

New cryptococcal and histoplasmosis tests

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DISCLOSURES

I have financial interest/arrangement
or affiliation with

Name of Organization

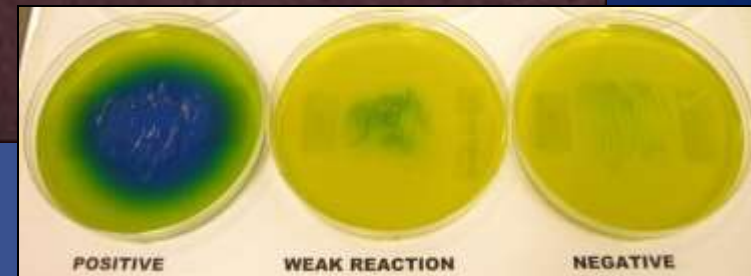
Relationship

Pfizer, Merck, Astellas
Basilea, Astellas

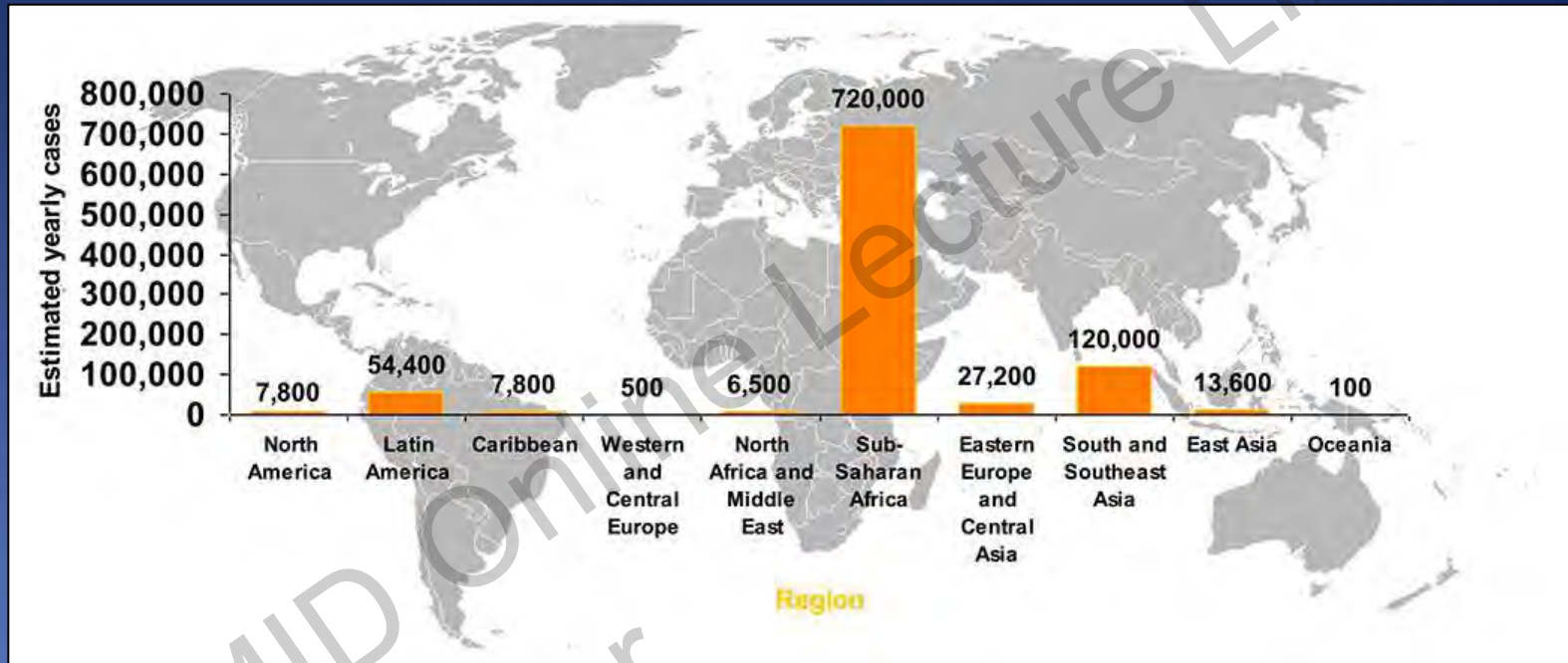
Research Support
Consultant

Cryptococcus: current diagnostics

- CSF India Ink preparation
~80% sensitive
- Culture – Standard media
 - CGB media + *C. gattii*
-- *C. neoformans*
- Cryptococcal antigen testing
(CrAg) >90% sensitive
 - Clear differences between developing world and elsewhere in type of test and utility



Cryptococcus: Epi and mortality



- 25 million in Sub-Saharan Africa with HIV
 - 4-12% infected with *Cryptococcus*
 - Responsible for 50% of HIV-related mortality
 - **0.5 - 1 million deaths annually** (TB ~ 350,000)

CrAg testing: current methods

CALAS – Overview

Stability/Storage

- 18-month shelf life
 - Refrigerated storage
 - Aliquoted pronase stored separately in freezer
 - Centrifugation step
-
- Subjective results



Positive

Negative

1+

2+

3+

4+

Time: 70 minutes

CrAg testing: current methods

ELISA based methods

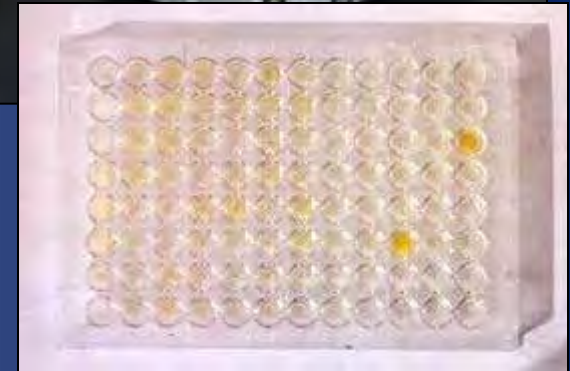
- Well established methods
- Automated
 - Although can be read visually...
- Objective interpretation

Time: 50 minutes

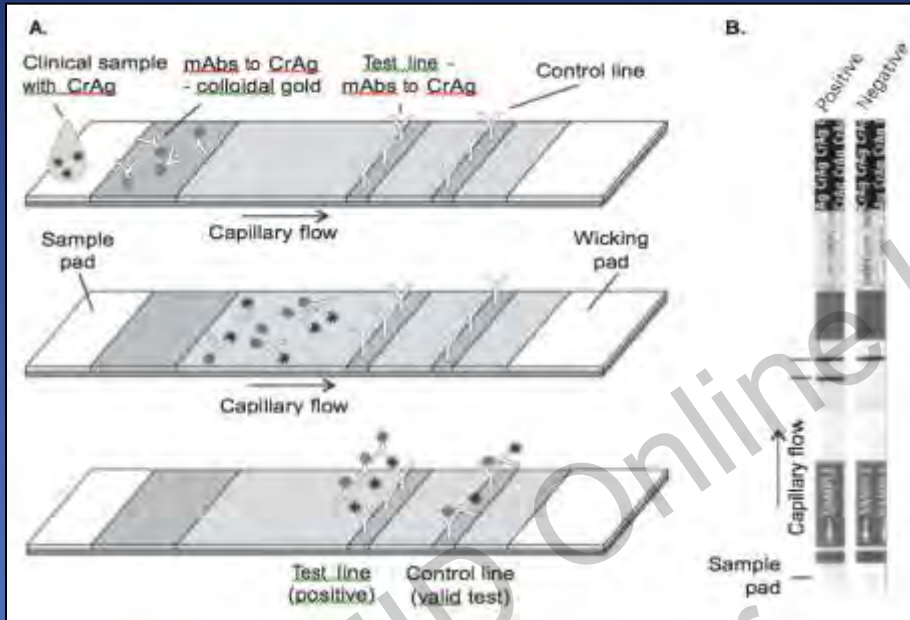
Does it really matter which method?

C. gattii – false negative CALAS

Very low titers – ELISA superior



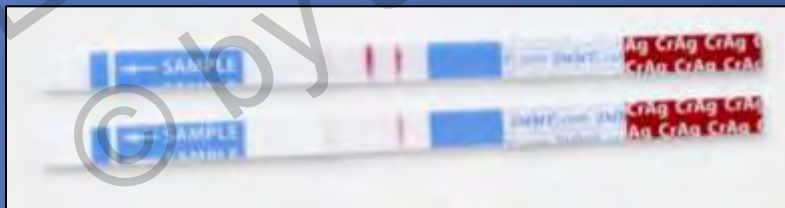
CrAg testing: new method - LFA



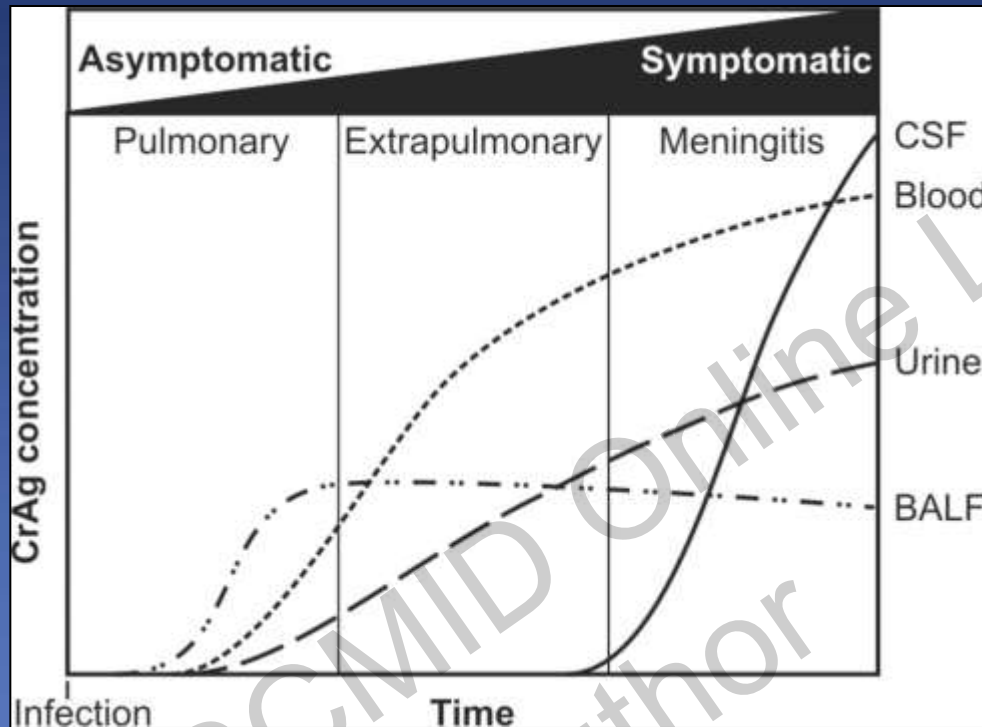
- Stored at room temperature
- No specimen pretreatment required - 40 μ L required
- **Results in 10 minutes**
- Qualitative and semi-quantitative
- Serum or CSF
- **Improved sensitivity serotype C**

Objective results

- 2 lines = positive
- 1 line = negative



Cryptococcus: asymptomatic screening



- Antigenemia precedes symptomatic disease
- CRAG positivity precedes symptoms of CM by median of 22 days (range, 5-234)
- Treatment during this time significantly improves mortality

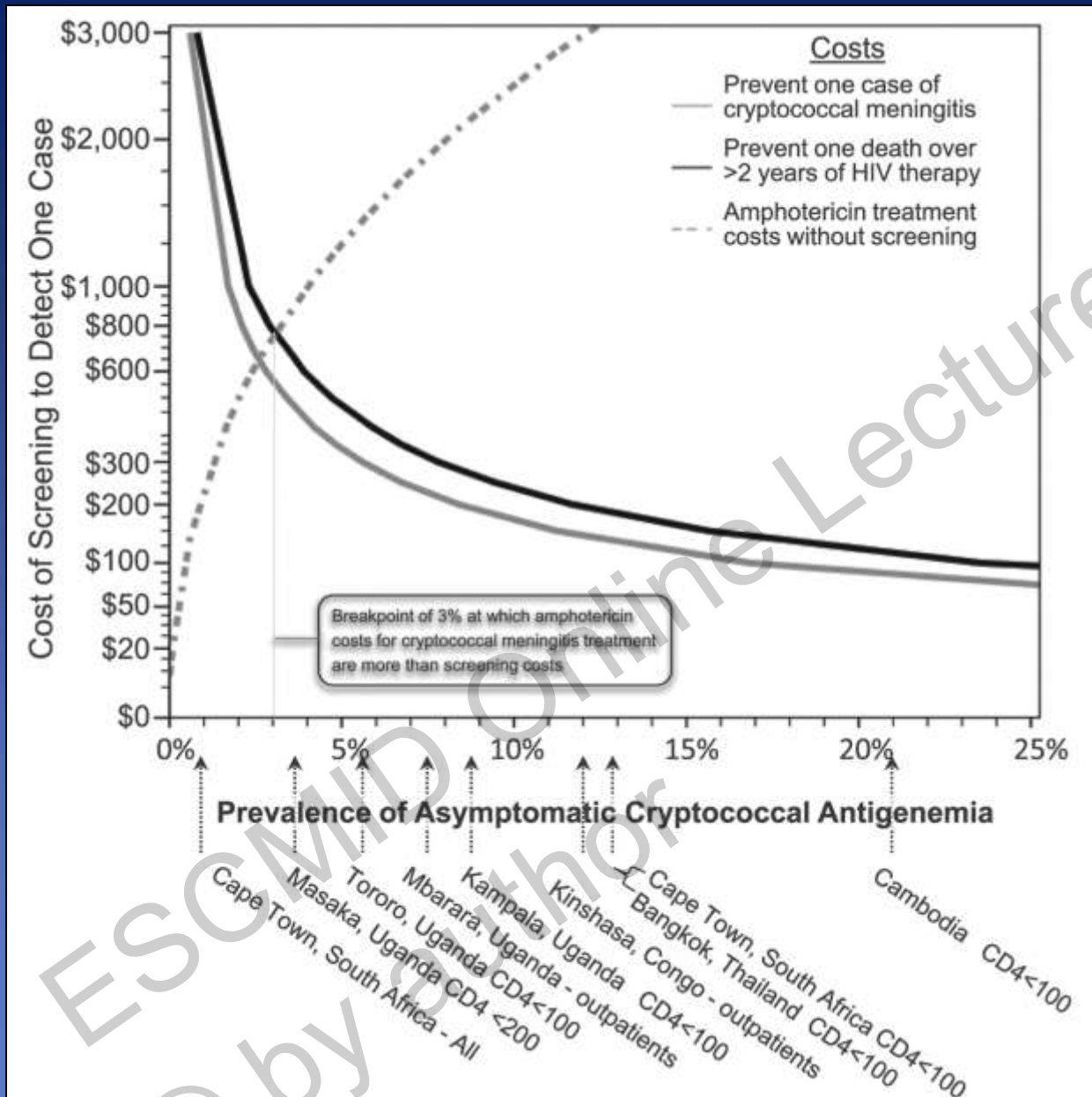
Kozel TR, *Expert Opin Med Diagn.* 2012 May;6(3):245-51.

French N, et al. *AIDS* 2002, 16:1031-38.

Case:

Diagnosis of Cryptococcus

- 22 y/o male seen in western Kenya is diagnosed with HIV.
- His blood is taken on clinic intake for routine testing and CD4 cell count, and reflex testing for CrAg if $CD4 < 100$.
- Reflex CrAg testing can be done on same sample sent for CD4 testing.

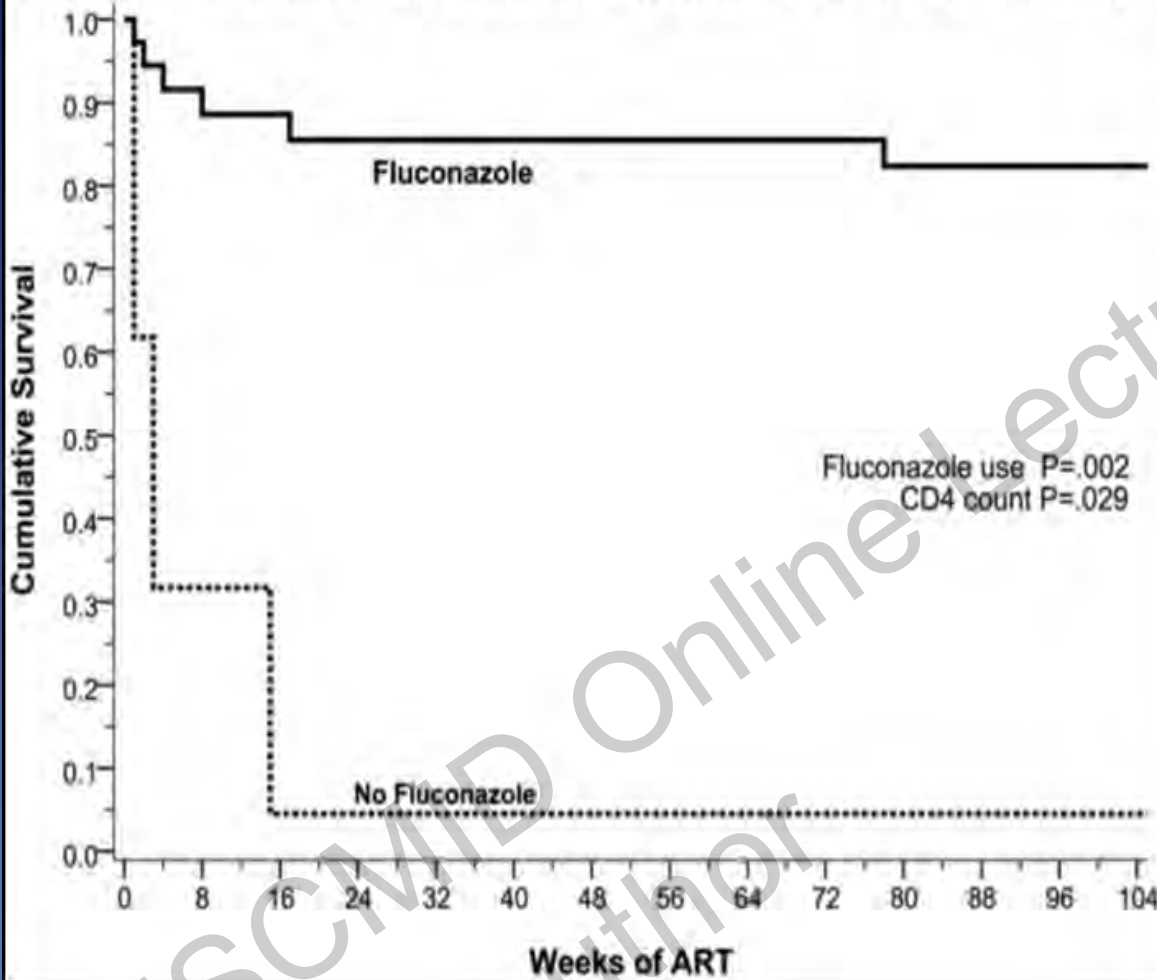


- Cost of screening varies
- Geographic differences in incidence
- 3% incidence screening < 14 days amphotericin

Cost Effective?

- Testing, medication, etc
- Using screening:
 - Incidence of 8%
 - 1 yr of fluconazole
 - \$21US/year of life gained
 - NNS to detect CrAg+ = 11
 - NNS to prevent death CM = 16

Survival in Persons with Asymptomatic Cryptococcal Antigenemia (CRAG+)



Jarvis JN et al. *J Int Assoc Physicians AIDS Care*. 2012;11(6):374-9.
Govender NP et al. *S Afr Med J*. 2012; 102(12):914-7.
Meya DB, et al. *Clin Infect Dis*. 2010 Aug 15;51(4):448-55.
Smith RM, et al. *PLoS One*. 2013;8(4):e62213

Compare NNS:
Colon CA: 1000
Breast CA: 543

Newer techniques

- **MALDI-TOF:** requires culture positivity
 - Can determine common species (not just crypto)
 - Expensive startup ~ \$160,000 to \$250,000
 - yearly maintenance costs roughly \$35,000 a year.
 - ~ \$0.10–\$0.50 reagent cost per sample
- **PCR:** useful primarily for epidemiology/typing
 - Little utility in clinical diagnostics
 - Whole genome sequencing

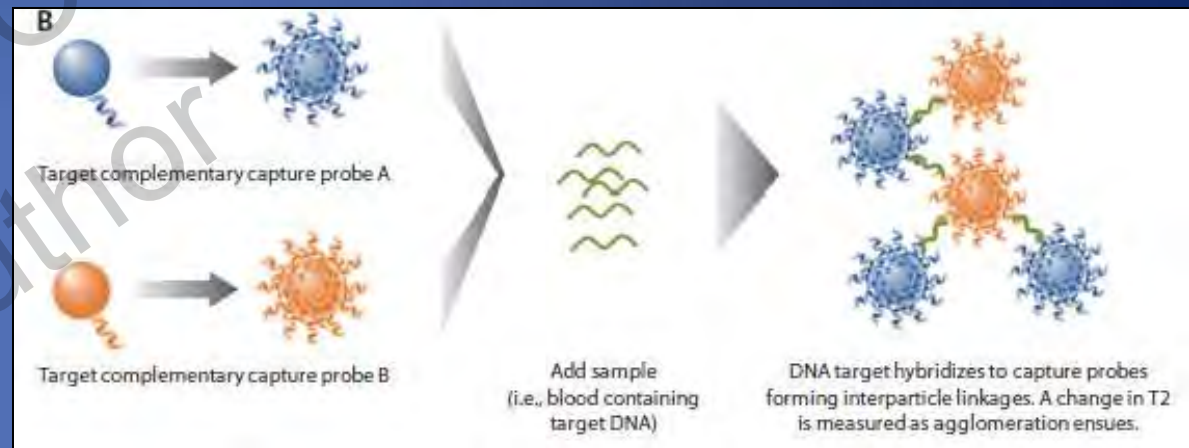
Fungemia diagnostics: the near future?

T2 Biosystems:

- Direct detection in clinical samples (blood, urine, CSF)
- Sensitivity = (1 CFU/mL)
- Cost?
- Adaptability?



Magnetic biosensor technology



Cryptococcus diagnostics/management: the future?

Non-invasive ICP assessment?:

Patients frequently refuse LP after diagnosis!

- Helpful to have non-invasive ICP assessment?

Multiple current methods – none 100%

- CT and MRI – high specificity, low sens
- Transcranial Doppler US – flow velocity
- Optic Nerve Sheath Diameter - \$\$\$ and expertise
- EEG
- Tympanic membrane displacement

Summary: new cryptococcal tests

Past: India Ink, culture, biopsy

Present: Latex agglutination/ELISA →
lateral flow assay

Future: T2 or similar systems using low volumes and direct patient samples

- Non-invasive methodologies for management/treatment

Histoplasmosis

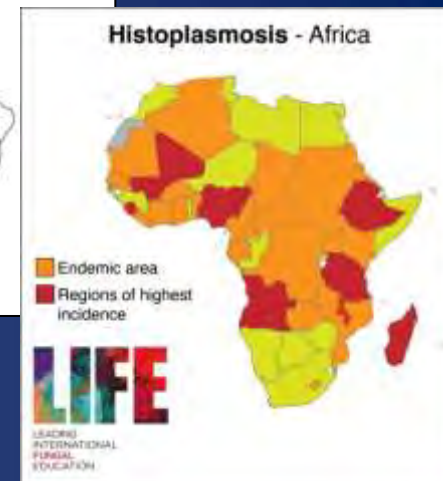
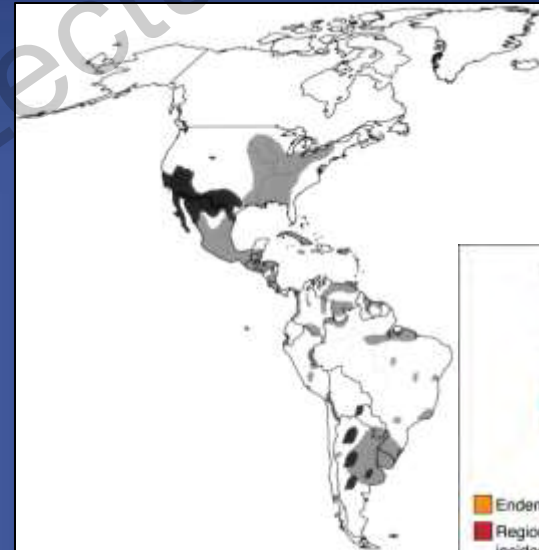
H. capsulatum - U.S., Central and S. America

H. duboisii - Africa

- Sporadic cases in Europe
SE Asia
Autochthonous cases
- Epidemiology studies ongoing

Estimated 500,000
cases/year

- Most are subclinical
- Skin-test positivity
- Small calcifications on CXR
- Coin nodules



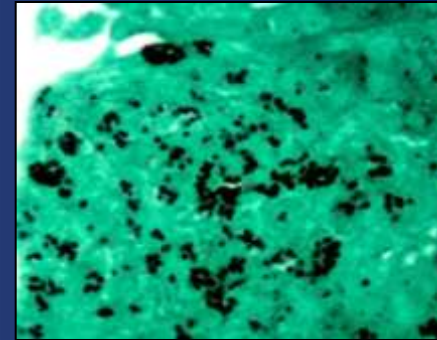
Histoplasma: Current diagnostics

Culture: Definitive, yet not always feasible

- Sputum: 15% in acute, 60-85% in chronic pulmonary
- Blood Culture: 50-70% with disseminated disease

Histopathology:

- Biopsy - Peripheral Smear (Buffy Coat)
- **Elevated LDH**
 - Helpful in disseminated disease
 - Diagnosis not made until (mean) 21.2 ± 2.6 days after admission



Histoplasma: Current diagnostics

Serology: helpful in subacute/chronic disease

- ID (M and H Bands)
- CF (Mycelia and Yeast)
- ELISA

Urinary and serum antigen:

- Sens/Spec: Varies by type of disease
- Very high sensitivity with disseminated disease
- Send out lab until recently – **newly available EIA kit**

PCR: limited sensitivity (7%)

- Helpful on tissue?
- Ocular histoplasmosis syndrome?
- Role in CSF?

Case:

Diagnosis of histoplasmosis

- 52-year-old female with ulcerative colitis maintained on etanercept presented in June of 2012 with cough, chest pain, and fatigue.
- “Spending most of day in bed”. Seen by her PCP and prescribed azithromycin - minimal improvement - followed by course of moxifloxacin for 10 days

Social History

- Lives in rural Missouri, stay at home mom

Exam: diffuse rhonchi throughout, no cutaneous or oral lesions

Case:

Diagnosis of histoplasmosis

Labs return 5 days later:

- **Histoplasma serum antibody:**

Histo Yeast Ab by CF: **1:128**

Histo Mycelia Ab by CF: **1:32**

- **Histoplasma urine antigen:**

Negative: **0.4 ng/mL (positive >0.6)**

Histoplasmosis: diagnosis

Antigen Sensitivity by disease type:

- Dissemination ~ 90%
- Acute ~83%
- Subacute ~30%
- Chronic pulmonary ~87%
- **Ab + Ag testing = 93% of patients positive**
- **Could disease have been anticipated in this patient?**
 - Latent TB screening required prior to immunosuppression...

Histoplasmosis: screening

Skin testing:

- Past studies have shown skin test positivity of 80% in Midwest U.S.
 - Histoplasmin not *currently* commercially available

IGRA:

- Development of Elispot assay, initial results suggestive of concept

Summary: new *Histoplasma* tests

Past: Culture, histopathology

Present: **New Ag Kit, Serum and urine antigen testing AND serology**

No role for PCR

Future: Screening for those from endemic area, anticipated immunosuppression

Conclusions

Advances in *Cryptococcus*:

- LFA: \$2 in resource limited areas, rapid
 - **Cost-effective screening/treatment**
- Improved sensitivity for *C. gattii*

Advances in Histoplasmosis:

- **New Antigen kit**, send out lab may be unnecessary
- **Use both Antigen and Antibody testing**
- IGRA test may soon be available