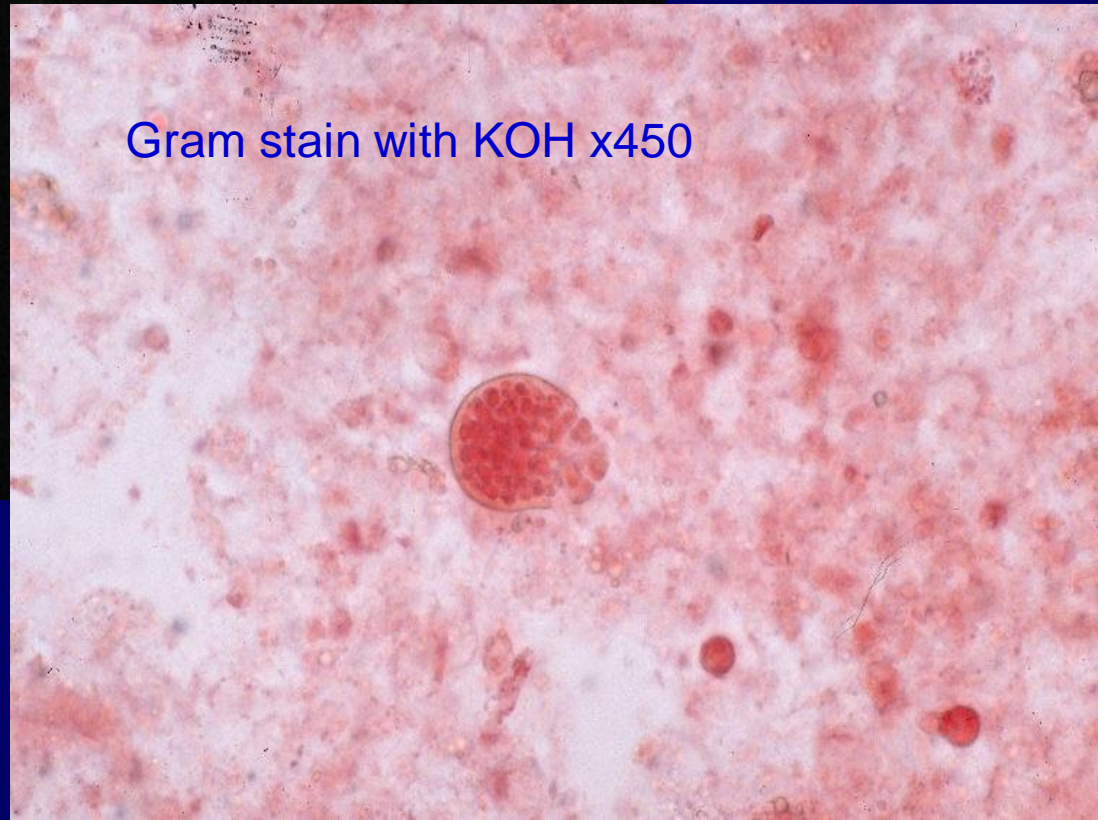
- **Direct**
 - Microscopy (spherules; endospores; mycelial forms)
 - PCR (Mayo Clinic)
 - urine, BAL (*Coccidioides* galactomannan antigen EIA; MiraVista Labs- Joe Wheat) CID 2008;47:e69
- **Serologic**
- **Skin Testing** (recently FDA approved – should be available early 2015)
- **Culture** (average time to recovery 4 days (2-16 days))

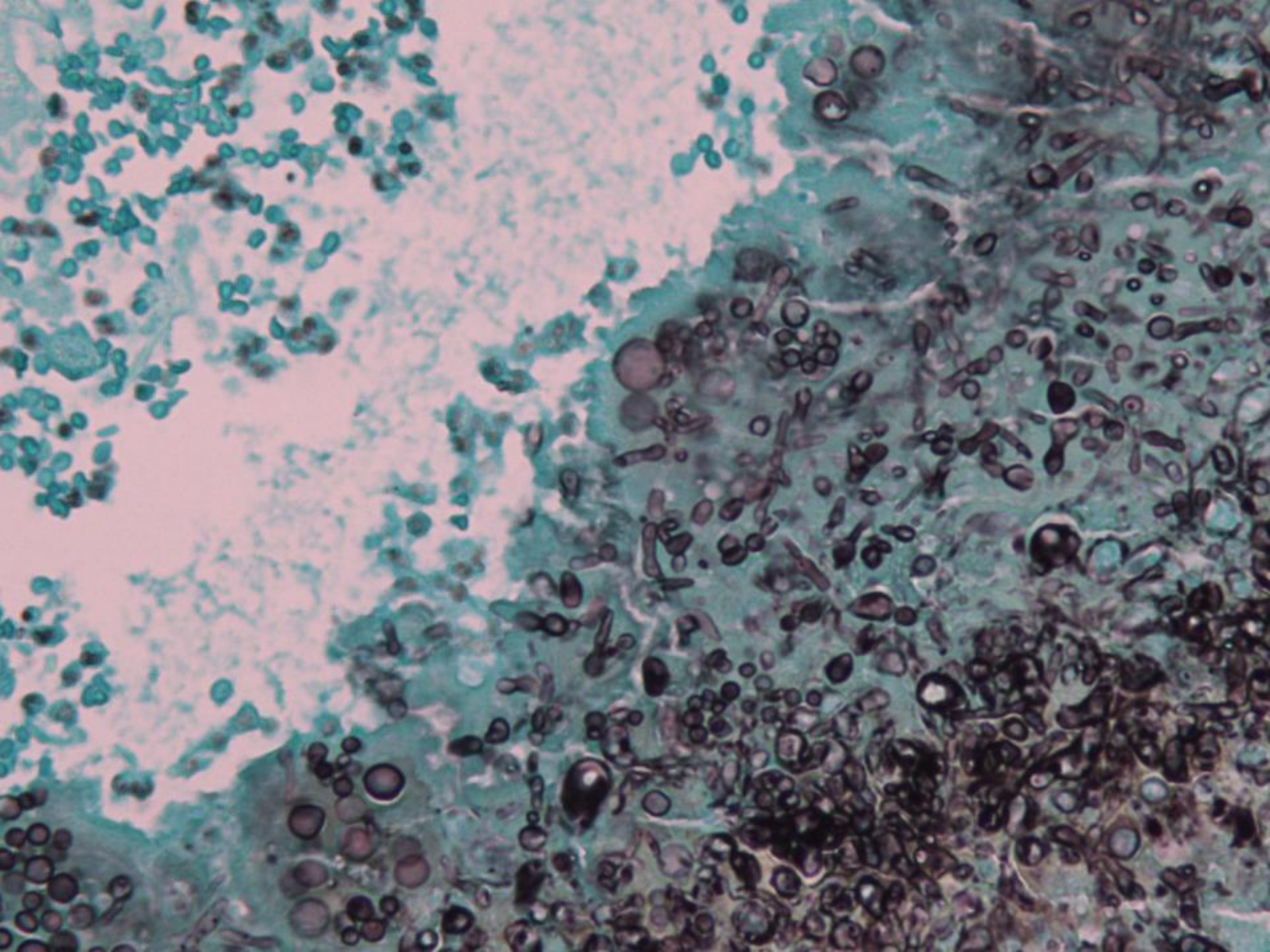
Calcofluor White fluorescent stain X450



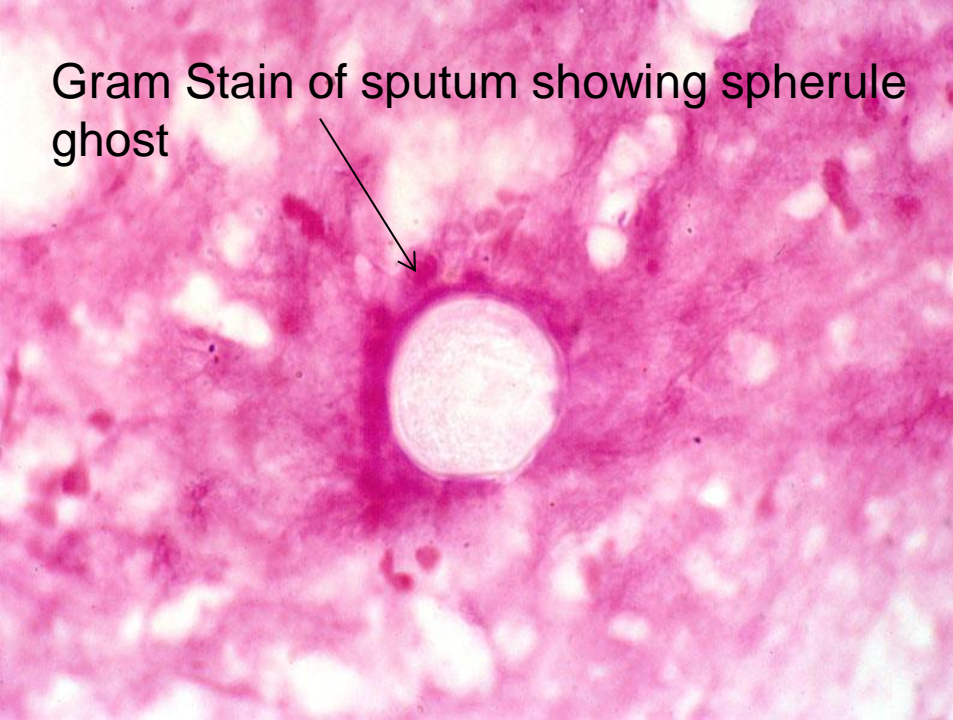
Spherules with
endospores

Gram stain with KOH x450





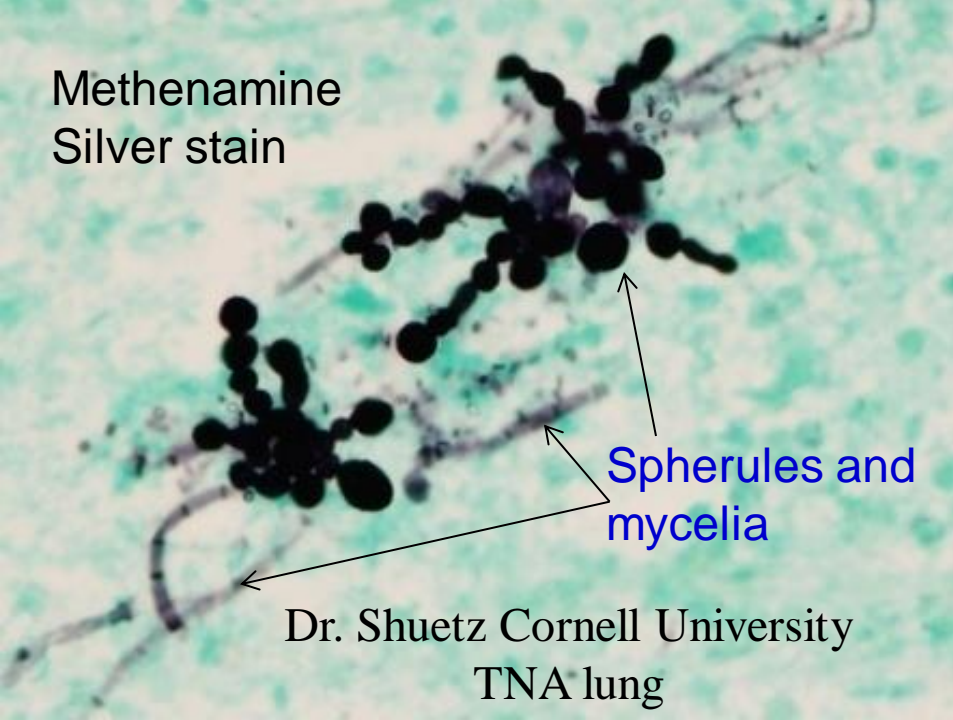
Gram Stain of sputum showing spherule ghost



Gram stain of KOH treated sputum showing mycelia



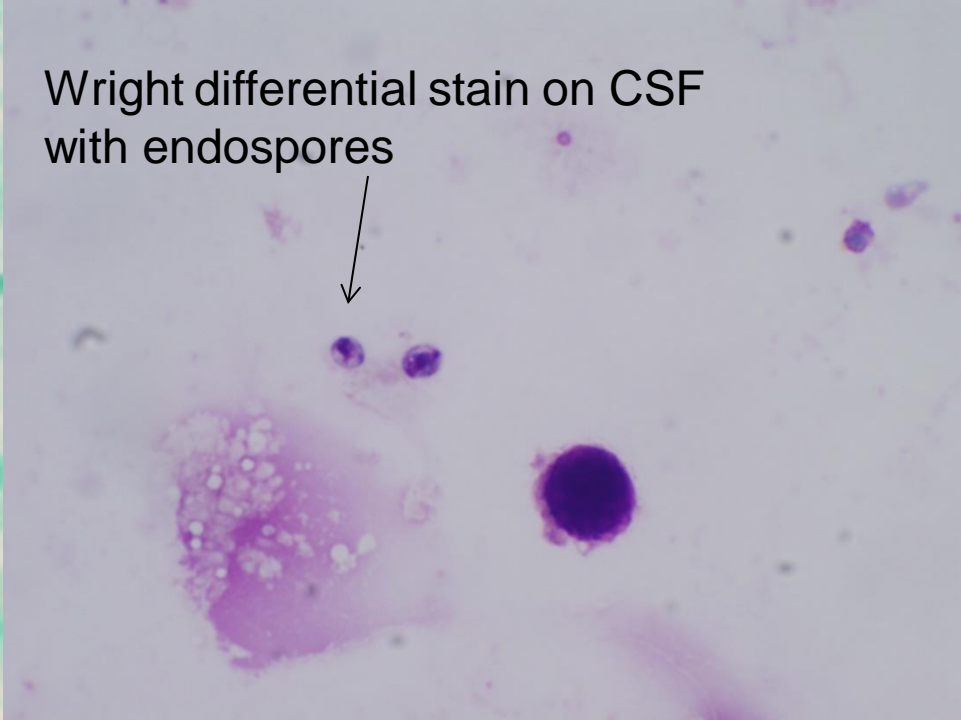
Methenamine Silver stain



Spherules and mycelia

Dr. Shuetz Cornell University
TNA lung

Wright differential stain on CSF with endospores



PCR Detection of *Coccidioides* spp.

- No commercially available kits
- Paucity of published studies
- Binicker, et.al. real-time PCR (J CM. 2007;45:173)
 - Respiratory (n=266): sens=100%; spec=98.4%
 - Fresh tissue (n=66): sens=92.9%; spec=98.1%
 - Paraffin tissue (n=148): sens=73.4%; spec=100%
 - CSF poor
- TGen (Flagstaff, AZ) and NY Dept Health: not FDA approved
 - Detection and separation of both species

Coccidioides Ag EIA

(MiraVista Diagnostics, Indiana; Joe Wheat)

- Rabbit anti-*Coccidioides* galactomannan Ab in microplate wells / EIA
- Evaluated 22 pts with severe pneumonia and 2 pts with disseminated disease
- Antigenuria detected in 70.8% using *Coccidioides* EIA and 58.3% using *Histoplasma* EIA
- Specificity : 99.4 (healthy individuals)
- X-reaction with other endemic mycoses: 10.7%

Coccidioidomycosis: Lab Diagnosis

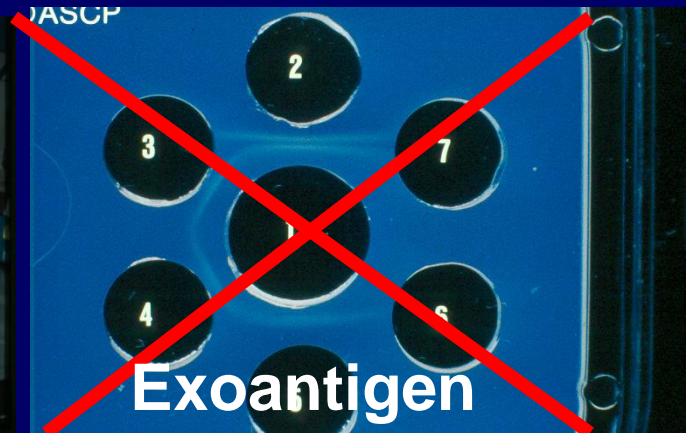
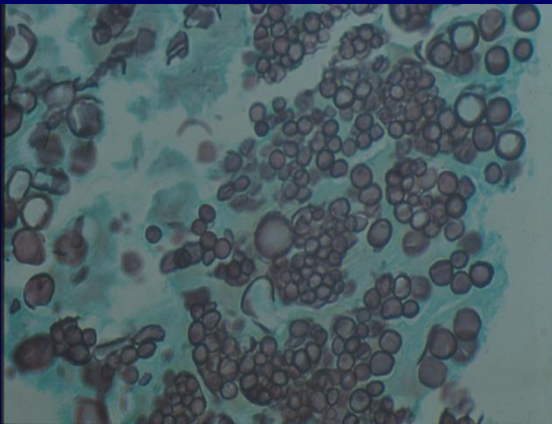
Saubolle and Sussland, unpublished LSA data, 2009

- Grows on almost all fungal and bacterial agar and broth media
- Incubation time (ambient air, 30°C) for 2-3 days to several weeks (at LSA/SQL lab average time to recovery 4 days, variation 2-16 days)
- **Recovery by culture within specimen type:**
 - Respiratory specimens (8.3%)
 - Other non-sterile body sites (2.5%)
 - Other sterile body site (2.1%); Bone marrow (2.6%)
 - CNS (0.9%); Blood (0.4%)
 - Urinary (0.6%)
 - Overall: Fungus culture – 71%; Bacterial culture: 29%

Coccidioidomycosis: Lab ID

Identification

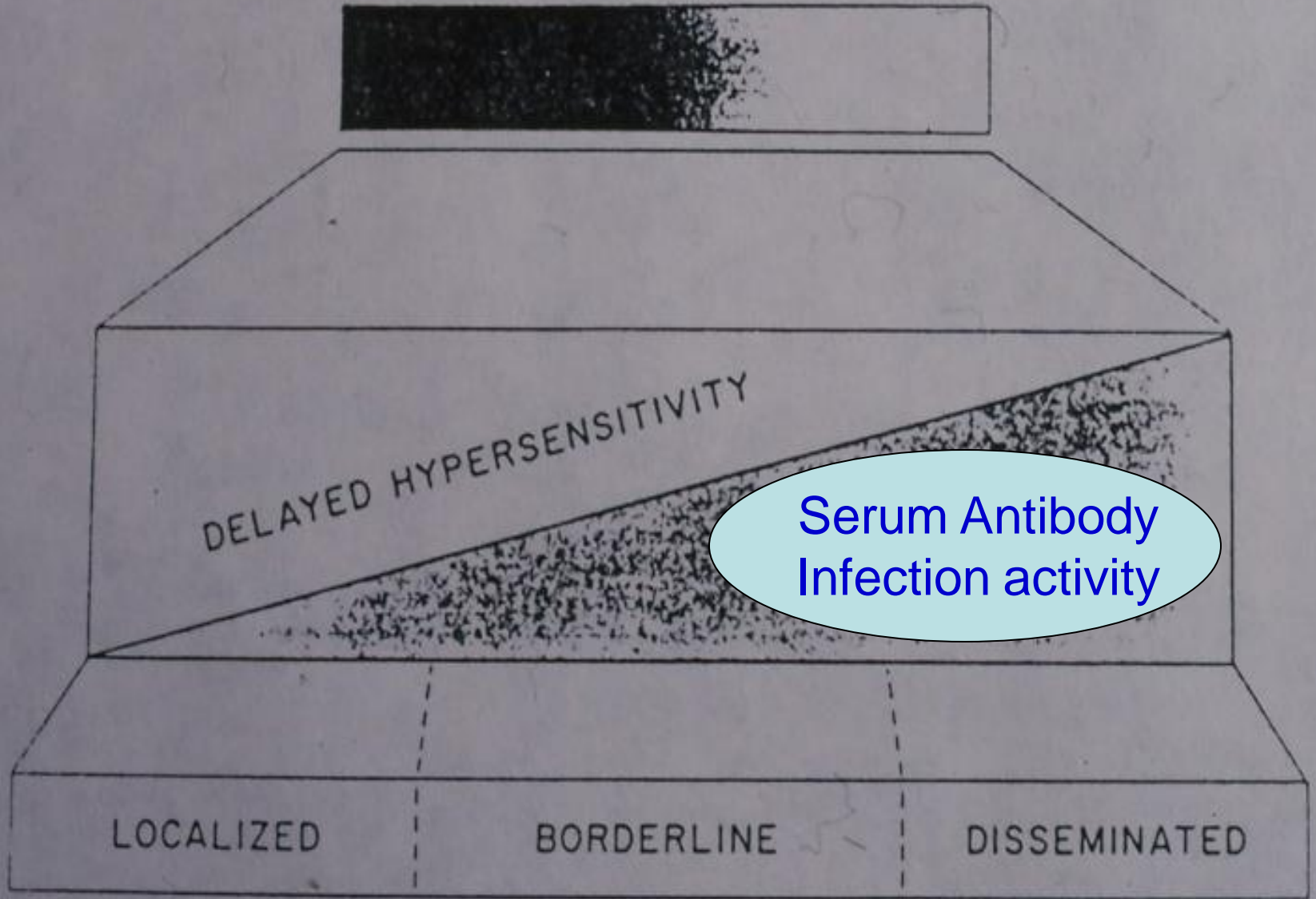
- Microscopic morphology of spherules (presumptive) in specimen
- Genetic Probe (Gen-Probe, San Diego) rRNA (will not differentiate species)



Coccidioidomycosis: Serologic Dx

- Serologic studies are less sensitive than often thought, especially in self-limited clinical cases
- Positive serologies are helpful, but negative ones cannot be relied on to rule out disease, especially early in disease process.
- False positive serologies can occur, especially with EIA IgM studies

Cell Mediated Immunity
Skin Test- Immunity



Serum Antibody
Infection activity

LOCALIZED

BORDERLINE

DISSEMINATED

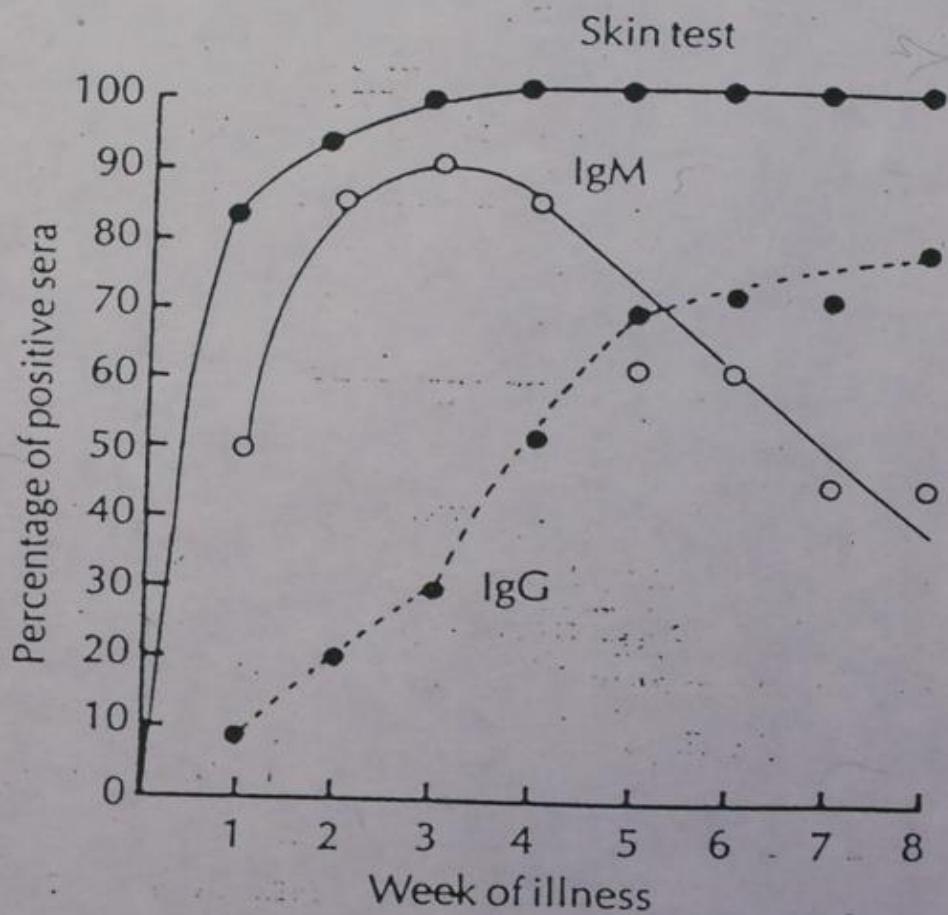


FIGURE 6. Chronology of conversion on coccidiodomycosis skin tests and serologic tests.

Coccidioidomycosis: Serologic Response

Cell mediated response (protective - immunity):

Skin testing with spherulin, coccidioidin (recently FDA approved- should be available early 2015; indicates past infection)

Humoral response (measures infection activity)

IgM (Tube Precipitin; polysaccharide is Ag): measurable earlier in acute phase usually between the first (50%) and third (90%) weeks of onset.

IgG (CF Ab; chitinase is Ag): becomes measurable between the 2nd and 28th week post onset. May remain for several months but is usually related to disease activity.

Coccidioidomycosis - Serologies

Humoral Ab (indicates level of activity)

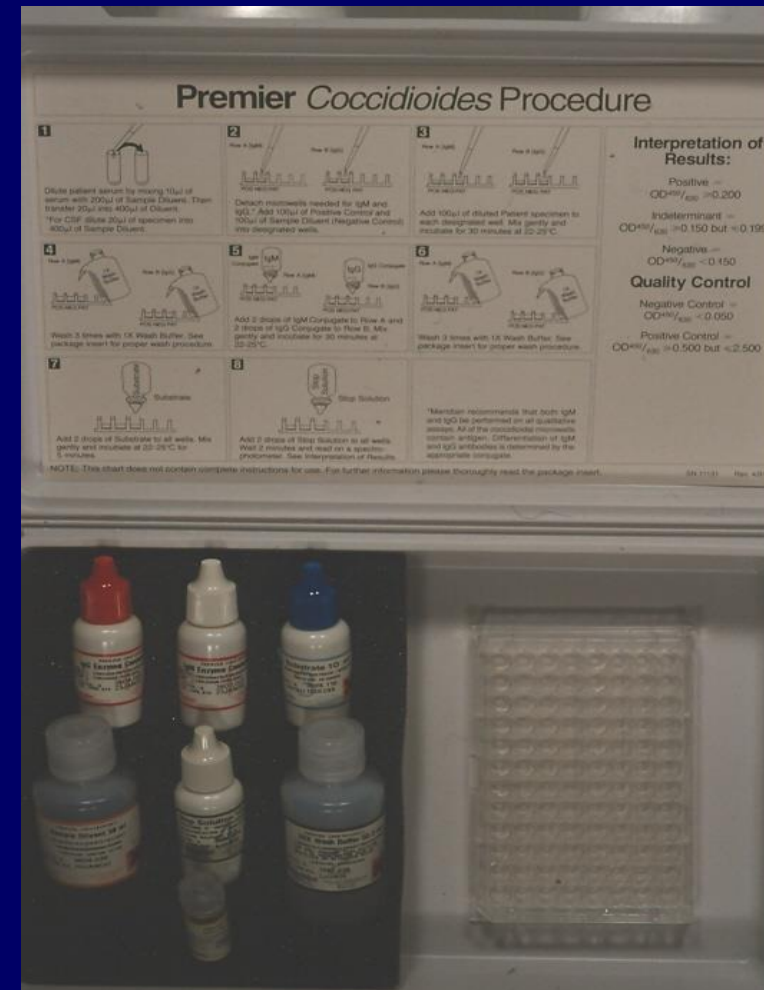
- Enzyme immunoassay (EIA)
 - IgM (Tube Precipitin, Precipitin Ab)
 - IgG (CF Ab)
- Immunodiffusion (IMDF)
 - IgM and IgG
- Complement fixation (CF; mostly IgG) :
increasing titers correspond with activity

Coccidioidomycosis: Laboratory Diagnosis

Serology

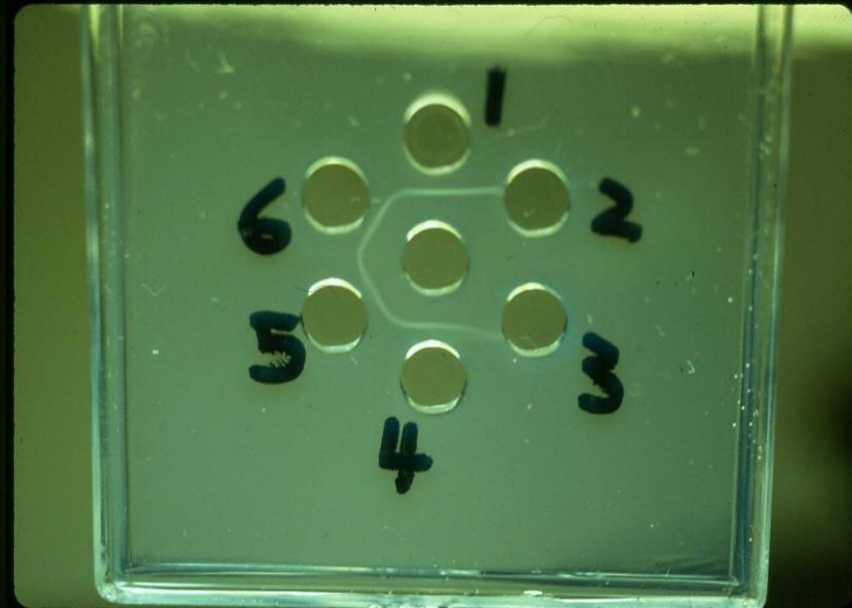
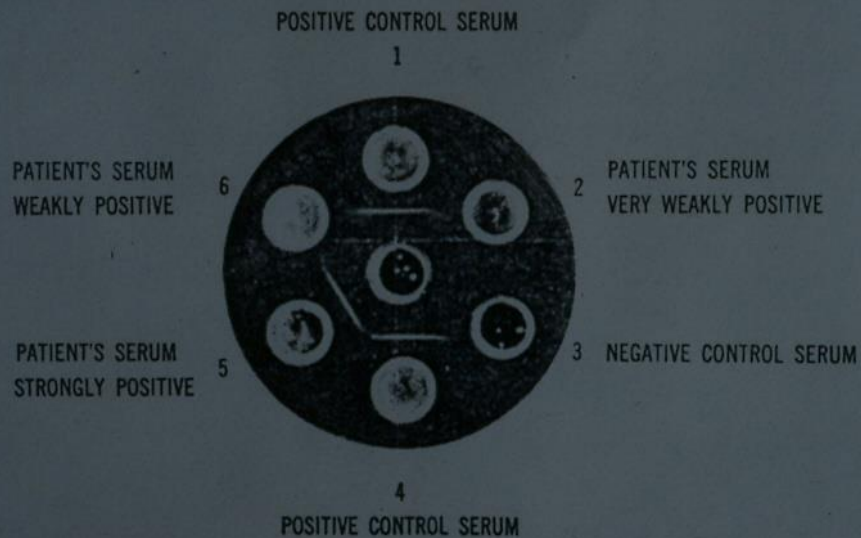
Enzyme Immunoassay (EIA)

- IgM (Tube Precipitin Ab)
- IgG (CF Ab)



Coccidioidomycosis: Laboratory Diagnosis

- Serology
 - Immunodiffusion (IMDF) : IgM, IgG



Coccidioidomycosis: Laboratory Diagnosis

- Serology

- Complement fixation (mostly IgG) : increasing titers correspond with activity (least sensitive, good to follow patients for disease activity)



Comparison of EIA, Immunodiffusion and CF Studies

<u>Study method</u>	<u>Sensitivity</u>
EIA IgG	79%
EIA IgM	63%
EIA Combined	83%
ID	71%
CF	64%

Polage et. al. Abstract F-005, ASM Annual Meeting, 2006

EIA IgM vs IgG Results

Blair, JE and JT Currier, Mycopathologia 2008;16677-82

- Of 706 total EIAs
- 37 (5%) EIAs on 28 pts had only IgM + (i.e. IgG -)
- Of the 28pts, there were no false + IgMs observed based on other laboratory data (other serologies, culture and histopathology)

Kuberski, T, J Herrig, and D. Pappagianis. J Clin Microbiol 2010;48: :2047-9

- 17 patients with EIA IgM + but IgG – studied by reviewing medical records
- 5 pts were coded out at discharge as Coccy based solely on IgM+ (IgG -) and none were judged as Coccy by chart review.
- Of the pts with both IgM and IgG +, 12 (80%) were judged to have coccy infection based on chart review

Comparison of EIA IgM and IgG results from two manufacturers (Kit A and kit B) using sera from the same patients divided among three laboratories (two in Arizona & one in California) – Sunenshine, Khan, Saubolle, Lancaster, et. al.-2014

A. 150 sera from confirmed cocci cases were selected retrospectively and frozen (Mike Lancaster, County Health Laboratory):

- Laboratory confirmed with ID and/or Complement Fixation (CF).
- Independently reviewed for clinical evidence of cocci by an infectious disease physician.

B. 50 remnant sera from CDC employees from non-endemic area (controls).

C. Percent agreement:

- Numerator: Number of times all three laboratories obtained the same result (all negative or all positive) for a particular test (IgG or IgM) using a particular test kit (Kits A or B) on a particular specimen.
- Denominator: 200 (total number of specimens); specimens with indeterminate results counted as “negative”.

Percent agreement for EIA IgM and IgG combined among the three labs:

- ❑ 85.5% for kit A (90% for IgM and 89% for IgG)
- ❑ 70.5% for kit B (67% for IgM and 81%, for IgG alone)

Sensitivity for EIA IgM and IgG combined:

- ❑ 68.5% for kit A
- ❑ 72.4% for kit B

Specificity for EIA IgM and IgG combined:

- ❑ 99.3% for kit A
- ❑ 91.3% kit B

Summary of Serological Assays

- Serological testing is the most commonly used diagnostic method and includes EIA, immunodiffusion (IMDF), and complement fixation (CF); sensitivity, specificity, and positive predictive value vary depending on laboratory methods
- EIA is the easiest and least expensive diagnostic test to perform, but sensitivity and specificity are not clearly defined
- Early in disease serologic studies can be falsely negative; additional testing 1-3 weeks later are crucial in suspected cases.
- False positives may occur, leading to additional diagnostic testing and unwelcome patient anxiety; repeat testing should be considered for EIA + & IMDF/CF- results
- IMDF may be used as a confirmatory test for positive EIA results by some laboratories as recommended by Kaufman et al.

Clinical Case 3

Slide 1

- 50 y/o male brought to ED with mental status changes and altered level of consciousness
 - Slow to respond, sluggish pupils, no neck rigidity
- X-ray showed moderate right sided pleural effusion with possible r-lung infiltrate as well as a VP shunt in place for hydrocephaly (inserted previous year)
- History for:
 - End-stage renal disease requiring hemodialysis
 - Hydrocephalus
 - Diabetes Mellitus requiring insulin
 - Hypertension
 - Coronary artery disease with a stent
 - Hep C Ab + and past history of drug abuse

Slide 2

- Differential diagnosis considered : VP shunt malfunction; possible metabolic encephalopathy due to uremia; community acquired pneumonia
- Started on Vanco and Pip-tazo for the possible CAP
- Lab data:
- Peripheral WBC of 12.8; pleural fluid WBC 700
- BCBs negative after 5 days
- CSF cloudy and submitted for culture

Slide 3 – more history

Previous admission 4 months earlier:

- with complaints of shortness of breath for a week prior to admission and a 25 lb weight gain over two months
- Chest X-ray showed bilateral opacities –considered to be pulmonary edema
- Found to have heart failure and renal disease and started on hemodialysis
- Was stabilized and released to be followed by nephrology and cardiology

Back to most recent admission:

- Differential Dx
 - VP shunt infection – meningitis
 - R lower lobe pneumonia
 - Cultures:
 - Urine – NG
 - Pleural fluid – NG
 - Blood x 3 - NG
- Serologies : Hep C +
- Aerobic cultures from VP shunt grew ??????

Most recent admission:

- Differential Dx
 - VP shunt infection – meningitis
 - R lower lobe pneumonia
- Aerobic cultures from VP shunt grew

Coccidioides spp

- CSF Coccy CF titer was 1-2 and 1-4 a week later
- Serum Coccy CF >256 (EIA IgM neg; IgG +; IMDF both +)
- BCBs remained negative
- Pleural fluid remained negative
- Patient started on AMB but switched to 800 mg IV Fluconazole for 8 week
- Released once stable to be followed by ID, cardiology, etc

- Clinical Diagnosis of Coccidioidomycosis often difficult as presentation can be protean
- Most presentations are of a respiratory nature but often can't separate from other respiratory infections
- At times patient does not realize that he/she has more than a virus until progression occurs
- It may take months before a true diagnosis is made
- Must have high degree of suspicion and must understand laboratory studies (pros/cons; shortcomings)

Coccidioidomycosis: Future Epidemiology

- Growing susceptible population
- Growing immunocompromised patient pool
- Expansion into desert
 - (record new home starts)
- Increasing travel and tourism
- Better education
- Possible expansion of geographic distribution in future

Conclusion: future of coccoy seems assured

CocciMarket

The END

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