27th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES LIVE

The Mucormycosis Workshop: Diagnosis from Bench to Bedside and Back Again

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Objectives

- To understand the importance of early diagnosis of mucormycosis
- To discuss the early diagnostic clinical and radiological manifestations of mucormycosis
- To review conventional microbiological and mycological methods for laboratory diagnosis
- To delineate new molecular and antigenic technologies for rapid diagnosis of mucormycosis

Why is Early Diagnosis of Mucormycosis Important?

- **Hypothesis:**
- Early intervention may **reduce or prevent**
  - Angioinvasion
  - Direct tissue injury of lung, brain, and sinuses
  - Extension into critical sites: eyes, brain, great vessels
  - Progression to dissemination
- Reduce need for or extent of surgical resection
- Reduce disfiguring surgery
- Reduce suffering (invasion of sensory nerve fibers)
- Improve outcome and survival

How Can One Improve Diagnosis of Mucormycosis?

- Recognition of Host Factors
- Assessment of Clinical Manifestations
- Use of Imaging Modalities
- Expertise in Histology/Cytology
- Enhancement of Microbiology Methods
- Advances in Molecular, Proteomic, Metabolic, and Antigen Detection

How Can Early Recognition of the Clinical Manifestations of Mucormycosis Improve Diagnosis?

(Petrikkos et al, Clin Infect Dis. 54 Suppl 1:S23-34; 2012)

- **There are no specific complaints by history**
- **Examples of initial clinical manifestations of Zygomycosis:**
- **<u>Diplopia in diabetic patient</u>**

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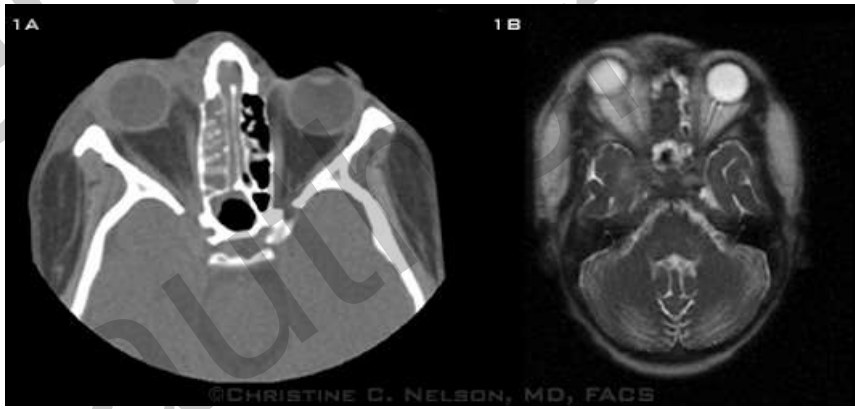

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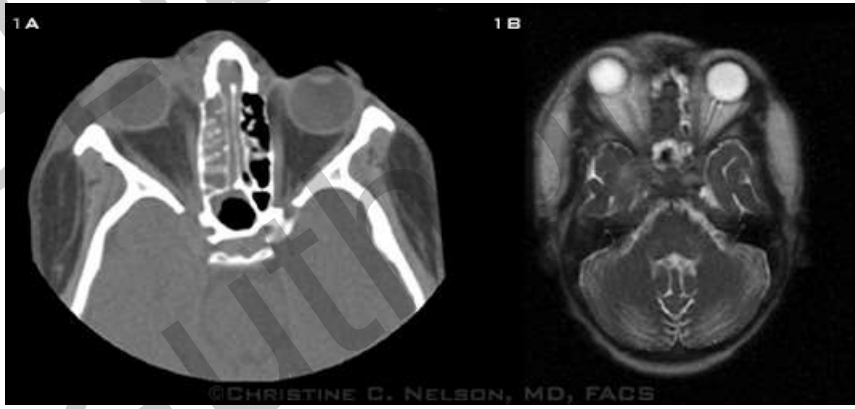
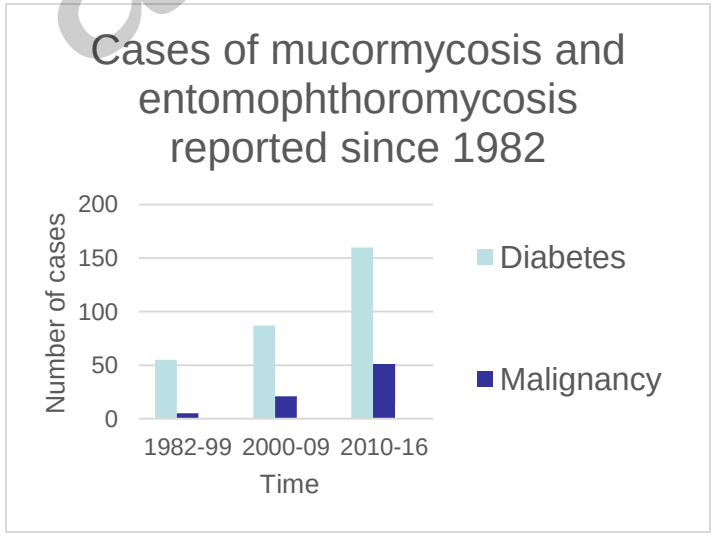
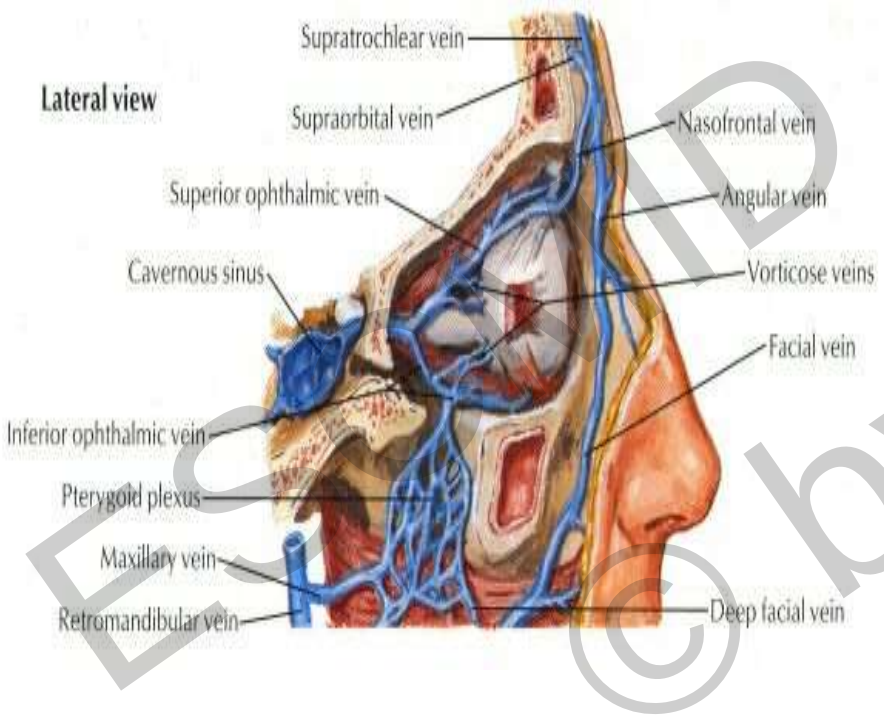
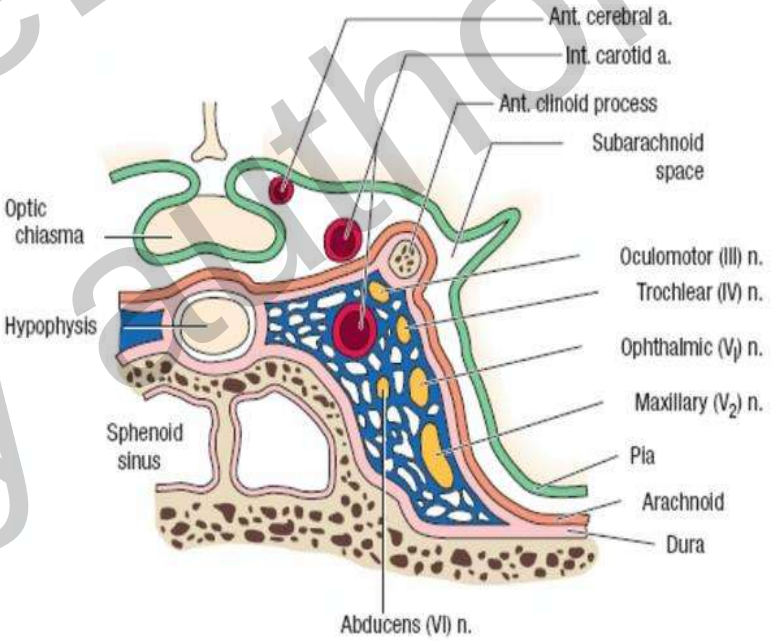


DIABETES MELLITUS AS THE MAJOR RISK FACTOR FOR MUCORMYCOSIS IN MEXICO: EPIDEMIOLOGY, DIAGNOSIS, AND OUTCOMES OF REPORTED CASES
Corzo-Leon et al Med Mycol (in press)



Orbital and Cavernous Sinus Anatomy





Diabetic Mucormycosis

How Can Early Recognition of the Clinical Manifestations of Mucormycosis Improve Diagnosis?

- There are no specific complaints by history
- Examples of initial clinical manifestations of Zygomycosis:
- Diplopia in diabetic patient
- Necrotic eschar in maxillofacial and sino-orbital tissues

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- Pleuritic pain in a neutropenic host
- Necrotic cutaneous lesion in immunocompromised patient

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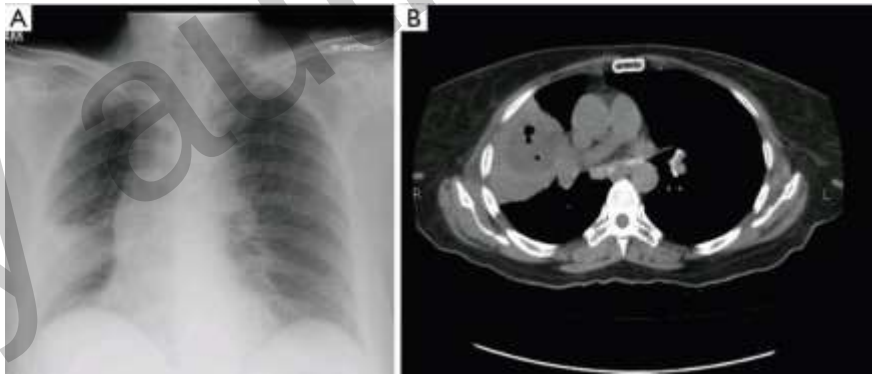


How May Diagnostic Imaging Facilitate Early Diagnosis?

- **Earlier detection of lesions than that of conventional CXR**
  - Presence of lesions already signifies thrombosis, infarction and tissue injury
- **Achieved with serial screening in high risk patients**
- **Limitations:**
  - Expense
  - Routine chest CT scanning does not account for paranasal sinuses
  - Non-specificity of signs
  - Cumulative radiation exposure of repeated CT scans, esp.. in children (580 mrem/chest CT)

Is Early Diagnosis Beneficial in Mucormycosis?

- Chamilos *et al* of MDACC analyzed the impact of delaying effective amphotericin B-based therapy on outcome among 70 consecutive patients with hematologic malignancy and Mucormycosis
- Delayed therapy resulted in a 2-fold increase in 12 week mortality rate compared with early treatment (82.9% vs. 48.6%)
- Delayed treatment was an independent predictor of poor outcome in multivariate analysis (OR, 8.1; 95% CI, 1.7-38.2; P = .008).

How Early Are CT Scans Able to Detect Lesions?

- **Detection of classical lesions**
  - **nodules,**
  - **halo signs,**
  - **wedge-shaped infiltrates**
  - **pleural effusions**
- **Already signifies tissue injury: hemorrhage, necrosis and edema**

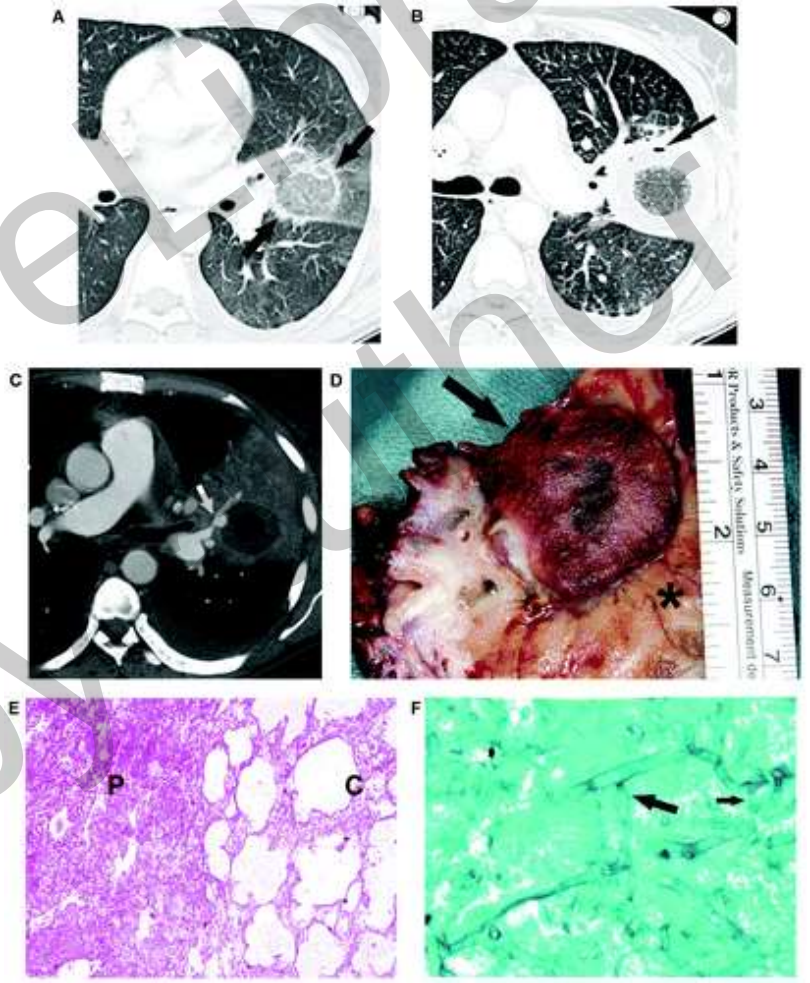
What are the Possible Clinical and Radiological Differences between Pulmonary Mucormycosis and Aspergillosis?

(Chamilos et al. Clin Infect Dis. 2005; 41:60-6)

- **Retrospectively reviewed the clinical and radiological characteristics at MDACC**
  - **16 patients pulmonary mucormycosis (IPM) at**
  - **29 contemporaneous patients with IPA**
- **Hematological malignancies: IPM (15/16) vs IPA (28/29)**
- **Independent predictors of IPM by logistic regression analysis:**
  - **concomitant sinusitis (OR, 25.7)**
  - **voriconazole prophylaxis (OR, 7.76)**
  - **multiple (10) nodules (OR, 19.8);**
  - **pleural effusion (OR, 5.07)**

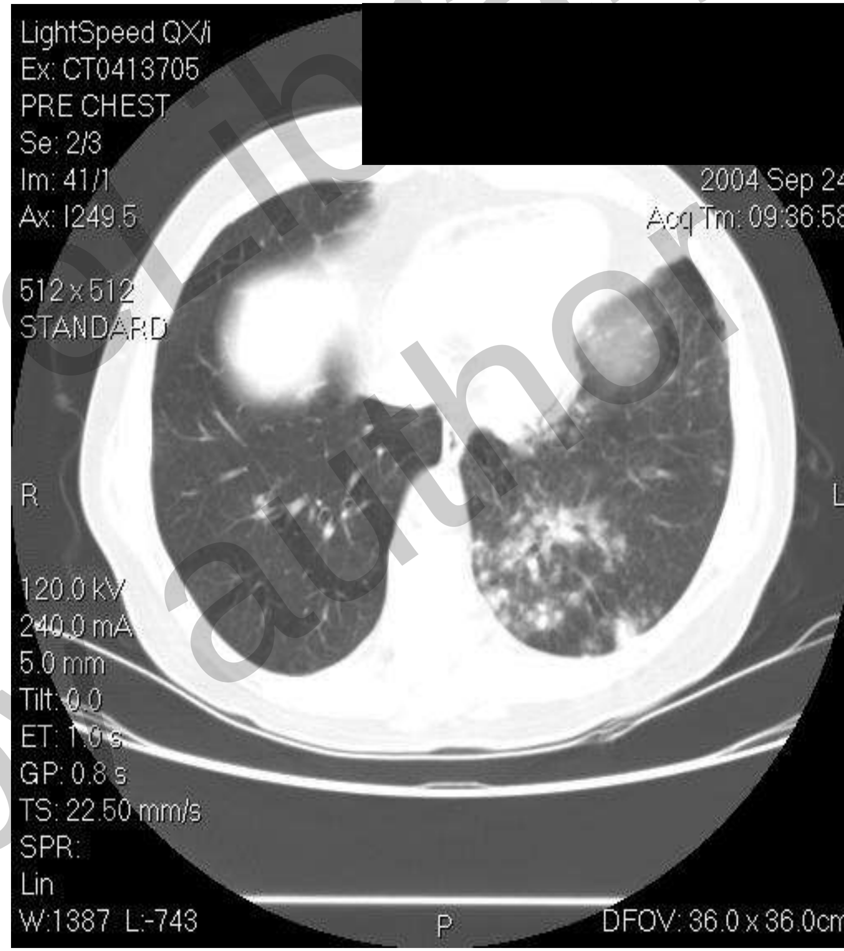
Reversed Halo Sign and Pulmonary Mucormycosis

- 189 patients with proven and probable fungal pneumonia:
  - 132 IPA
  - 37 mucormycosis
  - 20 fusariosis.
- RHS seen in 8 patients (4%)
  - 7 pulmonary mucormycosis
  - 1 IPA



Wahba et al. Clin Infect Dis. 2008; 46:1733-7

Lesions of Pulmonary Mucormycosis Vary over Time



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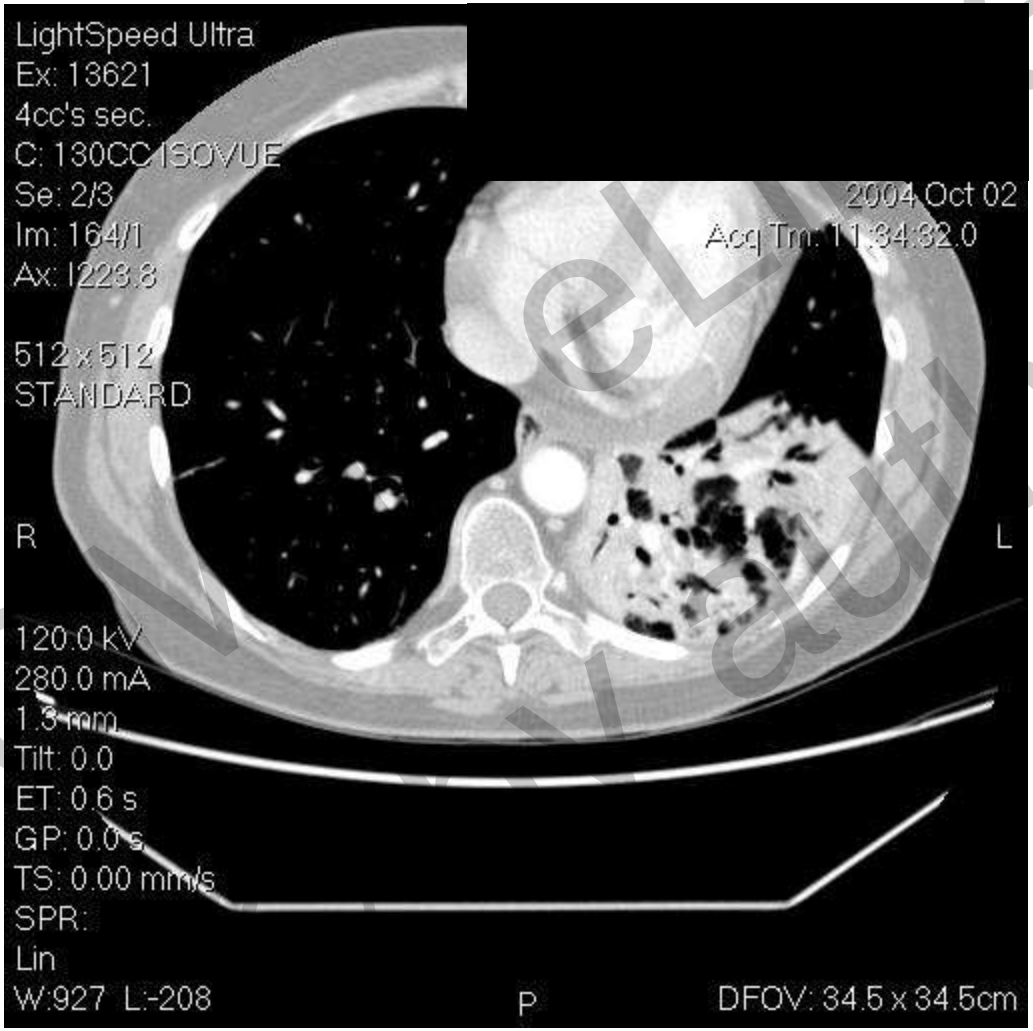

Radiopathological Correlation

- **Persistently neutropenic rabbit models of invasive pulmonary mucormycosis**
  - ***Rhizopus oryzae***
  - ***Rhizopus microsporus***
  - ***Mucor circinelloides***
  - ***Cunninghamella bertholletiae***
- **Serial CT scan imaging and histopathological analysis**

Radiopathological Correlation

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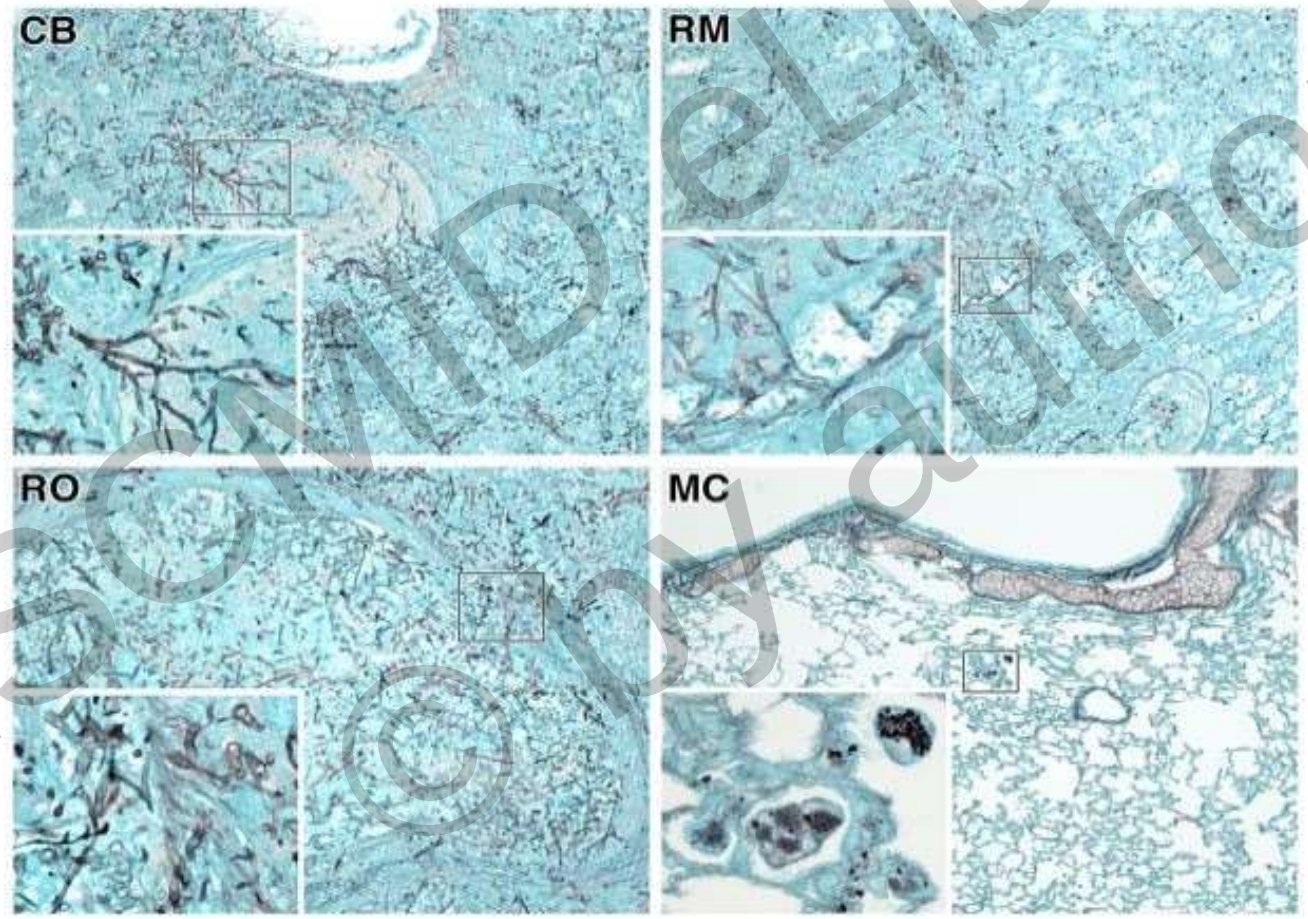
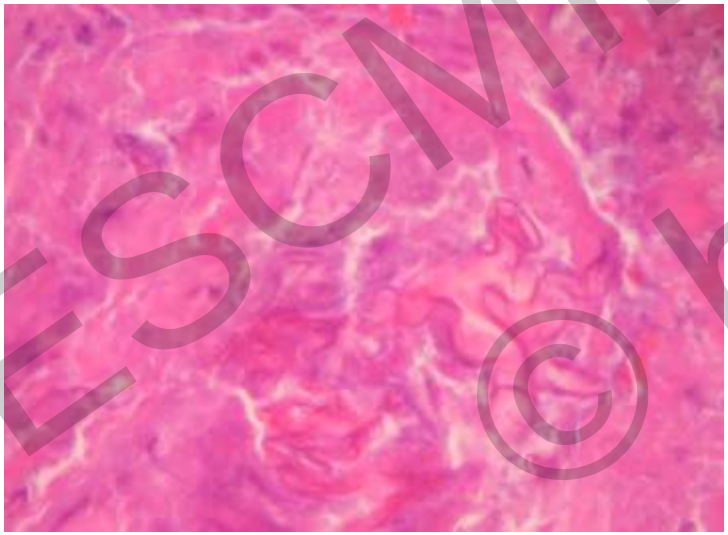
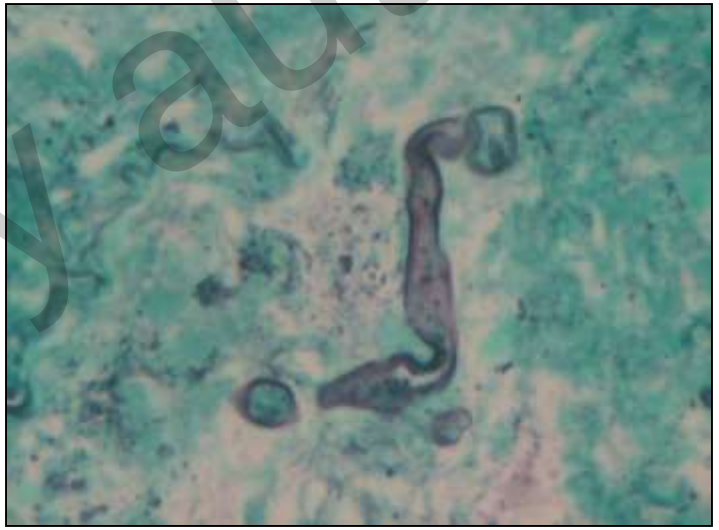


How May Histopathological Exam of Zygomycetes be Improved?

- We currently use GMS and PAS stains for suspected infections



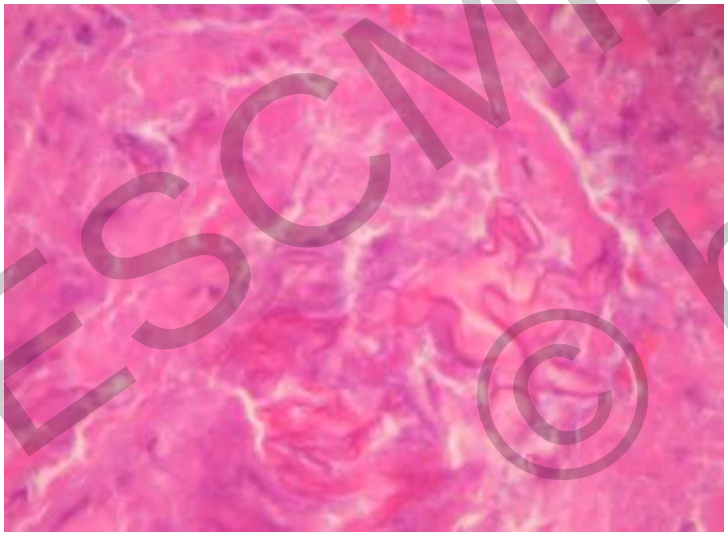
H&E stain



GMS stain

How May Histopathological Exam of Zygomycetes be Improved?

- Immunohistochemistry (paucity of reagents)
- FISH and *in situ* PCR



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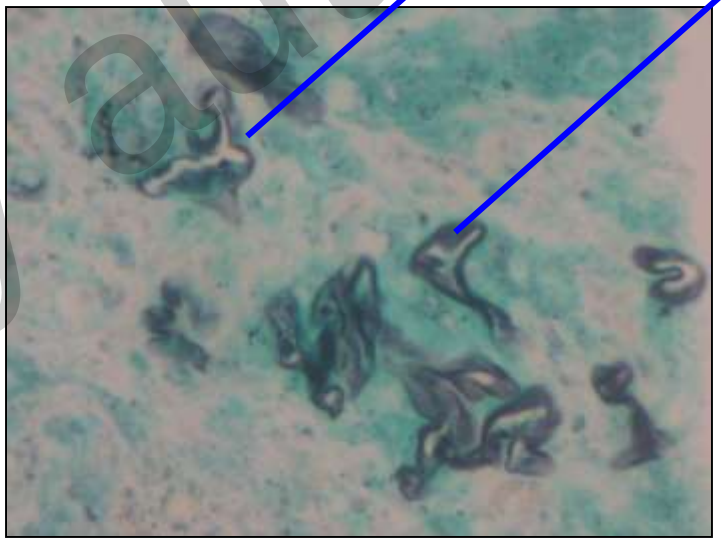
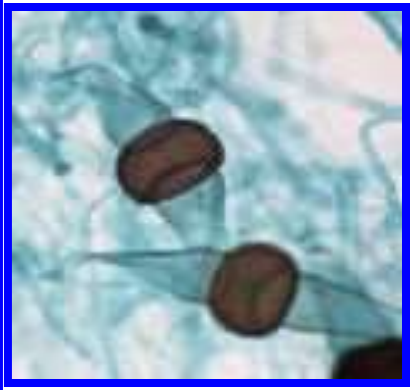


GMS stain

How May Microbiological Recovery of Mucormycetes be Improved?

(Kontoyiannis et al. Am J Clin Pathol. 2007;127:208-12)
(Walsh TJ, et al. Mycoses. 57 Suppl 3:2-7; 2014)

- Avoid homogenization and disruption of hyphae.
- Mincing of tissue specimen may yield better growth.
- Increased culture recovery of Mucormycetes at 37° C.



Hypothesis

Detection of molecular and antigenic biomarkers may improve the diagnosis of pulmonary and disseminated mucormycosis.

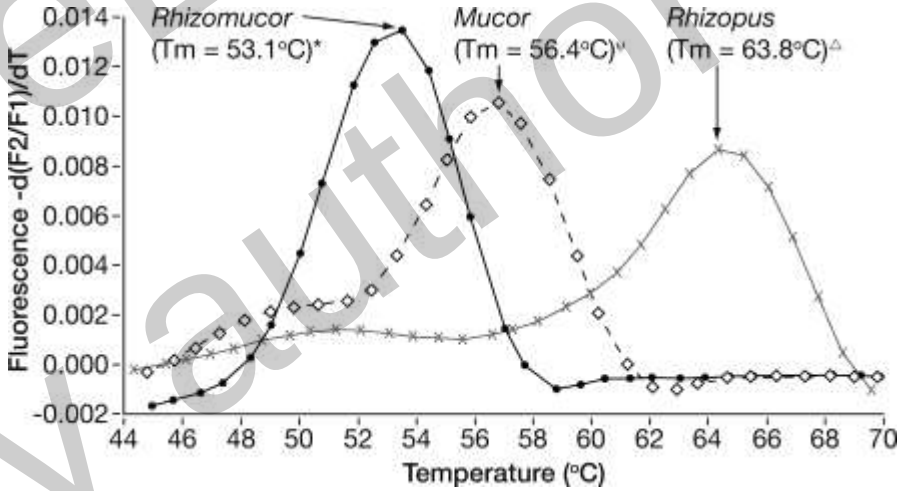
Novel Platforms and Targets for Laboratory Detection and Early Diagnosis of Mucormycosis

- Nucleic Acid Amplification Techniques
- Proteomics & Mass Spectroscopy (MALDI-TOFF)
- Metabolite Detection
  - LC-MS (soluble)
  - GC-MS (volatile organic compounds (VOCs)
- EIA
  - Galactofuranose Epitope-based cell wall components
  - Other cell wall epitopes

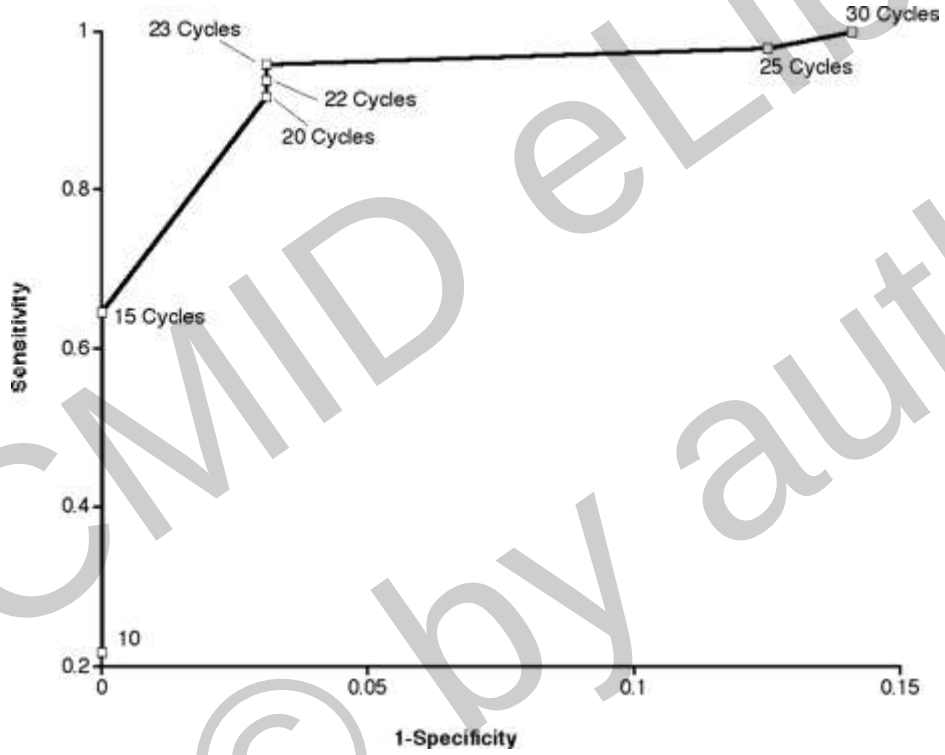
Molecular Diagnosis of Pulmonary Mucormycosis

(Kasai et al. J Clin Microbiol. 46: 3690-3702, 2008)

- **Detection of molecular biomarkers for *Rhizopus* spp., *Mucor* spp., and *Cunninghamella* spp.**
- **Quantitative PCR and melt curve analysis**
- **Plasma, bronchoalveolar lavage, and lung tissue**
- **Four models of experimental invasive pulmonary mucormycosis**



Design and Analytical Performance



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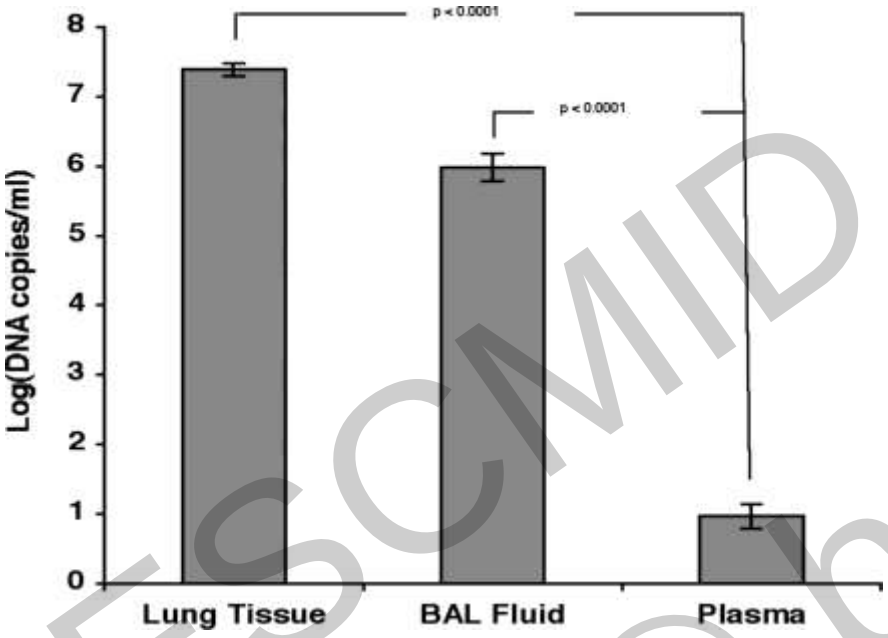
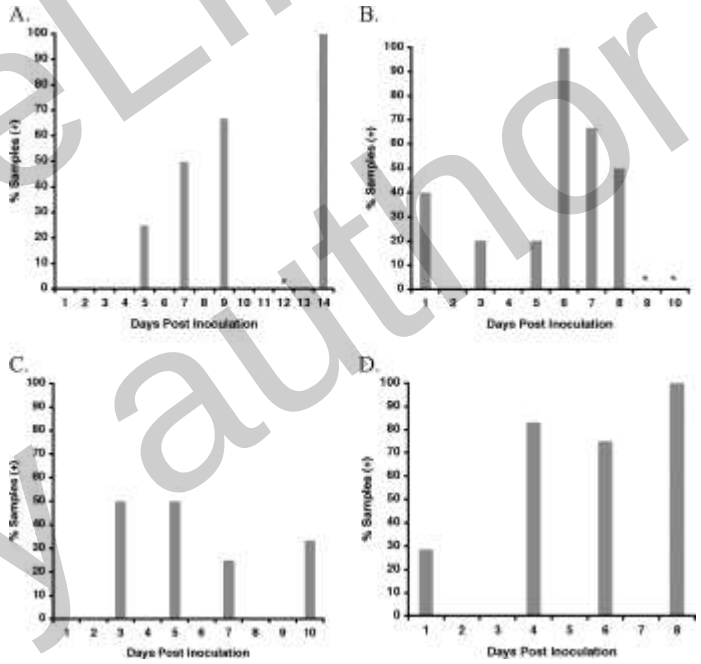




Translation to Patient Care

(Millon L et al. Clin Infect Dis. 2013;56(10):e95-101)

- **Millon *et. al* evaluated a combination of three quantitative PCR assays using hydrolysis probes targeting *Mucor/Rhizopus*, *Lichtheimia*, and *Rhizomucor***
- **circulating DNA in patients with mucormycosis confirmed by histopathological examination and/or positive culture.**
- **DNA from mucormycetes was detected in the serum of 9 of 10 patients with proven disease**

What Other Amplification Platforms May be Used?

(Buelow DR et al Medical Mycol. 50:775-80; 2012)
(Walsh TJ, et al. Mycoses. 57 Suppl 3:2-7; 2014)
(Springer J et al. J Med Microbiol. 2016;65(12):1414-21)

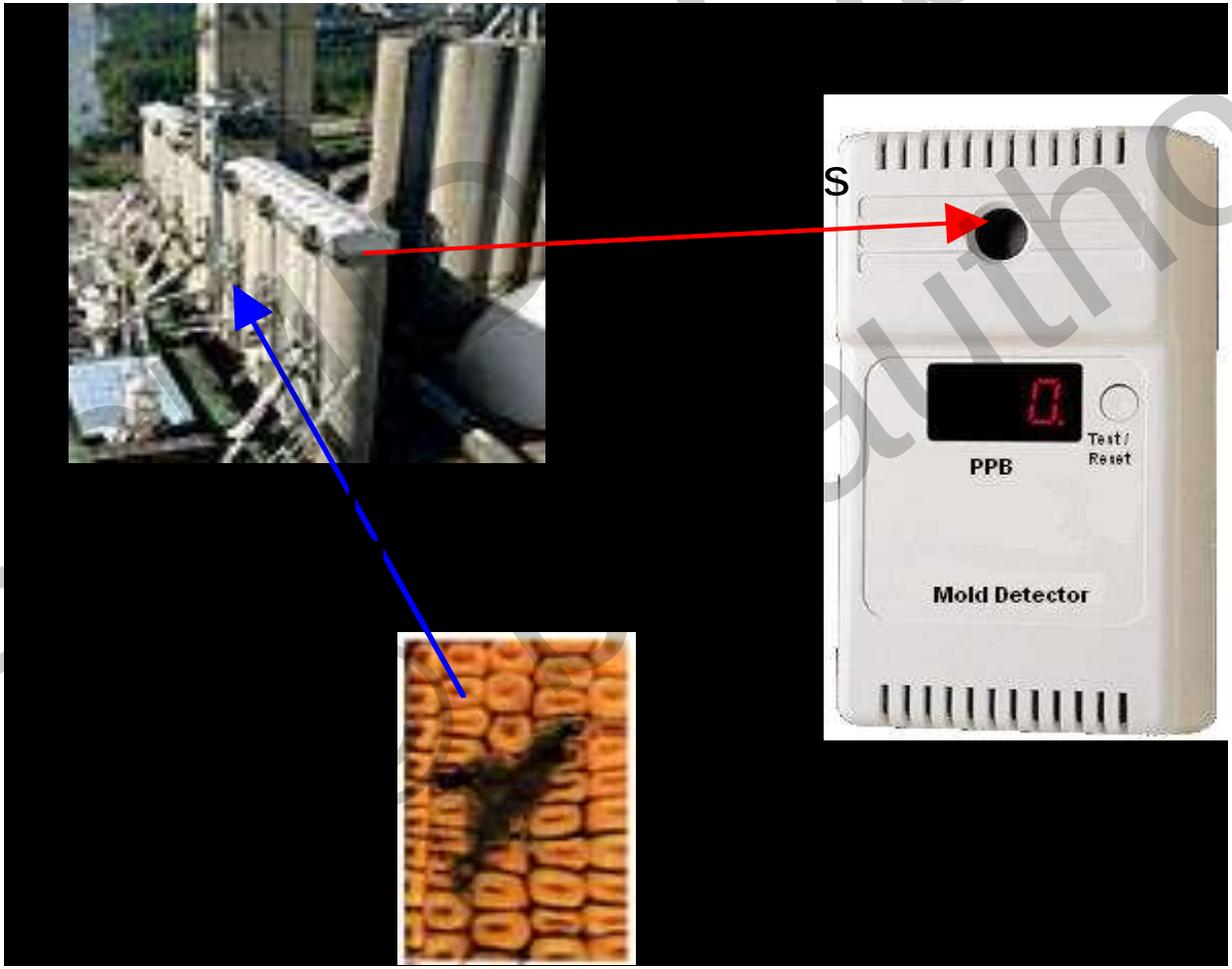
- **Repetitive sequence PCR and PCR-mass spectrometry**
- **Multiplexed PCR and liquid-phase array**
- **Electrospray ionization mass spectrometry**
- **Quantitative multiplexed detection using labeled primer PCR**
- **Probe based detection in tissue sections and serum**

What Other Laboratry Platforms May be Used?

(Walsh TJ, et al. Mycoses. 57 Suppl 3:2-7; 2014)

- **Antigen detection systems**
- **Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)**
- **Serum proteomic profiles**
- **Detection of Microbial Volatile Organic Compounds (MVOCs)**

Aflatoxin and Other MOVOCs are Rapidly Detectable by Programmable Nanocrystalline Technology



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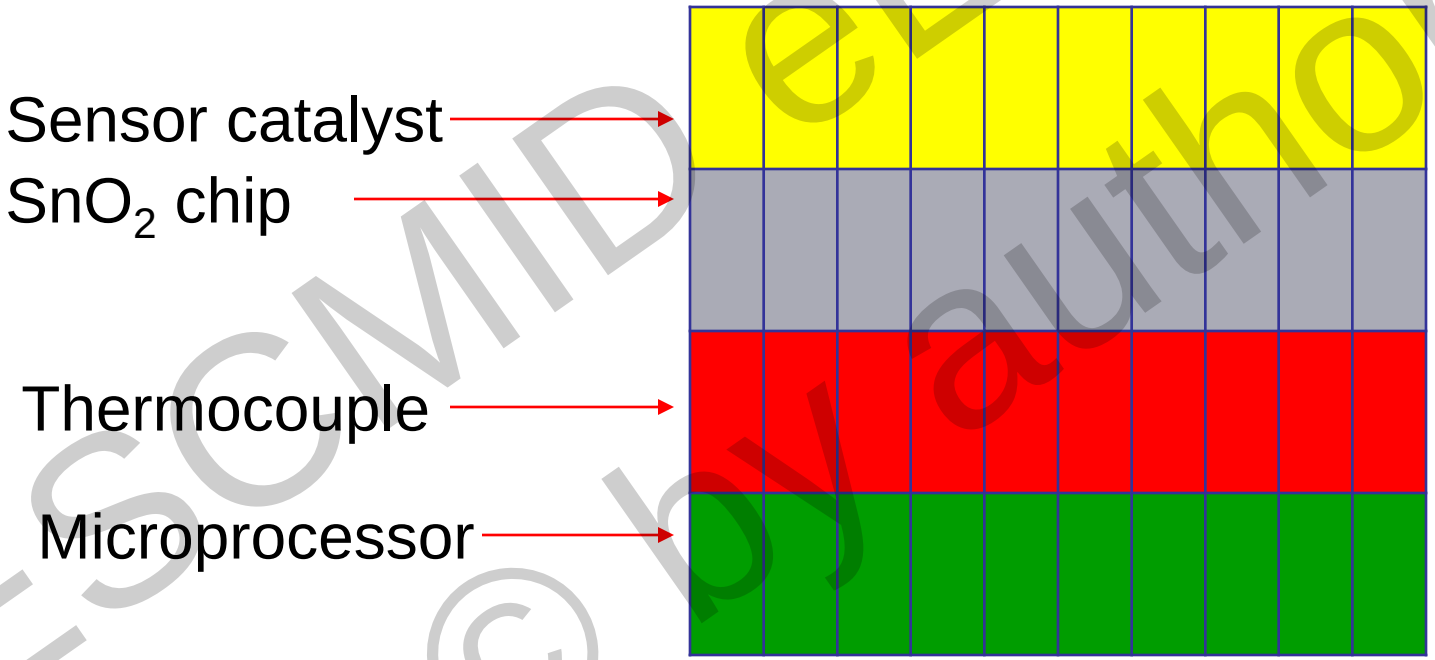

How Do We Develop New Laboratory Detection and Diagnostic Systems?

- **Biochemical and molecular plausibility**
- ***In vitro* detection**
  - **sensitivity and specificity established**
  - **methodological standardization**
- ***In vivo* studies in suitable and predictive animal models**
  - **Standardization of inoculum size, host response, tissue burden, and therapeutic effect**
  - **Controlled variability**
  - **Critical to understanding analytical parameters and in interpreting results from humans specimens**
- **Studies of human specimens**

How Do We Develop New Laboratory Detection and Diagnostic Systems?

- Analysis of human specimens
- Developed and managed through ISHAM Mucormycosis Working Group
- Critical link to strong epidemiological database
- Accelerate development and implementation

Future Directions

- In the absence of a major conceptual breakthrough of therapeutic intervention, early diagnosis will likely have the greatest impact in improving survival and outcome
- Strategies:
  - Epidemiological Risk Assessment Tool
  - Advanced Laboratory Diagnostic Systems

Need for Predictive Risk Model

- **Combining host factors in a predictive epidemiological model may provide a powerful tool**
- **Early initiation of antifungal therapy**
- **Selected population for increased Bayesian prior probability**
- **Increased PPV for use of advanced laboratory diagnostic tools**

Summary

- Importance of early diagnosis of mucormycosis
- Early diagnostic clinical and radiological manifestations of mucormycosis
- Conventional microbiological and mycological methods for laboratory diagnosis
- New molecular and antigenic technologies for rapid diagnosis of mucormycosis

Thank you!