Coccidioides Diagnostics

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Diagnostics

Culture

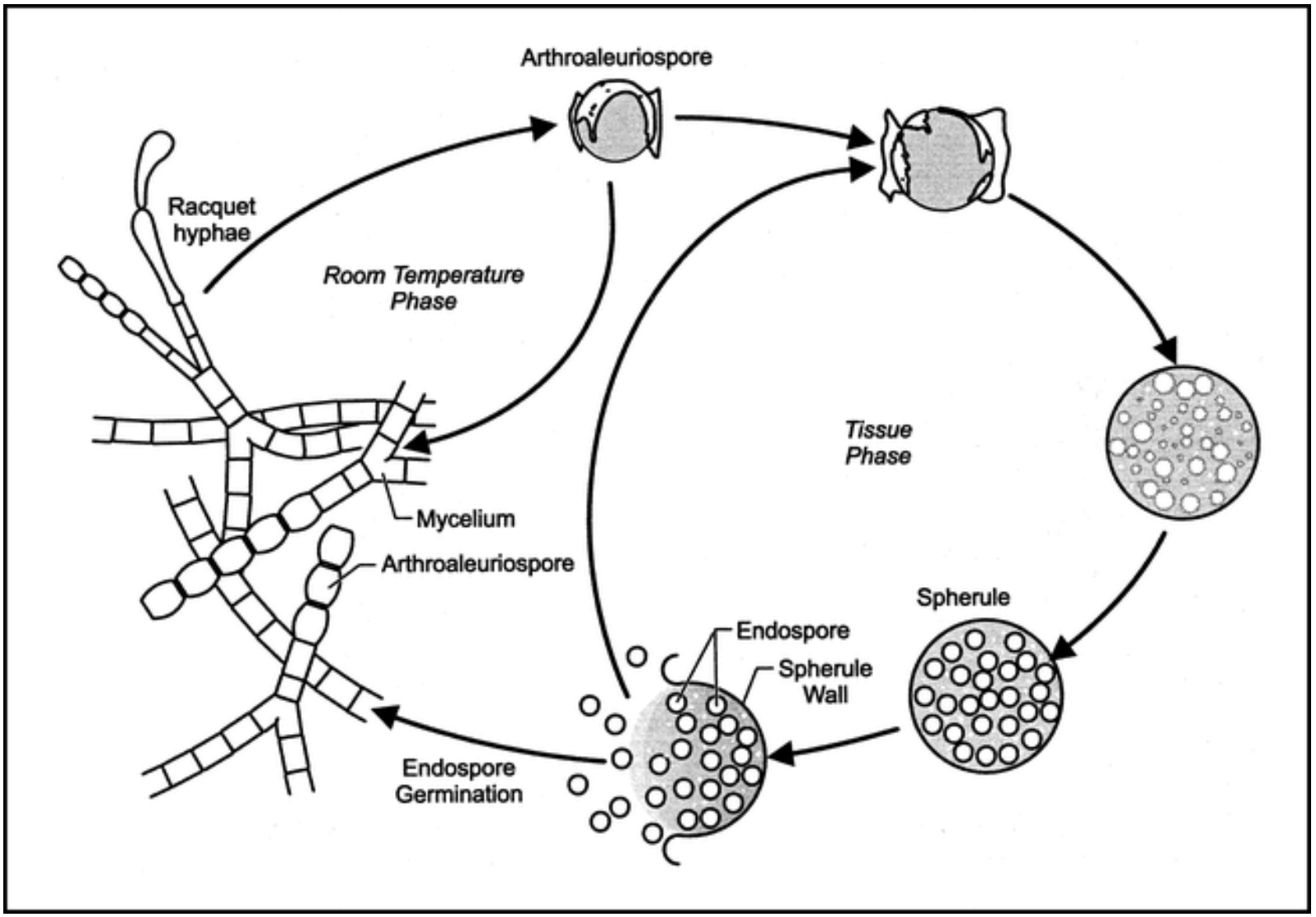
Histopathology

Antibody Testing

Antigen Testing

Molecular Techniques

Coccidioides Infection Cycle

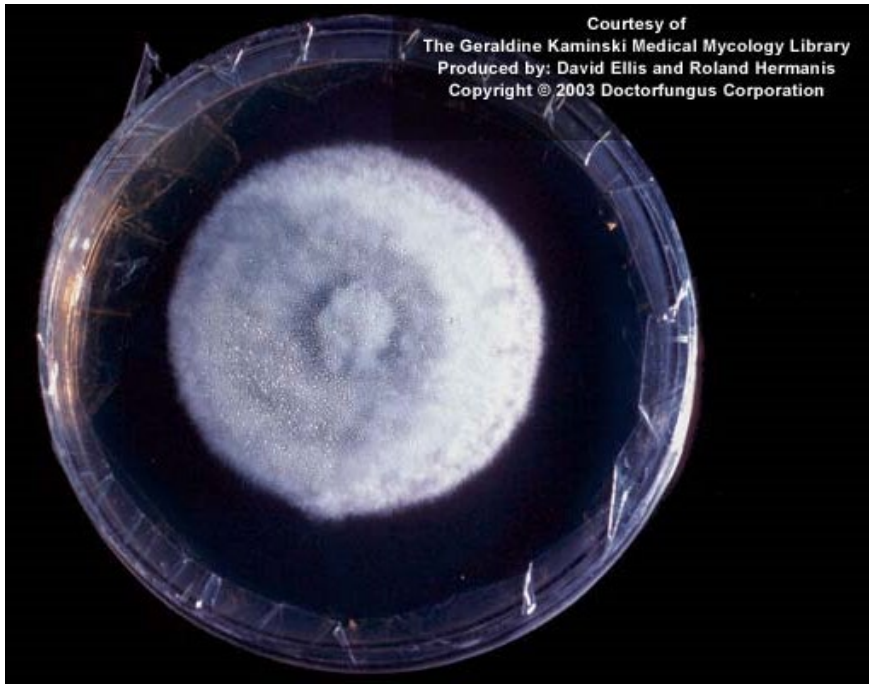


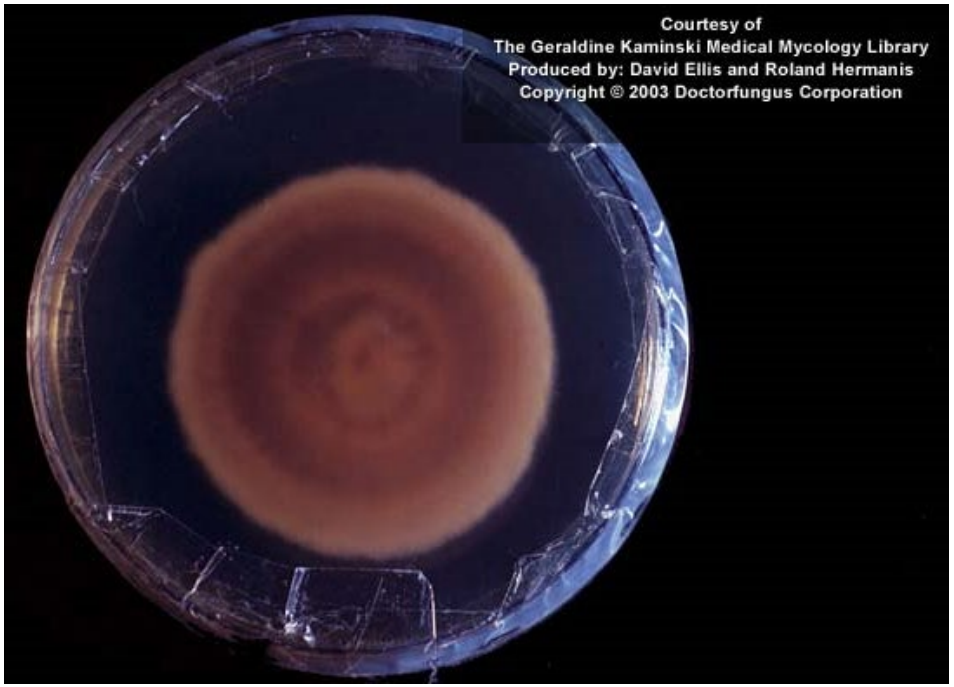
www.ajnr.org/cgi/content-nw/full/20/3/509/F1

Culture

Overall culture sensitivity estimated at less than 50%

- Pleural Fluid: 13%
- CSF: 30%
- Respiratory Specimens: 44%

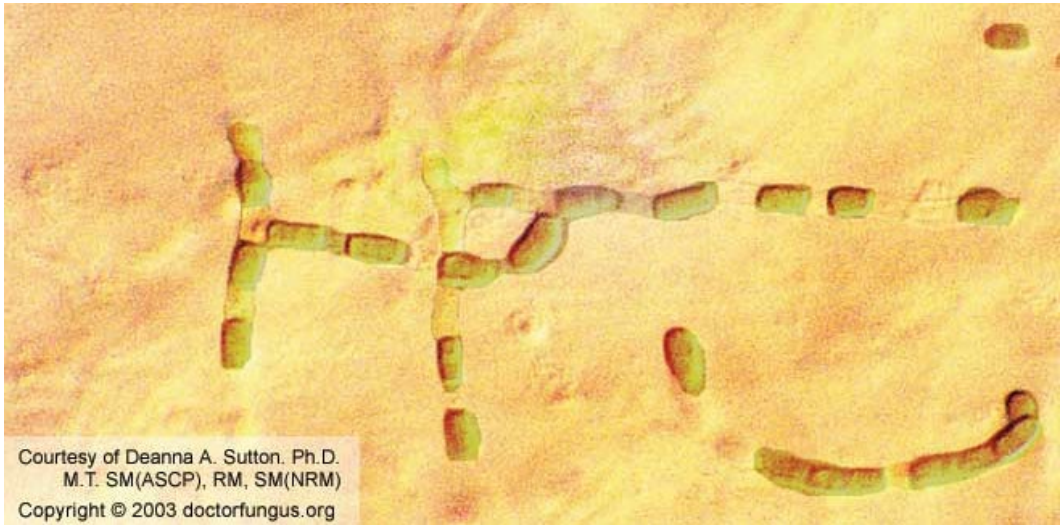




Rate of growth: Moderate; mature within 10 days.
Growth occurs in 3-5 days, but production of arthrocondia may take 1-2 weeks

Microscopic Features Using Tease Prep

Growth on potato dextrose agar at 25°C; color enhanced



Cultures exhibit coarse, septate, branched hyphae that produce thick-walled, barrel-shaped arthroconidia (3-4 x 3-6 µm) that alternate with empty cells.

Arthroconidia can be seen with Geotrichum or Malbranchea, so confirmation of ID is necessary (CAP requirement).

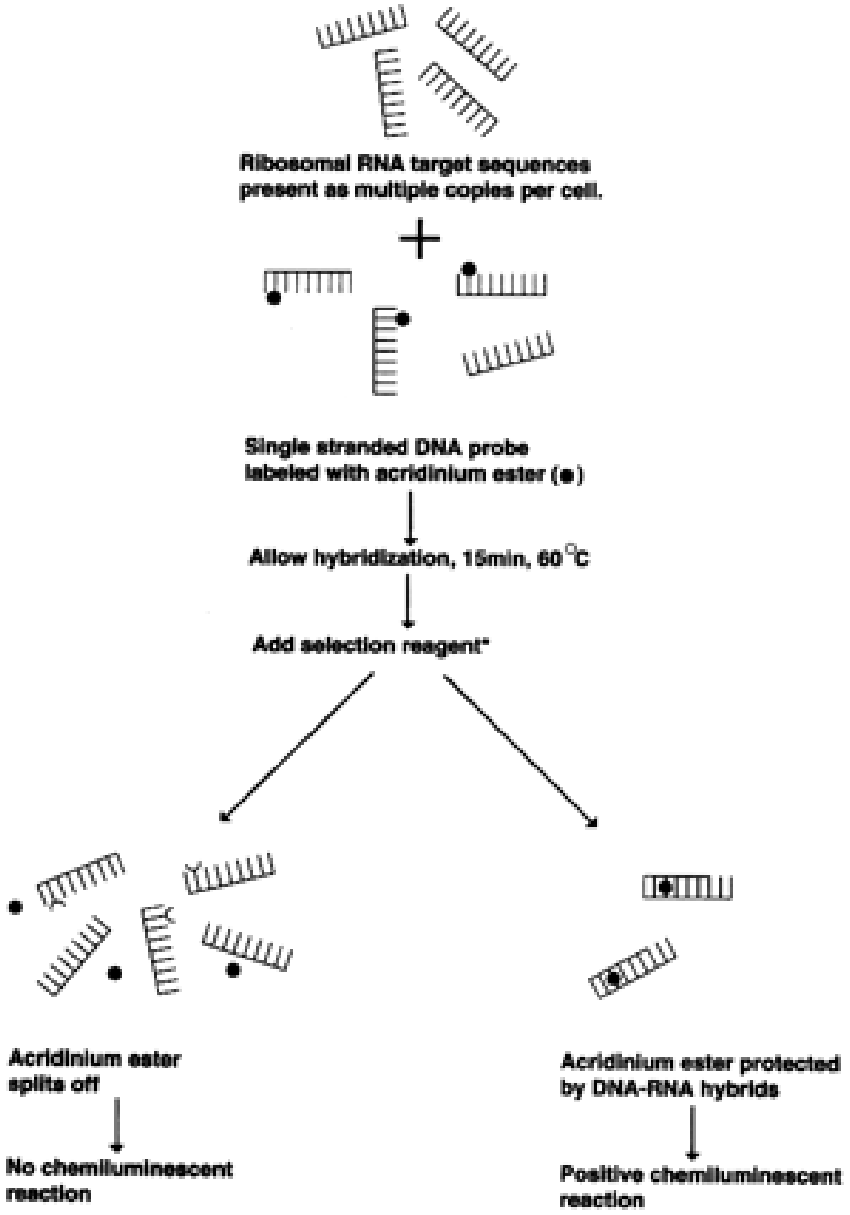
Fungal Culture Identification: Accuprobe

Uses a single-stranded DNA probe with a chemiluminescent label (acridinium ester) that is complementary to the ribosomal RNA of *C. immitis*.

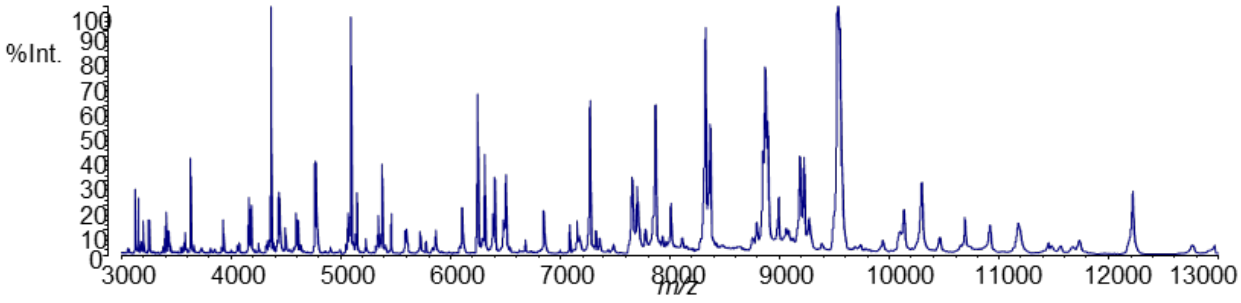
After the ribosomal rRNA is released from the organism, the labeled DNA probe combines with the target organism's rRNA to form a stable DNA-RNA hybrid.

The Selection Reagent allows for the differentiation of non-hybridized and hybridized probe.

The labeled DNA:RNA hybrids are measured with a luminometer.

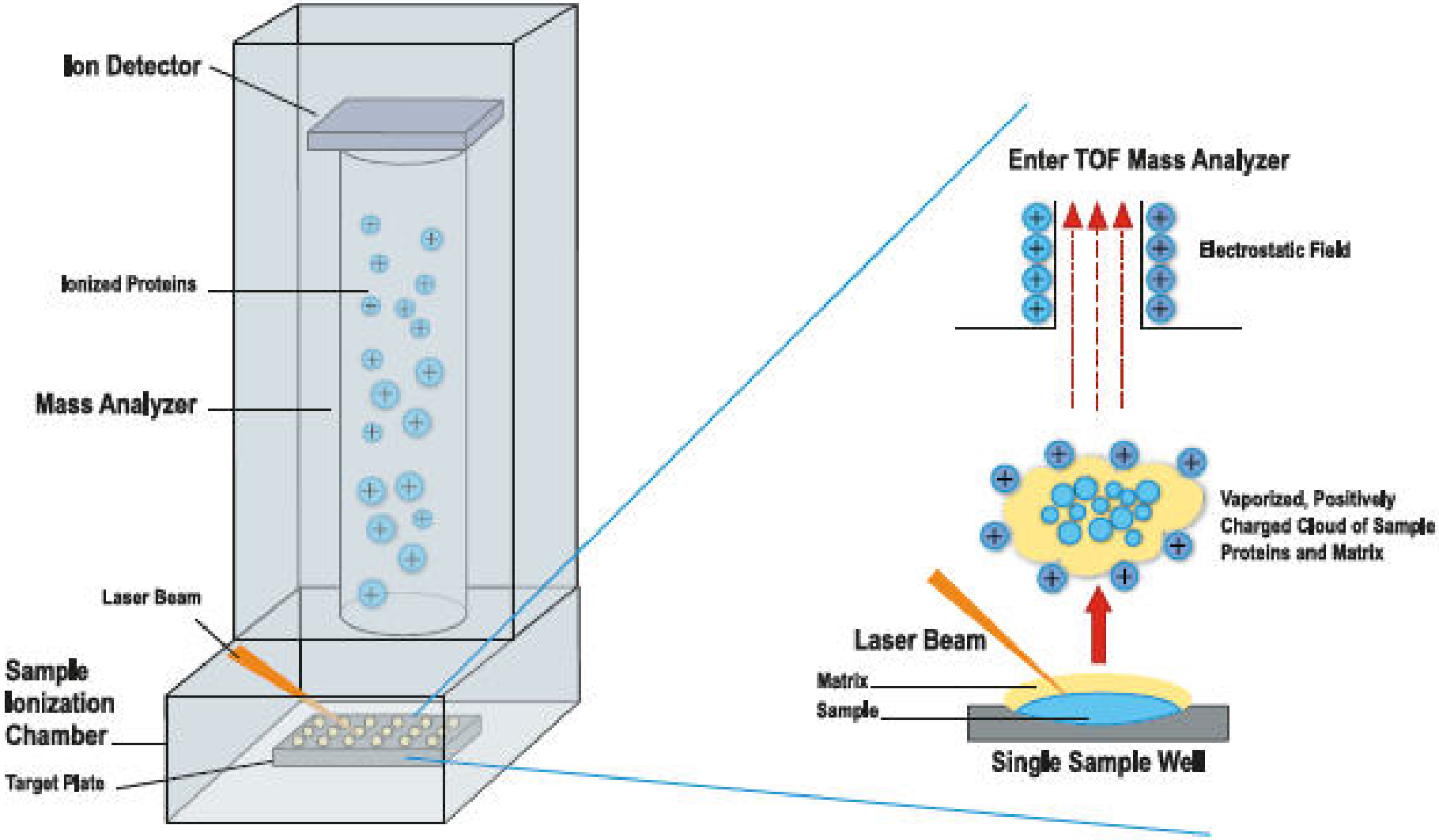


Fungal Culture Identification: MALDI-TOF



MALDI-TOF:

Matrix Assisted Laser Desorption Ionization Time Of Flight



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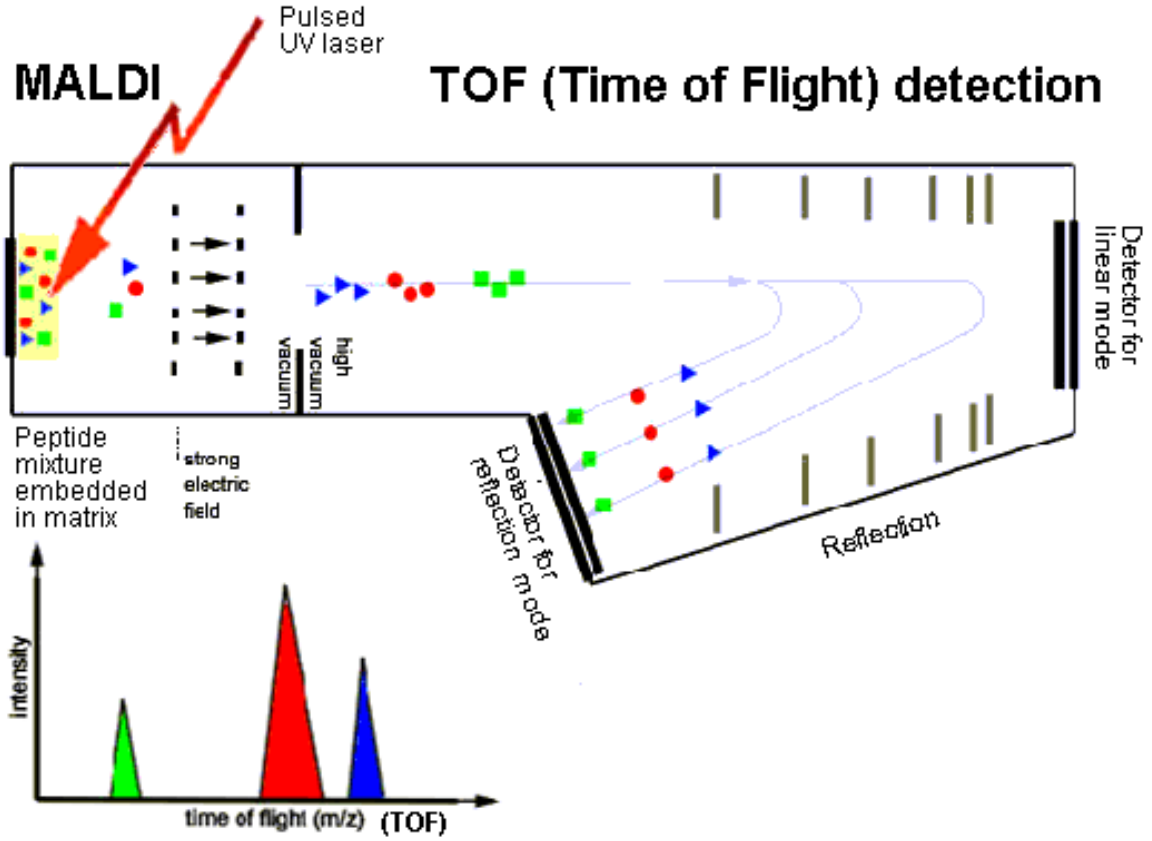
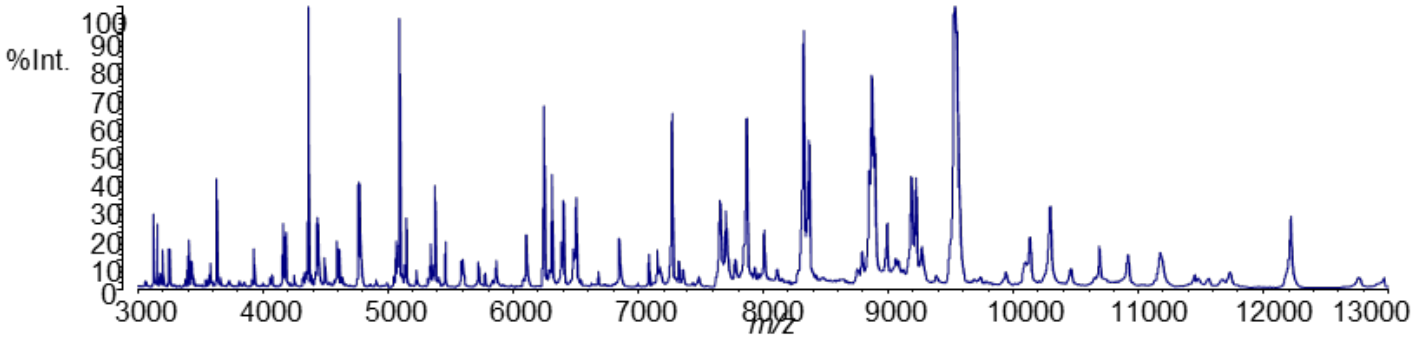
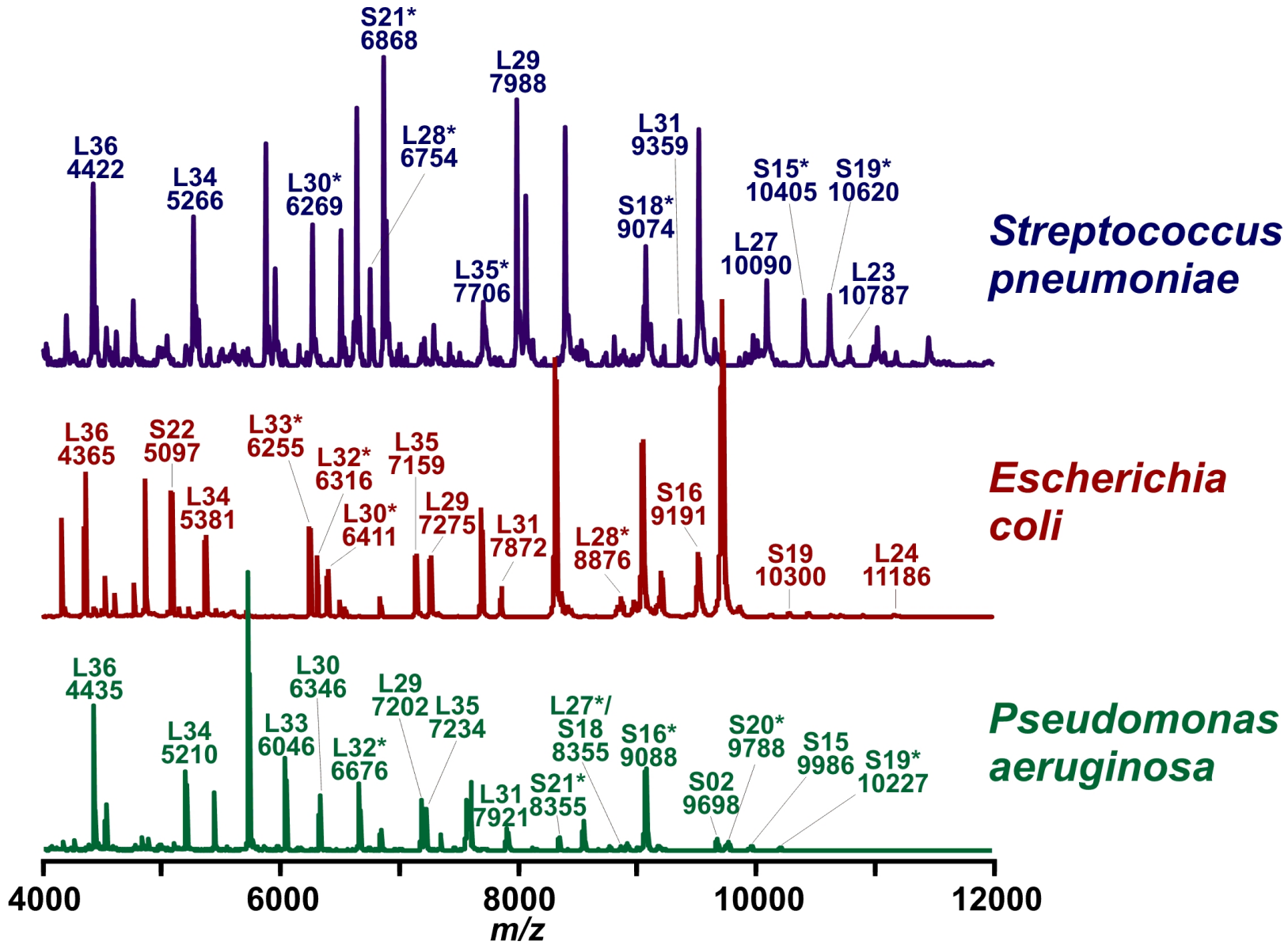




Difference Species – Different Patterns



VITEK® MS Expanded V3 Database now FDA 510(k) cleared (Molds)

Hyaline Molds

Aspergillus brasiliensis
Aspergillus flavus/oryzae
Aspergillus fumigatus
Aspergillus lentulus
Aspergillus nidulans
Aspergillus niger complex
Aspergillus sydowii
Aspergillus terreus complex
Aspergillus calidoustus
Aspergillus versicolor
Fusarium oxysporum complex
Fusarium proliferatum
Fusarium solani complex
Paecilomyces variotii complex
Penicillium chrysogenum
Pseudallescheria boydii
Scedosporium apiospermum
Scedosporium prolificans

Mucorales

Lichtheimia corymbifera
(AKA Absidia corymbifera)
Mucor racemosus complex
Rhizopus arrhizus complex
Rhizopus microsporus complex

Endemic (Dimorphic)

Blastomyces dermatitidis
Coccidioides immitis/posadasii
Histoplasma capsulatum
Sporothrix schenckii complex

Dermatophytes

Epidermophyton floccosum
Microsporum audouinii
Microsporum canis
Microsporum gypseum
Trichophyton interdigitale
Trichophyton rubrum
Trichophyton tonsurans
Trichophyton verrucosum
Trichophyton violaceum

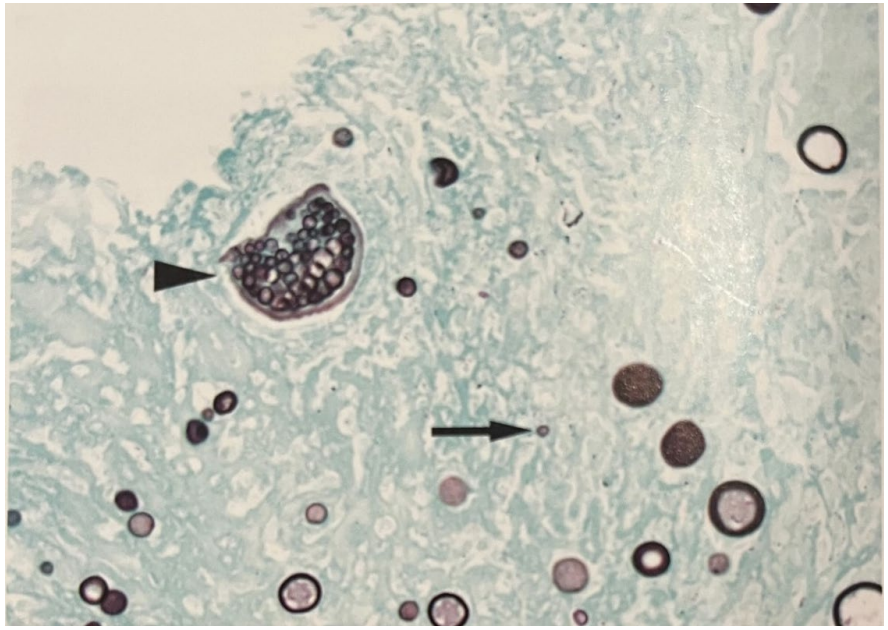
Others

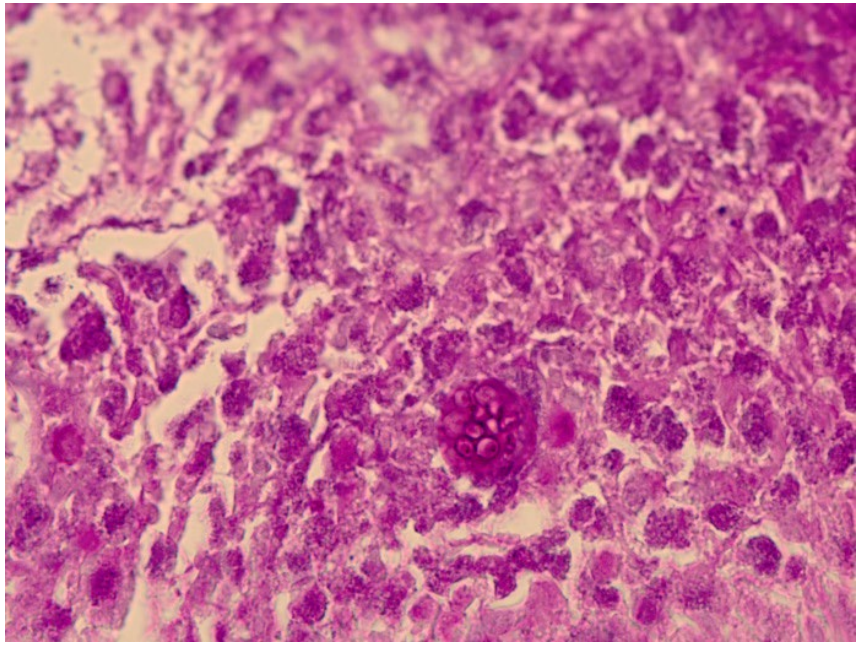
Acremonium sclerotigenum
Alternaria alternata
Cladophialophora bantiana
Curvularia hawaiiensis
Curvularia spicifera
Exophiala dermatitidis
Exophiala xenobiotica
Exserohilum rostratum
Lecythophora hoffmannii
Purpureocillium lilacinum
Rasamsonia argillacea complex
Sarocladium kiliense

Histopathology

Generally poor sensitivity (less sensitive than culture)
Specificity is high for spherules, low for endospores

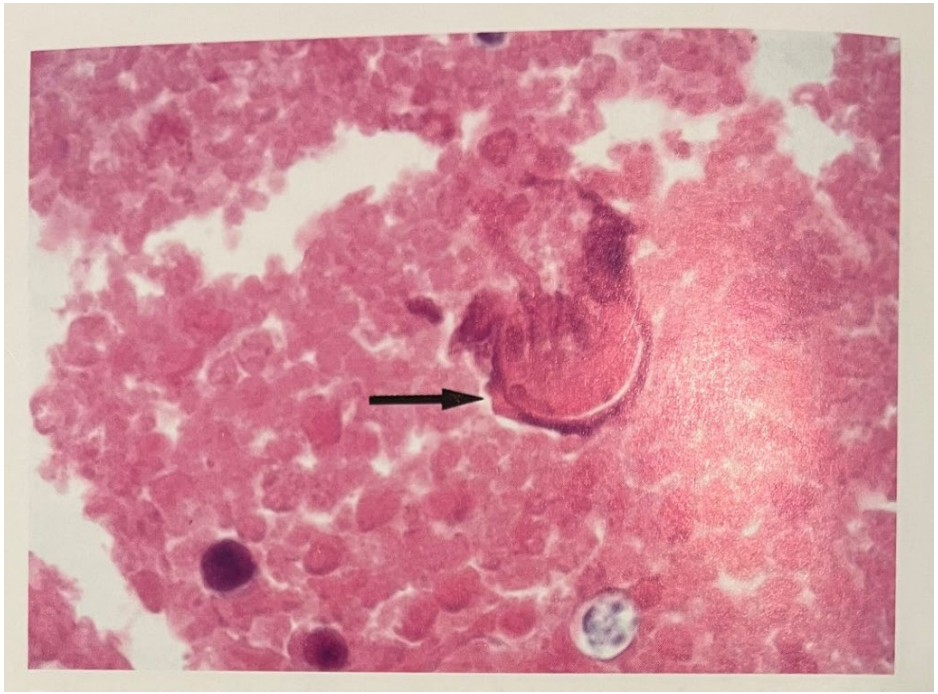
Thick walled spherules (100um in diameter) containing endospores (2-4um).

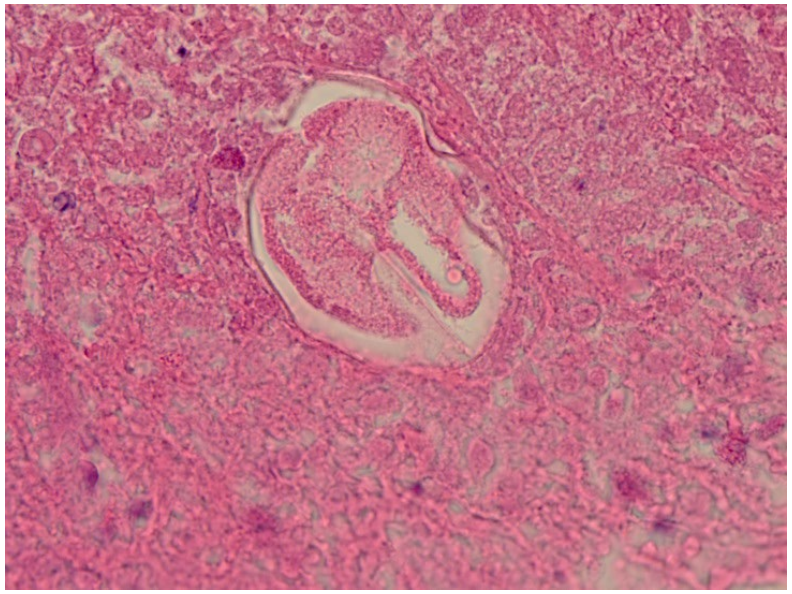


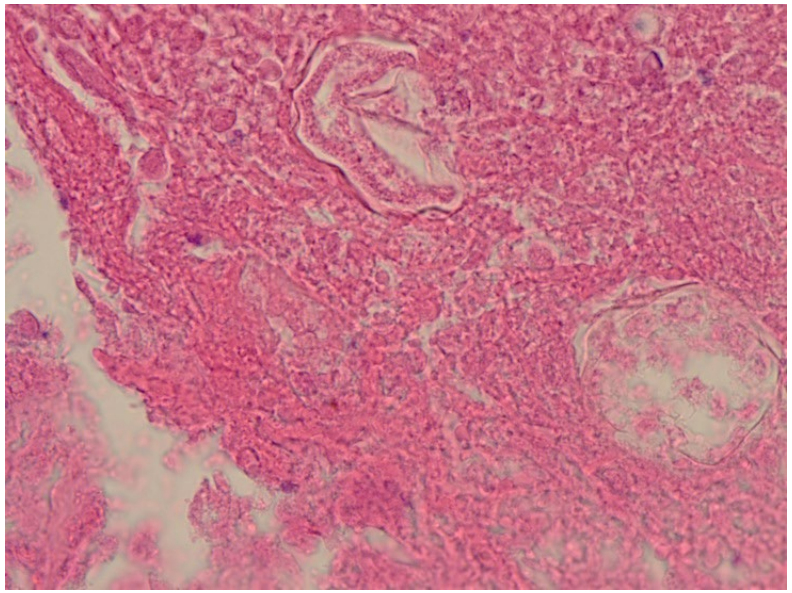


Histopathology

Spherules can be empty of endospores

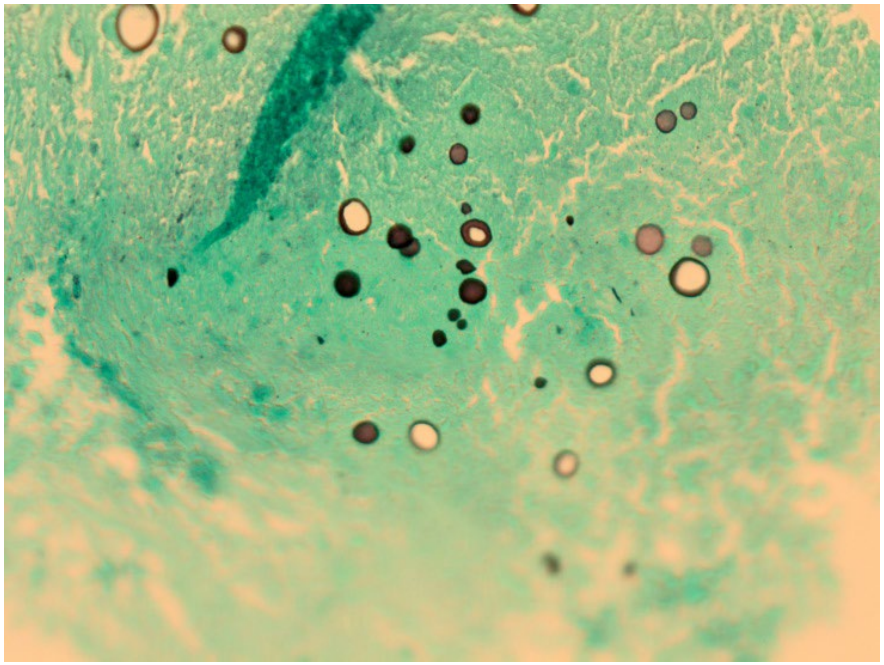


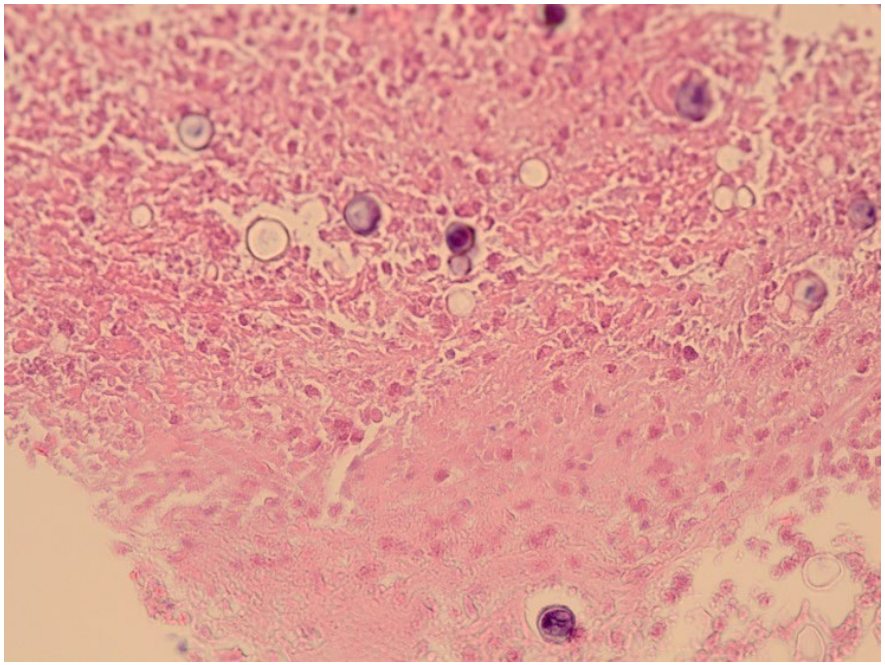




Histopathology

Endospores alone are not specific for cocci. Just look like yeast.





Histopathology

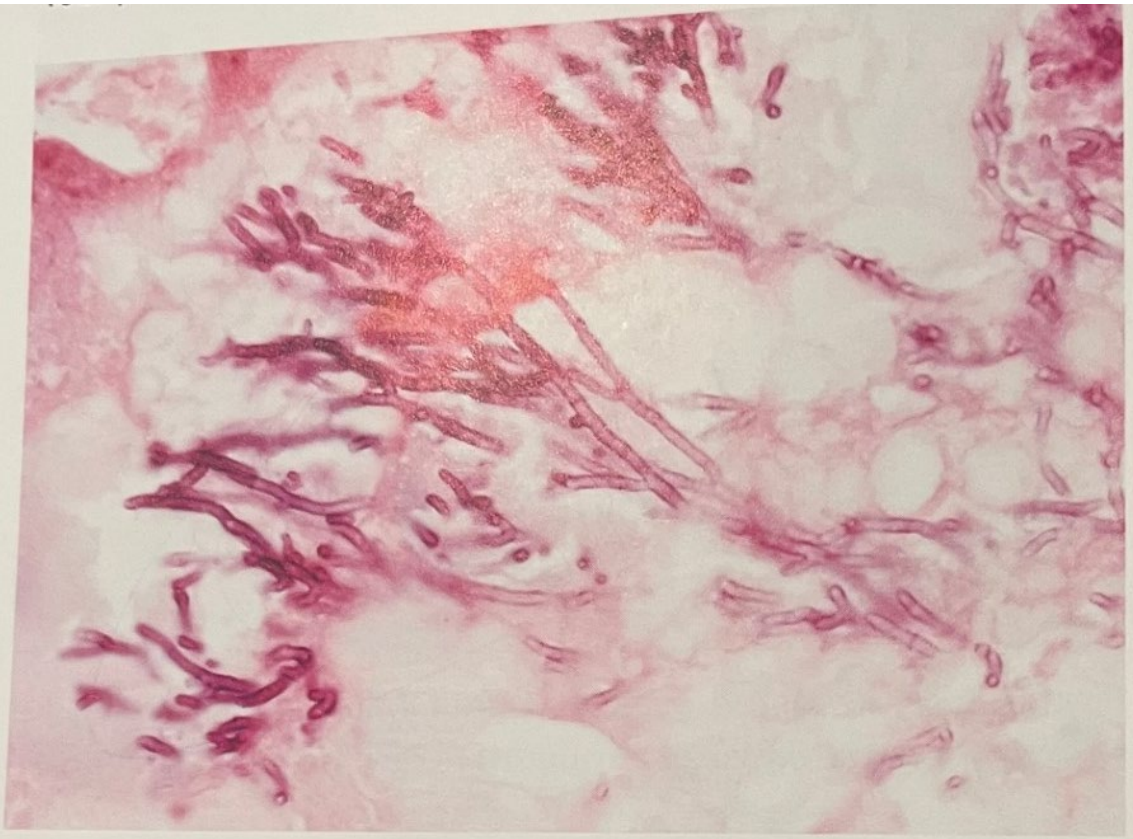
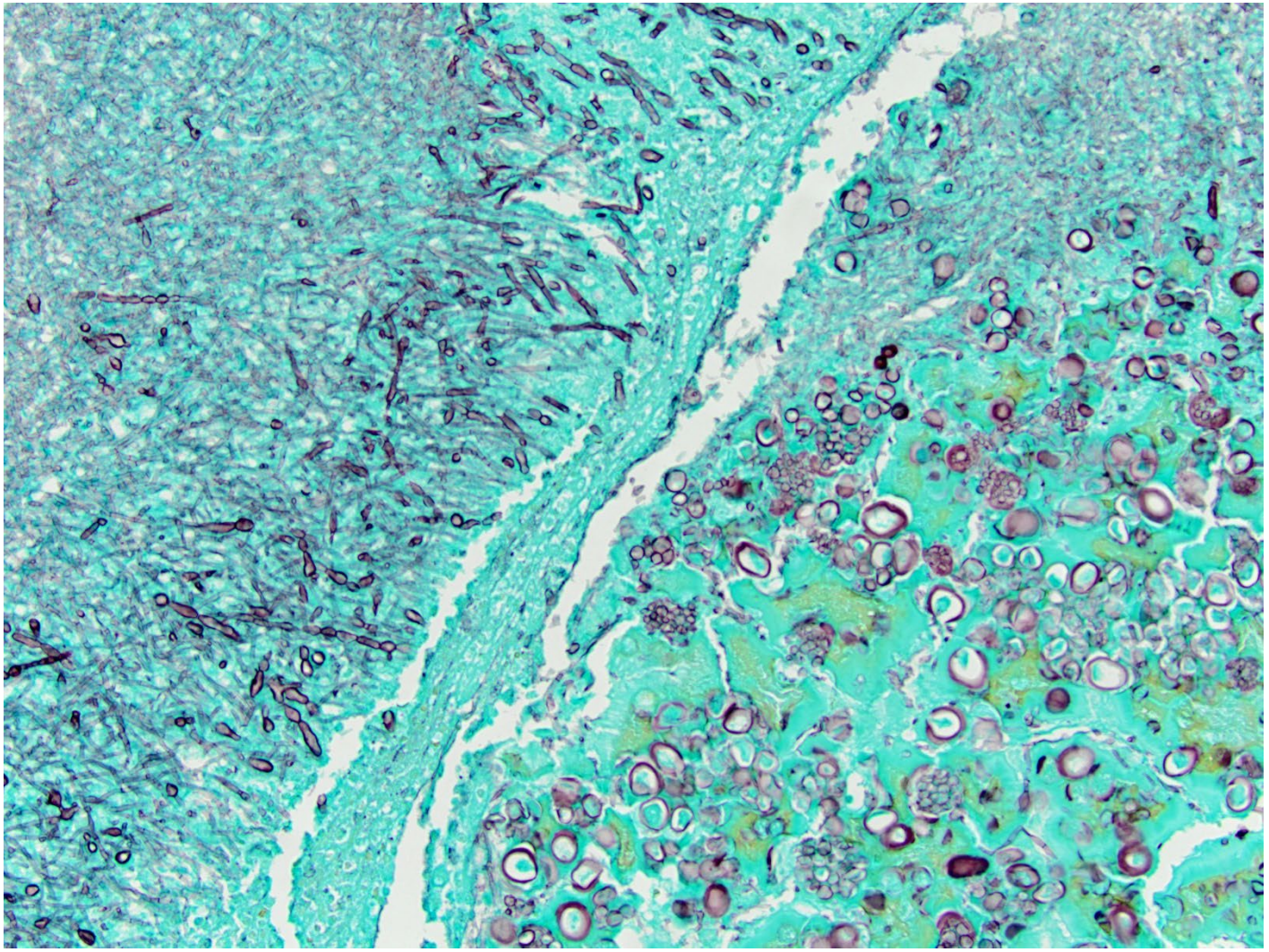


Figure 3.39. Although infrequently encountered, if *Coccidioides* reaches an airspace (eg, erosion through a bronchus), then hyaline septate hyphae, like those formed in culture, may be seen (H&E, 200X).

Histopathology



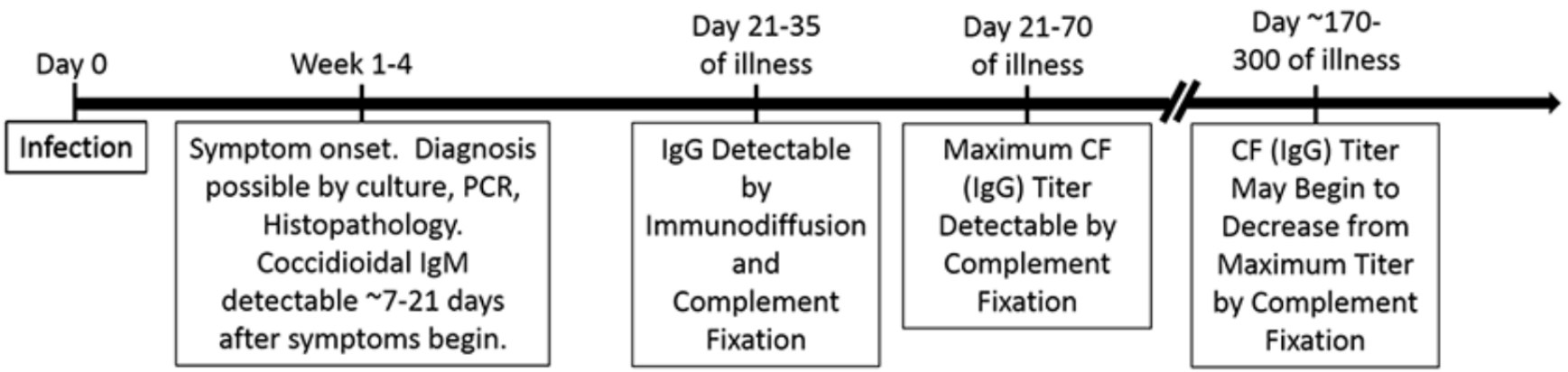
Cocci in a cavitary lesion

Serological Assays for Coccidiodes

C. immitis IgG/IgM antibodies by EIA
Sensitive Screening Assay (ng/ml)

ID-TP/CF Antibody by Immunodiffusion
Specific Confirmation Assay (ug/ml)

Semi-Quantitative Complement Fixation
Assay to determine dissemination and treatment effectiveness



Cocci IgG/IgM antibodies by EIA (screen)

Principle: Qualitative detection of IgM and IgG antibodies directed against tube precipitin (TP) and complement fixation (CF) antigens of *C. immitis* in serum.

TP is a 120 kDa glycoprotein; antibodies to TP antigen is interpreted as an indication of acute coccidioidal disease and is primarily an IgM response.

CF antigen is heat labile protein; antibodies to CF are typically seen during later stages of disease.

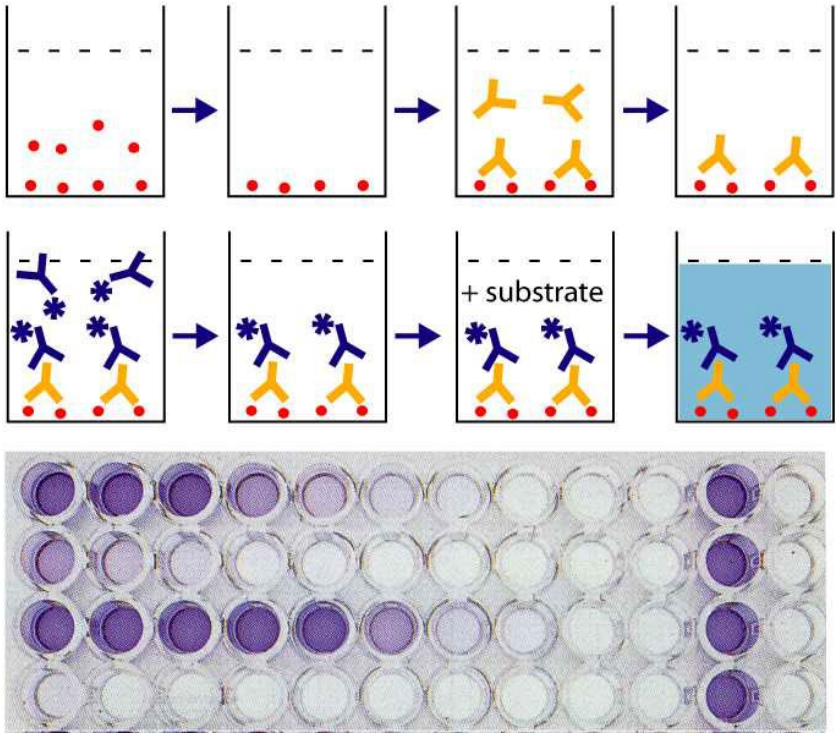
Specimen type: Serum

Results Interpretation:

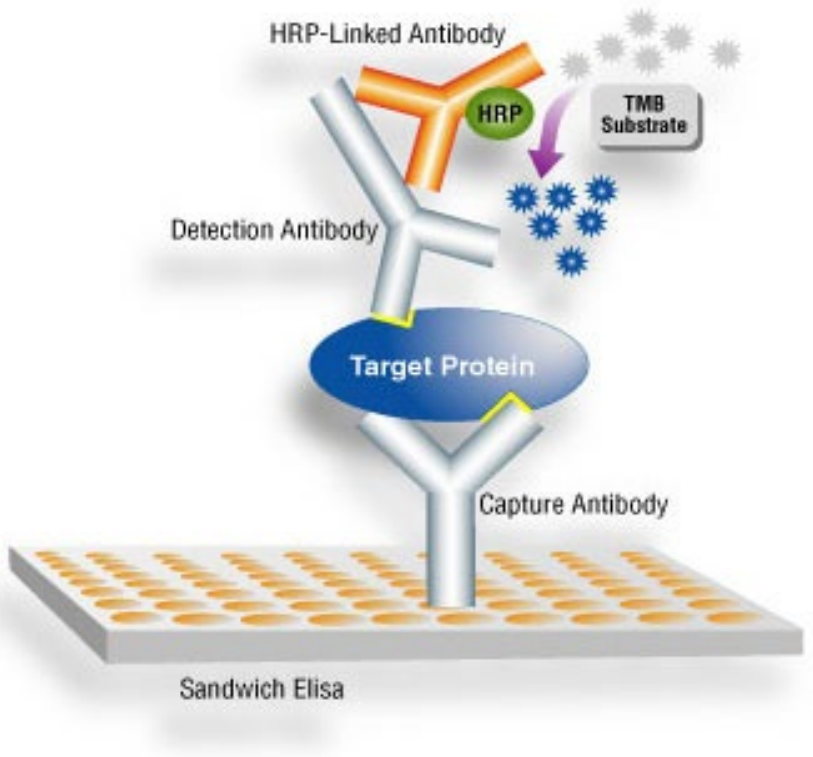
Negative = Absorbance Value <0.150
Indeterminate = Absorbance Value =0.150 but =0.199
Positive = Absorbance Value =0.200

IgM can exhibit a higher rate of false positivity – should be confirmed with Immunodiffusion
IgG has a lower rate of false positivity
Neither have good sensitivity within first few weeks of symtpoms

Cocci IgG/IgM antibodies by EIA (screen)



Antibody recognition

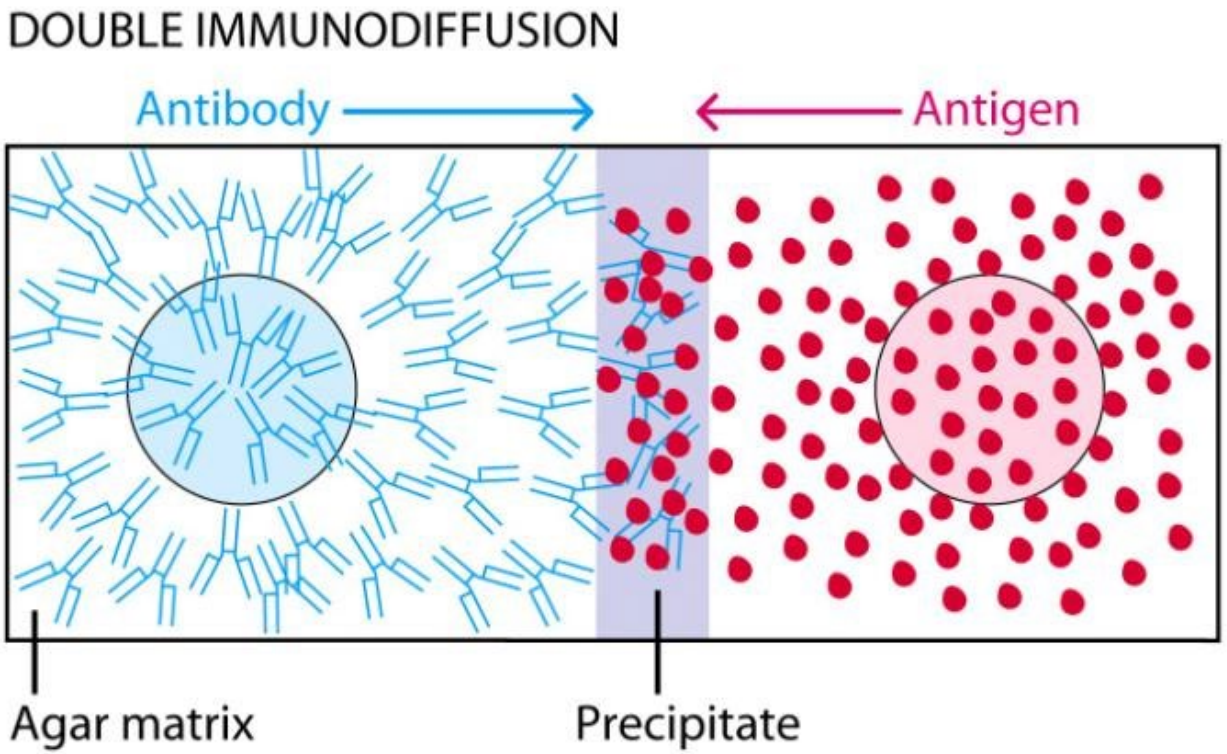


Antigen Recognition

Coccidioides Antibody Confirmation by Immunodiffusion

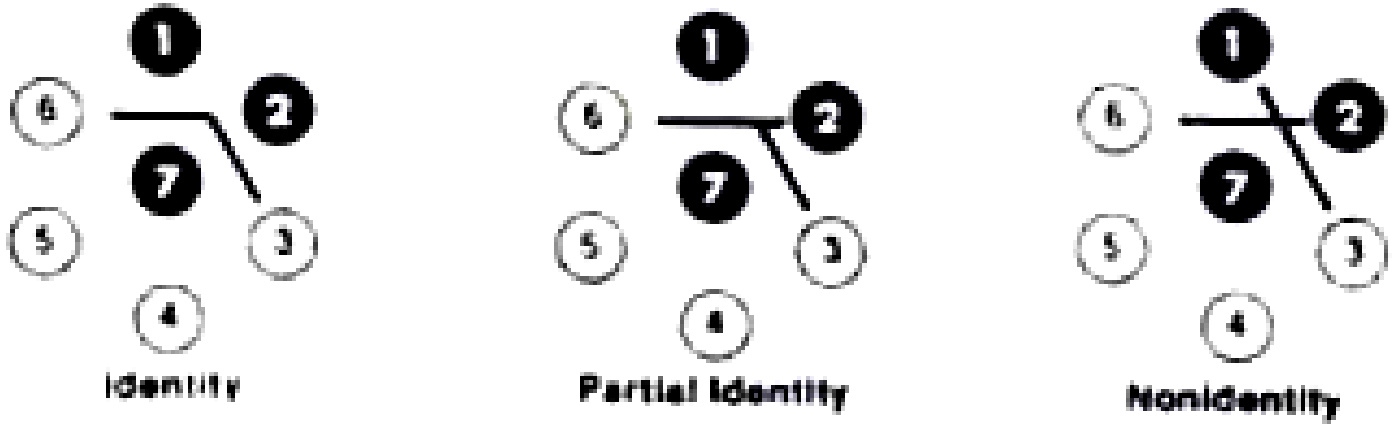
The immunodiffusion assay is based on the principle of double diffusion as described by Oudin and Ouchterlony.

An antibody and its homologous soluble antigen are placed in separate wells cut in an agarose diffusion medium and allowed to diffuse outward. Between the two wells, a concentration gradient of each of the reaction components is established ranging from antigen excess closest to the antibody well, to antibody excess closest to the antigen well.



http://www.sbs.utexas.edu/sanders/Bio347/Lectures/2006/Lecture%208%202006.htm

Coccidioides Antibody Confirmation by Immunodiffusion



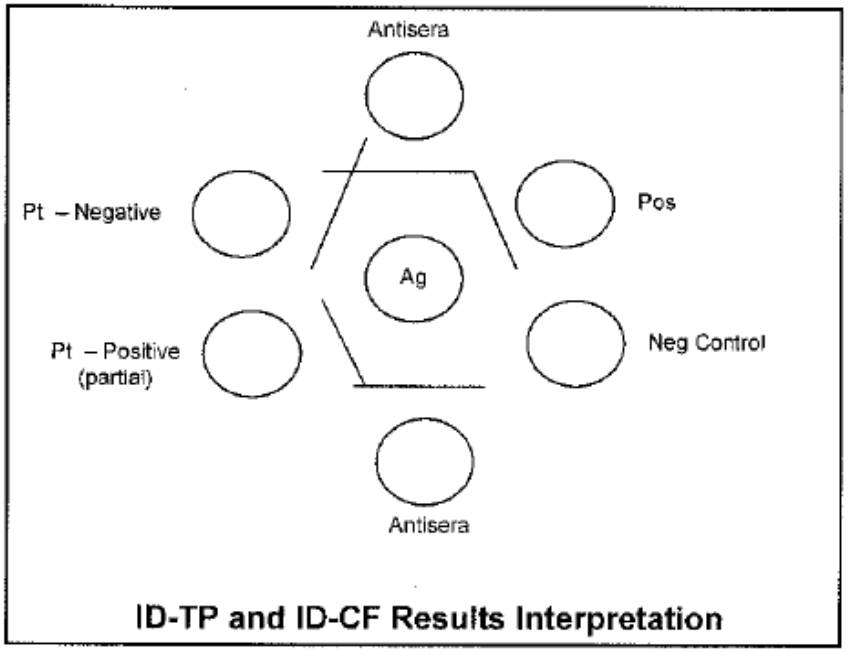
Results Interpretation: Well 1: Control positive serum
Well 2: Patient serum
Well 7: Antigen

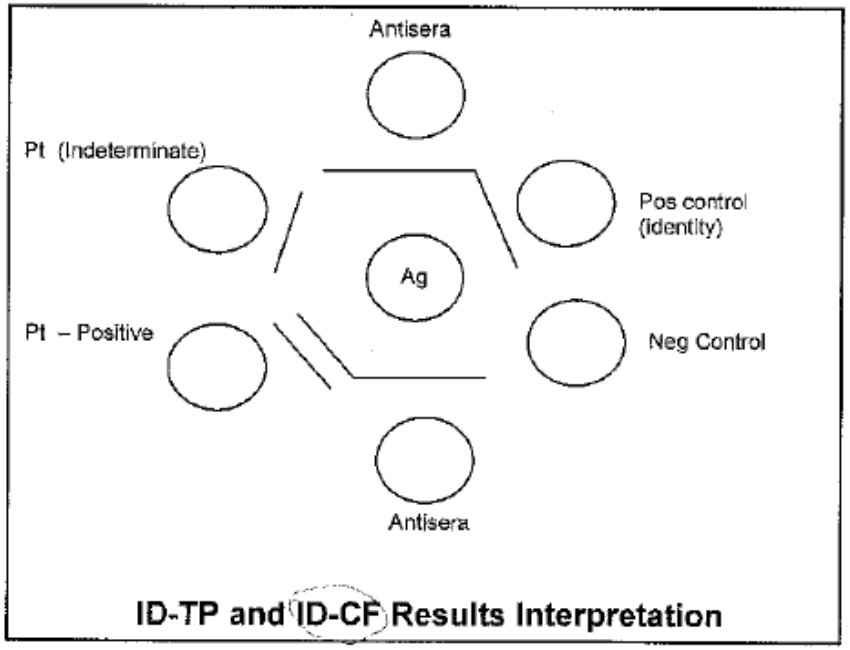
Identity, if the antigen antibody complexes are identical, the precipitin line form an unbroken line of identity with the known system.
Partial identity, reaction occurs when certain components of the antigens (or antibodies) are identical and other are not. The “spur” represents the components which are unrelated.
Nonidentity, occurs when antigen-antibody complexes are different. The resulting “X” or crossed reaction indicates that two unrelated complexes are present

Coccidioides Antibody Confirmation by Immunodiffusion

http://www.snv.jussieu.fr/bmedia/ATP/images/ouchb1.jpg

Coccidioides Antibody Confirmation by Immunodiffusion



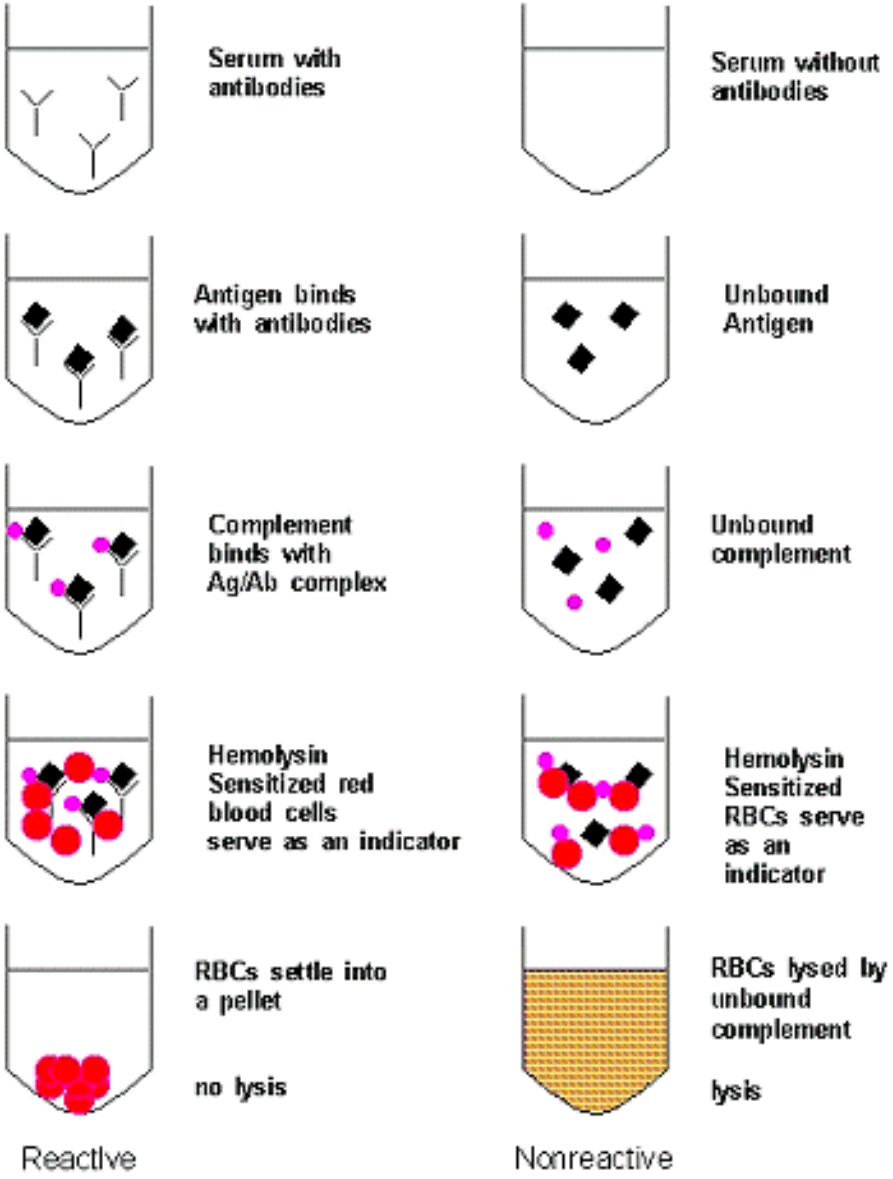


Positive – Identity or Partial Identity
Interpretation: Confirms the specificity of the EIA test
(ID = IgG, TP = IgM)

Negative – Non identity or no-line of precipitation seen
Interpretation – The EIA was a false positive, or the antibody level is too low for the test

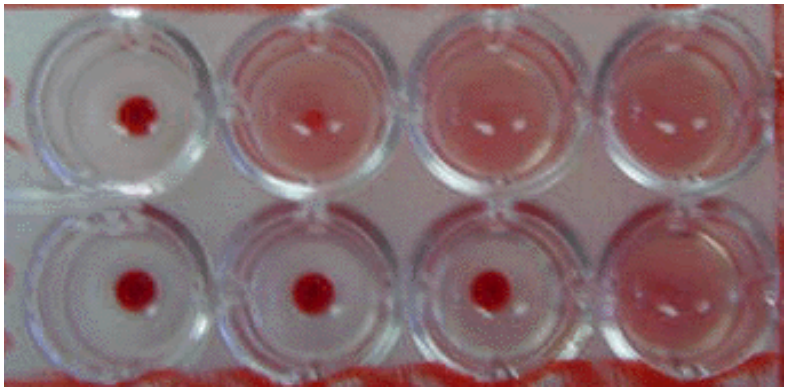
Indeterminate – No connection between the lines of precipitation
Interpretation – Unable to be determined

Semi-Quantitative Complement Fixation



Interpretive Data:

Any titer suggests past or current infection. However, greater than 30% of cases with chronic residual pulmonary disease have negative Complement Fixation (CF) tests. Titers of less than 1:32 (even as low as 1:2) may indicate past infection or self-limited disease; titers greater than or equal to 1:32 may indicate disseminated infection. Antibody in CSF is considered diagnostic for coccidioidal meningitis, although 10% of patients with coccidioidal meningitis will not have antibody in CSF.



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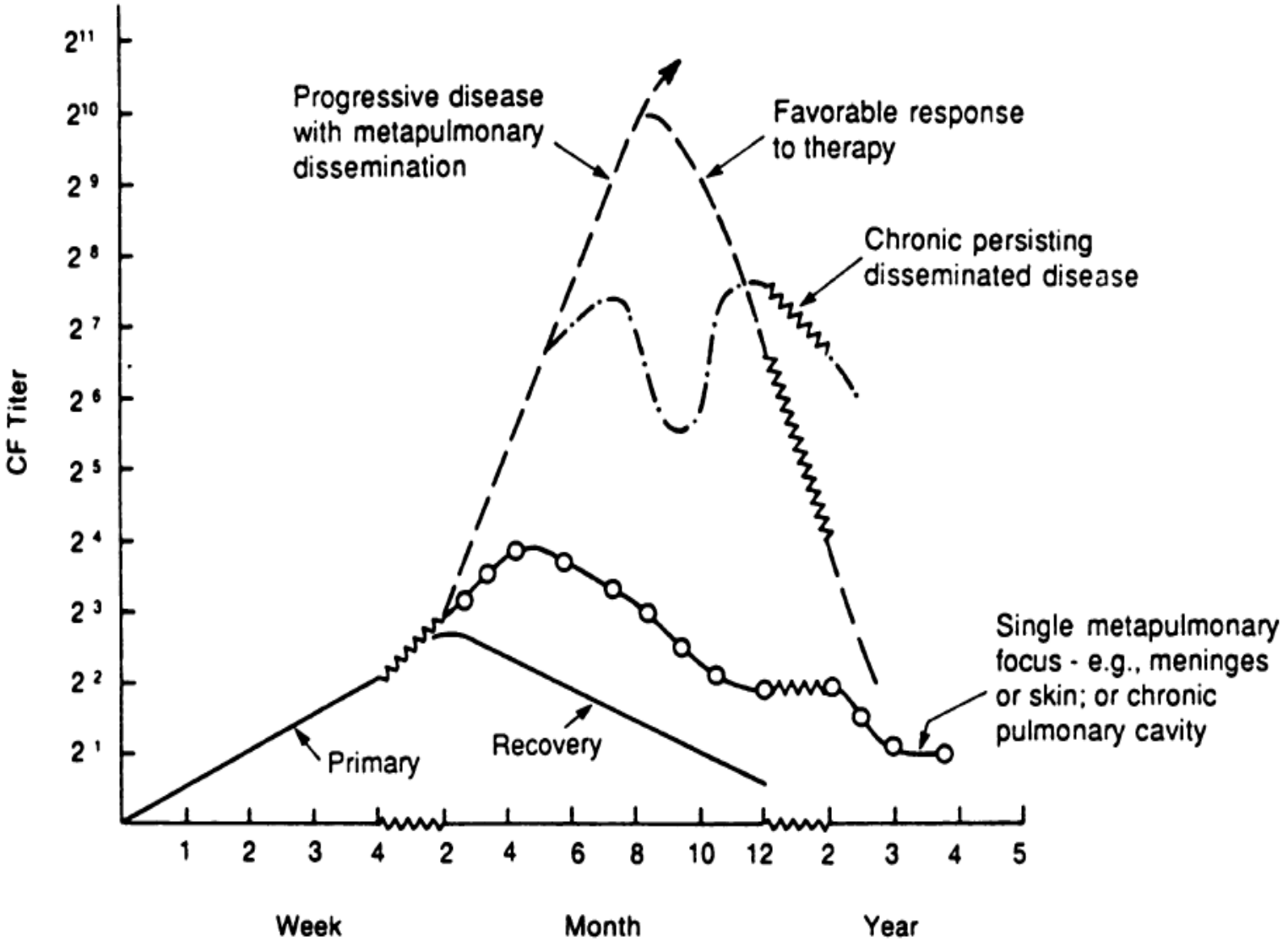
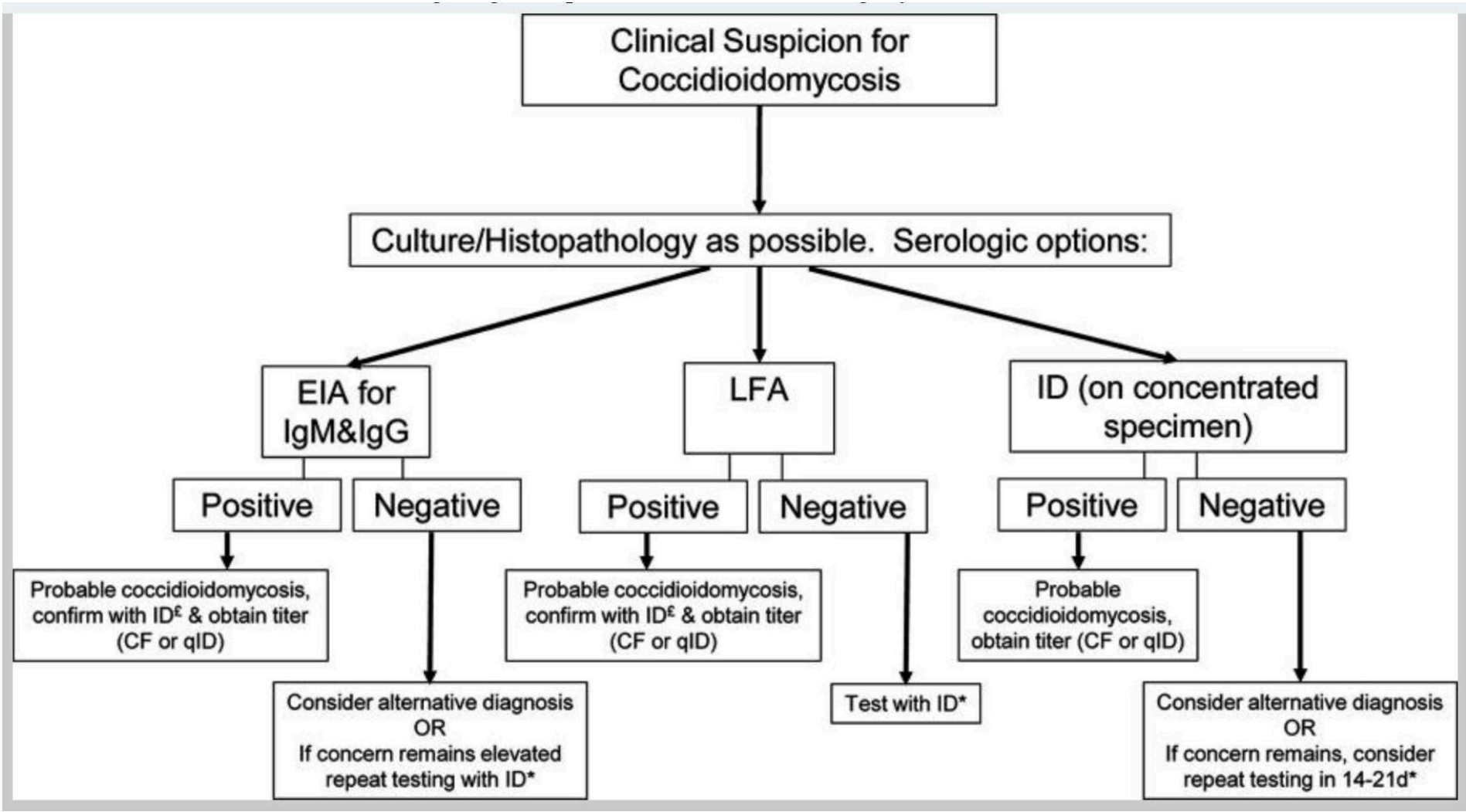


Recommendation for Serological Test Use



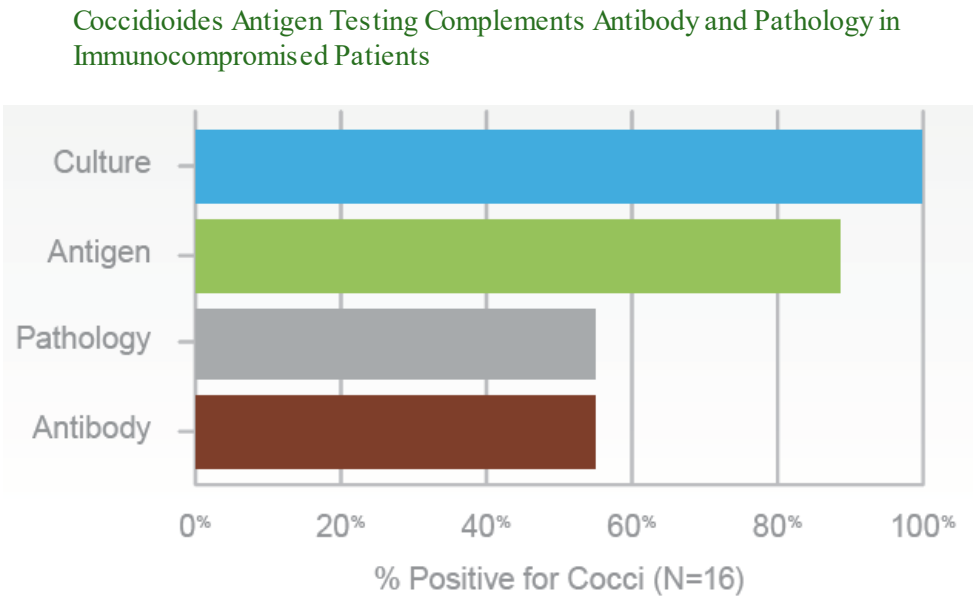
Cocci Antigen Testing (Reference Lab)

Testing available for urine, serum, BAL, CSF

Very few studies examining diagnostic impact

Several case reports and a case series show CSF antigen positivity in patients with cocci meningitis

Probably most useful in the immune-suppressed patient



1-3 Beta D Glucan shows poor sensitivity for cocci in serum
Has shown positivity in CSF for cocci meningitis patients

Diagnosing Invasive Infections: A Tale of Two Disciplines

Pathology

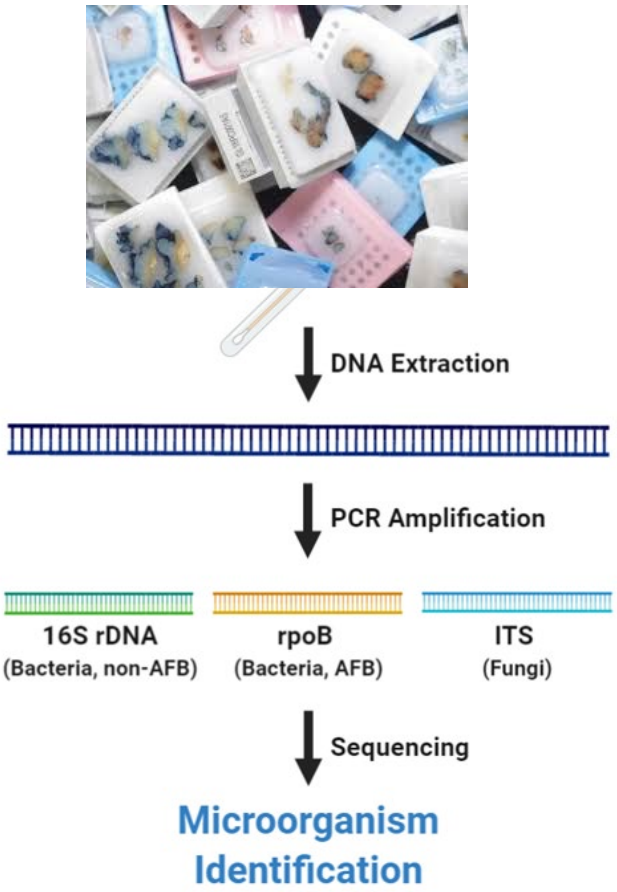
- FFPE slices positive for fungal stains (PAS, GMS)
- Morphological description

Microbiology

- Gram/fungal stain
- Culture

Organism Identification

Targeted Metagenomics

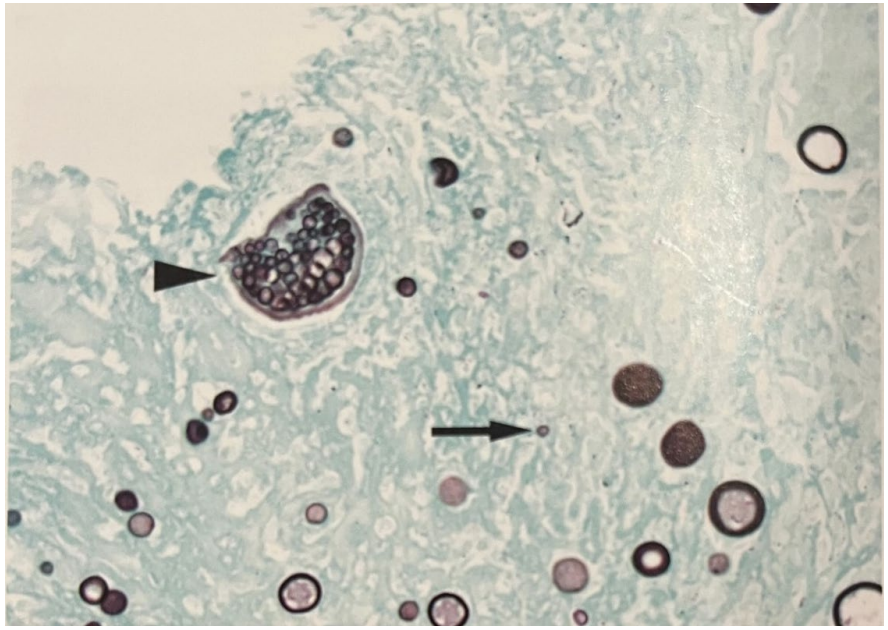


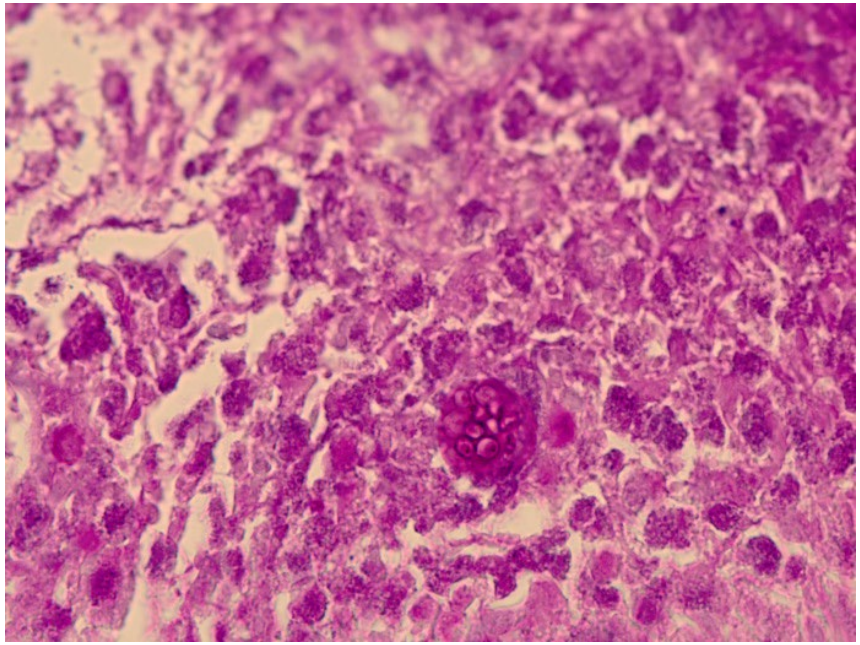
- **16S rDNA:** ribosomal RNA gene
  - Identification of bacteria (not AFB)
- **rpoB:** RNA Polymerase subunit beta gene
  - Identification of bacteria (including AFB)
- **ITS:** Internal Transcribed Spacer – region between 18S and 26S rDNA genes
  - Identification of fungi

Histopathology

Generally poor sensitivity (less sensitive than culture)
Specificity is high for spherules, low for endospores

Thick walled spherules (100um in diameter) containing endospores (2-4um).





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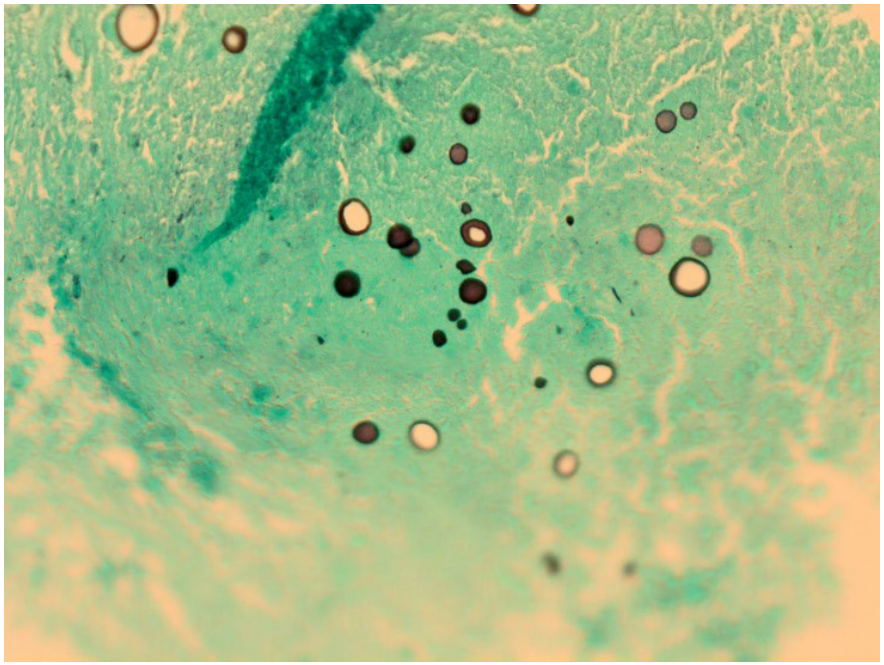
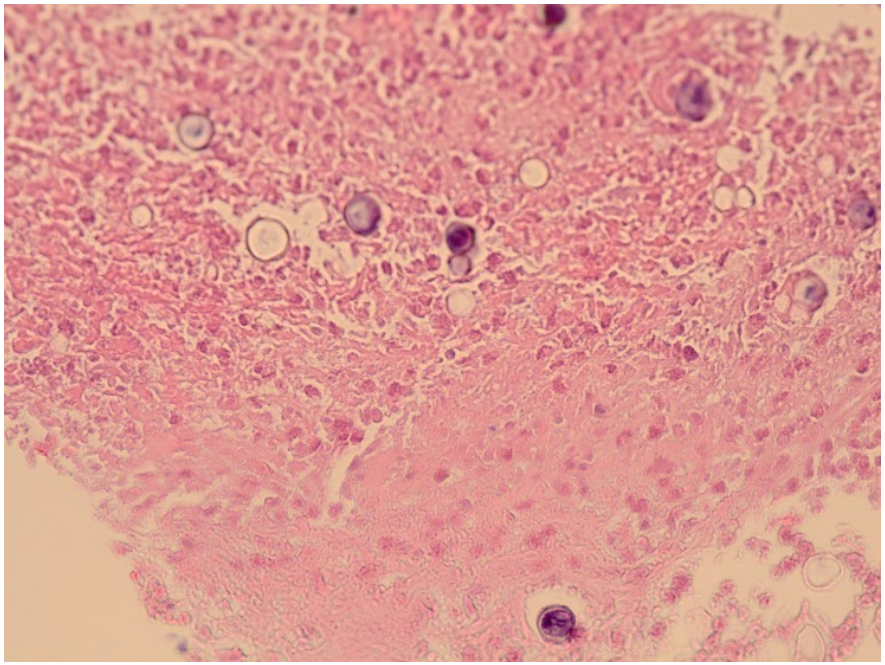




Histopathology

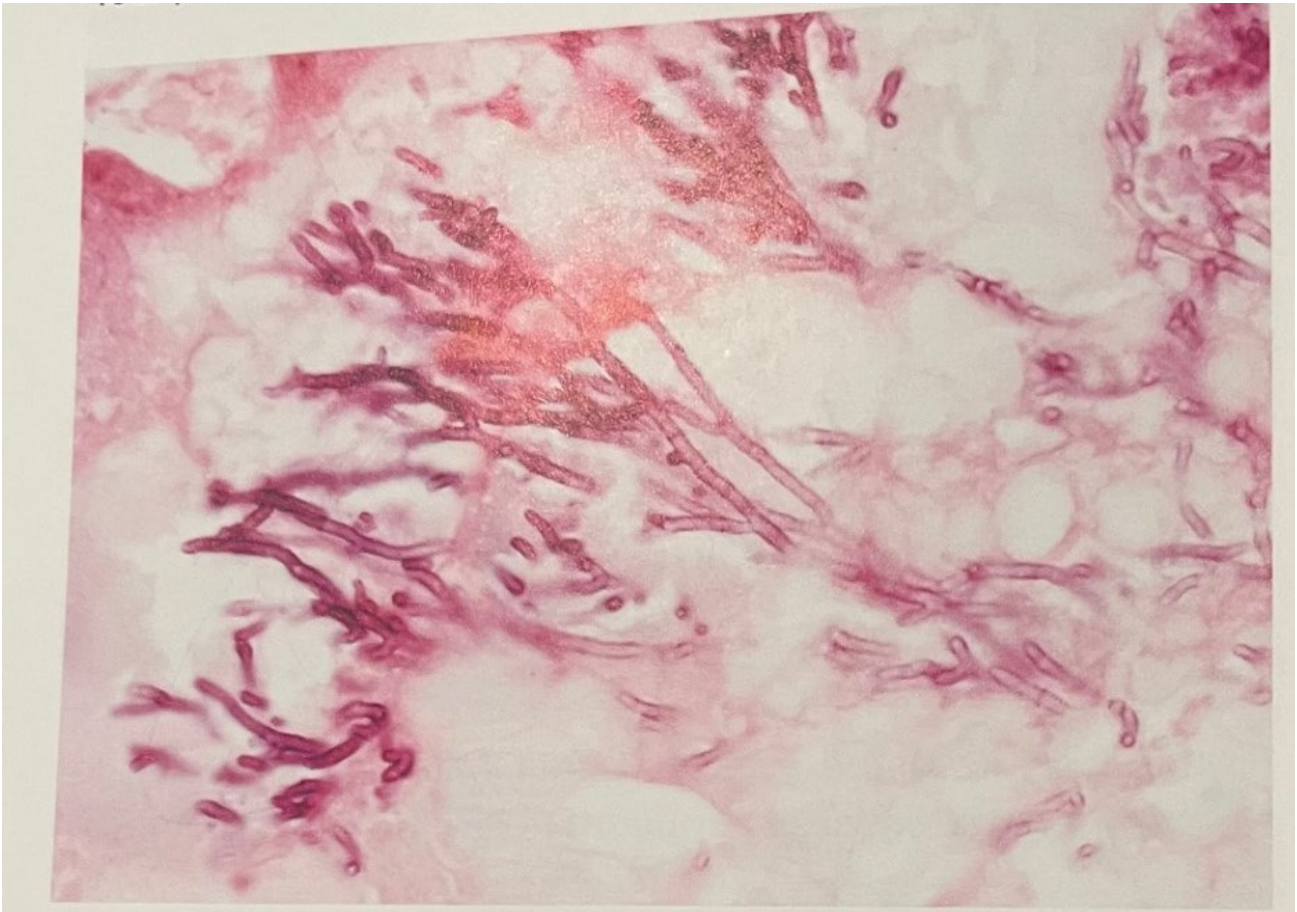
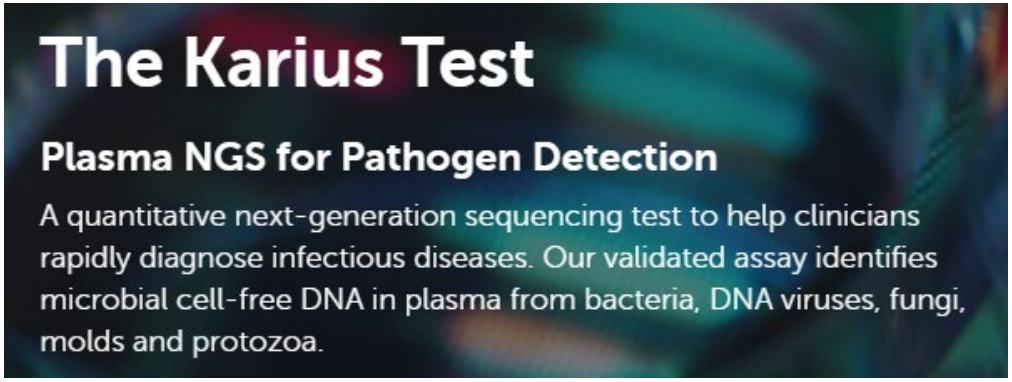
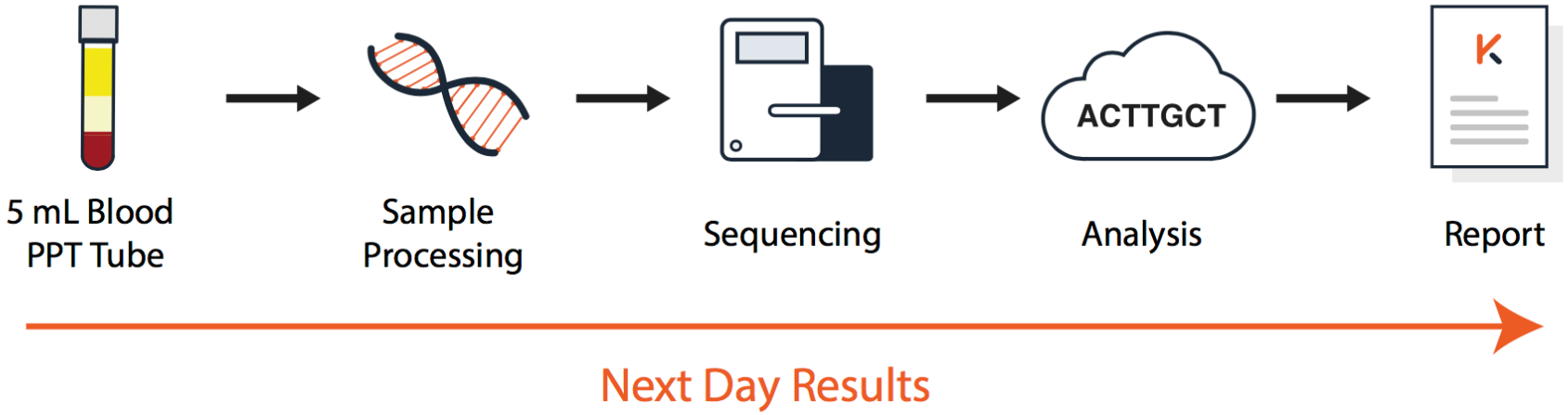


Figure 3.39. Although infrequently encountered, if *Coccidioides* reaches an airspace (eg, erosion through a bronchus), then hyaline septate hyphae, like those formed in culture, may be seen (H&E, 200X).

Karius Diagnostics
(NGS for Cell-free DNA in plasma)





Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nature Microbiology*

Patient characteristics (*n* = 348)	NGS positive	NGS negative	Agreement (%)	95% CI (%)
Positive by initial blood culture	59	4	93.7	84.5–98.2
Negative by initial blood culture	171	114	40.0	34.3–45.9
Positive by all microbiological testing	112	20	84.8	77.6–90.5
Negative by all microbiological testing	112	104	48.2	44.3–55.0
Positive by composite reference standard	169	13	92.9	88.1–96.1
Negative by composite reference standard	62	104	62.7	54.8–70.0

The composite reference standard includes the results from all microbiological tests (including the initial blood culture) performed within seven days of presentation and clinical adjudication. The NGS false negatives compared to initial blood culture included *Listeria monocytogenes*, coagulase-negative S. *aureus*, *Streptococcus agalactiae* and *Stenotrophomonas maltophilia* (this organism was not included in the NGS-test reportable range). NGS agreement with other methods was calculated as described in Supplementary Figures 7 and 8.

167 asymptomatic individuals tested. 22% were positive

Helicobacter pylori
Klebsiella pneumoniae
Haemophilus influenzae
Enterobacter cloacae complex
Aureobasidium pullulans
Bacteroides ovatus
Micrococcus lylae

Fusobacterium necrophorum
Agrobacterium tumefaciens
Pseudomonas putida
Escherichia coli
Streptococcus mitis
Rothia mucilaginosa
Pseudomonas fluorescens
Gemella haemolysans

Staphylococcus aureus
Corynebacterium kroppenstedtii
Aeromonas caviae
Pseudomonas oryzihabitans
Human herpesvirus 4
Acinetobacter radioresistens
Debaryomyces hansenii
Lactobacillus plantarum
Neisseria gonorrhoeae

Clinical Infectious Diseases

MAJOR ARTICLE

Clinical Impact of Metagenomic Next-Generation Sequencing of Plasma Cell-Free DNA for the Diagnosis of Infectious Diseases: A Multicenter Retrospective Cohort Study

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Retrospective multicenter study of clinical impact of Karius results on 53 immune compromised patients

Positivity rate was 61%.
50% of positives had more than 1 organism

Karius result had:
Positive impact on care in 7% of cases
Negative impact on care in 4% of cases
No impact in 87% of cases

The most impacted patients: Neutropenic children with invasive mucor infections

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MINIREVIEW

Review of Clinical and Laboratory Diagnostics for Coccidioidomycosis

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