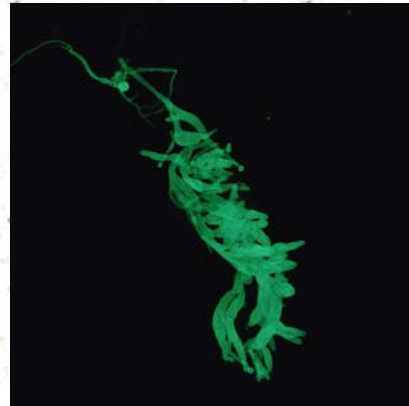
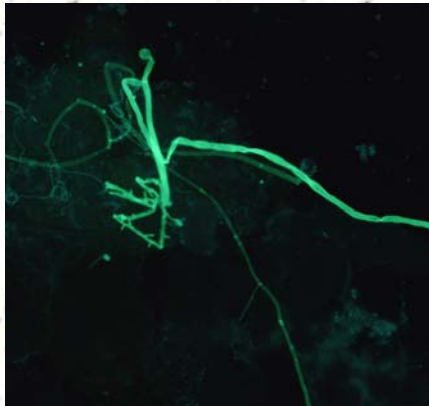




**MMSA**  
Medical Mycological  
Society of the Americas



## A recent Calcofluor KOH in the Mayo Clinic Mycology lab



### Inside this issue:

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## Tibia Wound Tissue – Fungal Smear, KOH/Calcofluor White, 20X

**How would you interpret this?**

Continued on Page 2

## How do you know what this is?

- Broad, ribbon-like hyphae (irregular walls)
- Aseptate (zygomycetes can be pauci septate)
- Often right-angle branching

## What are some of the genera associated with this type of morphology

*Rhizopus, Mucor, Rhizomucor, Absidia, Apophysomyces, Saksenaea, Cunninghamella, Basidiobolus, Conidiobolus*

## The Patient: 14 year old boy

The young boy arrived at the emergency room on by ambulance within 1 hour after an accident. He lived on a farm and his boot was caught in a corn auger. He had severe trauma to his ankle, femur (bone exposed), groin; with heavy bleeding. His leg wounds were grossly contaminated.

Pediatric General Surgery Service performed emergency trauma surgery to stop bleeding and Vascular Surgery tried to save his leg.

His antibiotic coverage started with Zosyn, and Pediatric Infectious Disease Service added fluconazole.

The patient was intubated, sedated for pain control and had issues with renal function, hypotension, tachycardia.

Two days later blood cultures grew a yeast; and his leg tissue cultures grew yeast and filamentous fungi. Ambisome was added to his antibiotic coverage. Four days later the patient went back to the Operating room for debridement.

The next day susceptibility testing on the yeast gave the following MIC's: voriconazole = 0.06 µg/mL, itraconazole = 0.12 µg/mL, amphotericin = 0.25 µg/mL.

The Pediatric Infectious Disease Service changed the antifungal to voriconazole.

Patient remained febrile and hypotensive.

The next day the mycology lab called the physician to report a positive fungal culture on leg tissue growing yeast & filamentous fungus resembling a zygomycete.

Pediatric Infectious Disease Service immediately stopped voriconazole, restarted ambisome and added posaconazole.

Final culture results were:

***Candida pelliculosa*** (doubtful identification on API at 48 hrs, excellent at 72 hrs, 100% D2 sequence match)

***Geotrichum klebahnii*** (low discrimination at 48 & 72 hrs on API, 99.94% D2 sequence

match) and ***Mucor*** sp. (morphologic identification and 99.73% D2 sequence match).

The patient went back to surgery for extensive wound debridement but was stable at the time of publication.

Thanks to: Nancy Wengenack, Director of Mycology and Mycobacteriology Laboratory Mayo Clinic, Rochester, MN.

Questions: e-mail

[wengenack.nancy@mayo.edu](mailto:wengenack.nancy@mayo.edu)

These organisms are highly invasive, grow rapidly, and delays in diagnosis can be associated with significant morbidity and mortality for the patient



From **LabLink** Michigan Department of Community Health

Bureau of Laboratories “Quality Laboratory Science for Healthier People and Communities

**Botrytis Species**

*Botrytis* species have worldwide distribution and are plant pathogens, commonly infecting ornamental plants, vegetables and fruits.

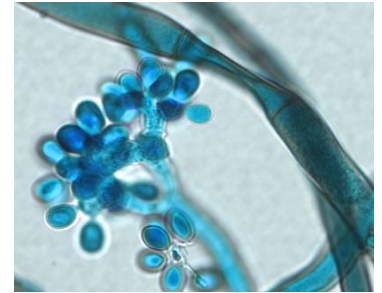
They are frequently referred to as *Botrytis* blight or more commonly as “the grey mould” due to appearance on plant material. Infected plants have masses of grey spores on the dead or dying leaves, stems, flower buds, or bulbs. The most common species is *Botrytis cinerea*. This species is most notable for causing two types of infections in grapes.

The first, called grey rot, results from wet or humid conditions re-

sulting in the loss of grapes. The second type of grape infection caused by dry conditions following wet ones is typically called “noble rot.” Noble rot is beneficial as it can result in sweet desert wines such as Sauternes. *B. cinerea* has been reported to cause infection in humans; a hypersensitivity pneumonitis called “winegrower’s lung.” *Botrytis* colonies are hyaline at first becoming grey or brown with age. Colonies become powdery due to the large masses of spores.

Microscopically, conidiophores are erect, brown and branching, and produce terminal

swollen ampulae (sporogenous cells). Conidia arise simultaneously on each ampulla formed on short denticles. Conidia are globose to ovoid and may be hyaline or dematiaceous. Large black sclerotia are frequently formed.

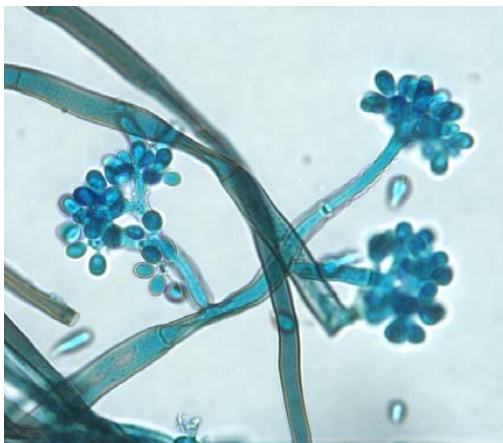


References:

1. Barron, George. 1977. *The Genera of Hyphomycetes from Soil*. Robert E. Krieger Publishing Company, Huntington, N.Y.
2. *Botrytis Blight*,
3. *Botrytis cinerea*, [http://en.wikipedia.org/wiki/botyrtis\\_cinerea](http://en.wikipedia.org/wiki/botyrtis_cinerea)
4. Domsch, K.H., Gams, W., Anderson, Traute-Heidi. 1993. *Compendium of Soil Fungi*. IHW-Verlag,

*LabLink*, the Michigan Department of Community Health, Bureau of Laboratories newsletter. Please feel free to share this with others who might have an interest in the laboratory news.

Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/ Mycology Unit



Michigan Department of Community Health



Jennifer M. Granholm, Governor  
Janet Olszewski, Director

# FOCUS 79 on FUNGAL infections

March 4 – 6, 2009, Fort Myers, Florida

**March 4 – 6, 2009**

Sanibel Harbour

Fort Myers, Florida

**Chairs**

*Elias J. Anaissie, MD*

University of Arkansas for  
Medical Sciences

Little Rock, Arkansas

*Michael G. Rinaldi, PhD*

University of Texas Health  
Science Center at San Antonio  
San Antonio, Texas

to a current problem in patients involving  
fungal infections (in the broadest sense) in  
contemporary medicine

- Candidate must submit a well-written, concise and accurate abstract
- Candidate must be registered for the meeting and agree to be present during the scheduled presentation times

March 4 – 6, 2009, Fort Myers, Florida

**IMPORTANT DATES**

**Discounted**

**Registration Deadline**

January 7, 2009

**Abstract**

**Submission Deadline**

January 22, 2009

**Abstract Notifications Mailed**

February 11, 2009

**Abstract Presenter**

**Registration Deadline**

February 18, 2009

**Regular**

**Registration Deadline**

February 25, 2009

**Conference Dates**

March 4 - 6, 2009

Email: [meetings@imedex.com](mailto:meetings@imedex.com)

[www.imedex.com](http://www.imedex.com)

## Navigating Molecular Diagnostics in Medical Mycology

Specific identification is important to patient care because it affords evaluations of the therapeutic agent by helping with pharmacodynamics and patient treatment.

Species specific PCR, Broad range PCR, Post-amplification detection, use of signature sequences. Signature sequences can be used for *in situ* hybridization, PCR associated probes and arrays. An example given was "FISHing for Fungi" which employs direct hybridization of a labeled probe to a nucleic acid target with subsequent microscopic visualization. Two commercially available probes detect *C. albicans* (Green) vs. Non-*C. albicans* (Clear), *C. albicans* (Green), *C. glabrata* (Red) or the "traffic light" which *C. albicans* / *C. parapsilosis* (Green), *C. tropicalis* (Yellow), *C. glabrata* / *C. krusei* (Red) from positive blood cultures.

A general overview of nucleic acid amplification (PCR and all the derivatives) presented principles: species-specific detection with examples of *Aspergillus*, *Candida*, *Coccidioides*, *Histoplasma capsulatum*, *Pneumocystis*, and *Cryptococcus neoformans* and using internal transcribed spacer region and large ribosomal subunit genes.

Some advantages of real time PCR include amplification and detection in the same tube, decreased chance of amplicon contamination, rapid thermocycling, fluorescence generation and detection occurs in "real-time" with no need for post-amplification analysis. Some of the limitations of PCR included cost, dedicated resources, volume.

The principle of broad range or multiplex PCR linked with post-amplification analysis uses broad-range or multiple species-specific primers for amplification which flank regions that contain *signature* sequences, and afford the identification of the selected fungi. Post-amplification methods of detection are used to determine the *signature* sequence and this allows identification of regions that can differentiate numerous targets of interest. Ways to detect *signature* sequences include probe hybridization, Southern blotting, "real-time" analogies, reverse hybridization, DNA sequencing, traditional Sanger Sequencing, pyrosequencing, solid phase microarray hybridization, fluorescent or bioelectric arrays. Also liquid phase microarray hybridization, fluid arrays, DNA profiling, probe hybridization with traditional Southern blotting: It works, but is too time/labor consuming.

Multicolor, real-time hybridization affords up to six colors, can include an internal amplification control, can obtain quantitative information about each pathogen.

Reverse hybridization, sequencing by termination, method of detection of the various length fragments, fluorescent dye labeled primers, capillary electrophoresis and ultra-thin disposable gels allow traditional Sanger sequence to determine the entire sequence of the amplified product and then it is compared the derived sequence with those in a *reliable* sequence database.

Advantages: Thorough and becoming cost-effective and

user-friendly. Particularly useful when the amplified product is derived directly from a specimen or from a "sterile" mold.

Disadvantages: Cost: Equipment and reagent, Dedication: Expertise and resources, Databases: Buyer beware: these are not all created equally. utilization fees are charged by commercial vendors.

Short-Length Sequence Based Identification (Pyrosequencing) Principle: Variable regions exist that contain genetic information that is useful for naming or categorizing microorganisms. The sequence of these regions may be determined by pyrosequencing. Advantages to Short Length Sequencing, Limited sequences to proof-read Less expensive equipment c/w Sanger sequencing, Fast; may follow SYBR green PCR; easy-to-perform; Disadvantages: Limited read lengths, Database issues the same as with Sanger sequencing Limited experience with this technology; Reliance upon a single vendor; Solid Phase Microarrays Principle: Oligonucleotide probes are bonded to a site on a solid material; The amplified product hybridizes with the appropriate probe as determined by Watson and Crick base pairing rules. This complex is detected, and the nature of the amplified product is thereby determined.

Example of a Solid Phase Array: Bioelectric Microarrays; Liquid Microarray Principle:

Probes are bound to beads that contain a signature. The amplified product is mixed with beads/probes under

Presented at the  
Annual Meeting,  
Boston, Mass. June  
2, 2008  
Gary W. Procop

## *Navigating Molecular Diagnostics in Medical Mycology*

*Continued from previous page*

conditions that favor hybridization. The liquid bead/probe – amplicon mixture is streamed and interrogated by a laser. The nature of the amplified product is determined by the bead/probe to which it has hybridized.

Liquid Microarray Technology  
Microarrays Advantages: Numerous determinations simultaneously May follow broad range and/or multiplex PCR  
Disadvantages: Cost (but this is changing) Too much information (but this is changing) Test volume limitations Likely most applicable at larger cen-

ters.

DNA *PRO*filin Molecular Mycology It is here...but the best is yet to come. Dependant on traditional methods for optimal design. Will complement traditional methods. Ancillary, very helpful tests, for the more rapid and highly accurate detection of serious fungal infections in the immunocompromised host.

Contact Gary Procop directly with comments and questions.

Abstracted by L. Hall

## *A tree fungus produces Diesel Fuel Naturally*

### *Green Energy*

A Tree Fungus Produces Diesel Fuel Naturally

This is an interesting article that talks about

*Gliocladium roseum* being a source of energy.

It is on the Hardy diagnostics web site and can be found at the URL

<http://www.hardydiagnostics.com/articles/Gliocladium-Biofuel.pdf>

*Gliocladium* as a biofuel?

Check this article out on the WEB!

## *Check out the Web site: New features*

Norman Goodman has a lovely presentation of MMSA the early years on the web site.

<http://www.mycologicalsociety.org/>

Document Display, the early years

These were photos taken by

Dick Kruse...a nice trip down memory lane.

Art DiSalvo has put together a photo gallery of the MMSA presidents, Rhoda Benham awardees and Billy H Cooper awardees. It is still

“under construction”...even though the photos are different sizes, they are fun to look at. Refinements should happen soon!

## MMSA Division F Symposium Philadelphia, PA ASM 2009

### Granulomatous Host Defenses against Endemic Mycoses of the Americas

Time and Place to be determined.	
Author's Name	Presentation Title
<b>Angela Restrepo</b>	Paracoccidioidomycosis: Current concepts in epidemiology, host response, diagnosis, and treatment
<b>Kieren Marr</b>	Granulomatous responses and epidemiology of <i>Cryptococcus gattii</i> infections
<b>George Deepe</b>	Granulomatous inflammation in host defense against histoplasmosis
<b>Bruce Klein</b>	Host-pathogen interactions in granulomatous host defenses of blastomycosis

**Summary:** Paracoccidioidomycosis, histoplasmosis, cryptococcosis and blastomycosis share in common a prominent granulomatous host response. This symposium will review the mechanisms of granuloma formation, macrophage response, and immunoregulation in host response to *Paracoccidioides brasiliensis*, *Cryptococcus gattii*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis*. Understanding this response has implications that affect not only treatment of the infections but understanding basic host-microbe mechanisms of interaction. New advances in immunology are shedding light on our understanding of the basic mechanisms to control granuloma formation and resolution/persistence. Ultimately, this interplay between the host and pathogen share a common feature with many other infectious agents in that this latent/persistent infection can become a nidus of reactivation during immunodeficiency. The four fungi covered in this session represent four major endemic mycoses of North and South America.

**Upon completion of this session, participants should be able to:**

- describe the similarities in the granulomatous responses to these four endemic mycoses
- identify the key differences in the granulomatous responses to these four endemic mycoses
- describe the current state-of-understanding for each of these mycotic agents of the mechanisms of host-pathogen interaction that lead to persistent/latent infection

**[info@mycologicalsociety.org](mailto:info@mycologicalsociety.org)**

# What's Happening?

**March 4-5, 2009 Focus on Fungal Infections 19** Sanibel Island, Florida

Hotel Location

<http://www.imedex.com/appweb/announcements/A047-01.asp>

**March 14, 2009 , A walk thru the Fungal Jungle**

1 Day Workshop, Jacksonville, Florida. Contact Jane Hata, Director Microbiology, Mayo Clinic Jacksonville [hata.donna@mayo.edu](mailto:hata.donna@mayo.edu)

**May 17-21, 2009 American Society for Microbiology Annual Meeting, Philadelphia, PA**— Workshops of interest: 2 day Mycology (for beginners) and 1 day on Nocardia

**May 25-29, 2009 ISHAM Congress, Tokyo, Japan**

A major event during the next few years will be the ISHAM Congress in Tokyo, May 25-29, 2009, with the theme: "Medical Mycology in the 21st Century: Scientific Base and Anticipated Challenges". The organizers have managed to offer this congress at a very moderate fee. Available hotels on site in the Shinjuku area are in all classes, ranging from high quality to the famous Japanese business hotels at modest prices. The costs of the congress thus can be kept quite limited, making this great event in reach for everyone. For information, click on <http://www.congre.co.jp/isham2009/> or go to the Mycological Happenings section of the ISHAM website.

## Summary of Focus 19 – MMSA Affiliation from Tom Walsh

[1] The title of the announcements will include the phrase, **“in affiliation with the Medical Mycological Society of the Americas”**

The brochure also will include the MMSA logo

### [2] **“Medical Mycology 101”**

This is an introductory workshop from MMSA that is designed to provide basic understanding of diagnostic medical mycology. The course is intended for residents, fellows, technologists, or other professionals who seek a foundation in medical mycology. The course complements the preceding highly successful and well established “Mycology Workshop.”

### [3] **Noon Symposium**

**“Clinical Implications of Recent Mycological Research in Cryptococcosis: An MMSA Symposium.”**

- The Cryptococcal Capsule in Human Infection. Arturo Casadevall
- Innate Host Defenses: TLRs take their toll on *Cryptococcus neoformans*. Stu Levitz
- Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. Joe Heitman

What can we take from bench to bedside to improve the care of patients with cryptococcosis? John Perfect

This symposium can be sent as a webcast to approximately 16,000 physicians in the Imedex directory.

[4] Application materials for membership in MMSA will be offered to attendees of Focus 19.

See announcement on  
Page 4!

Hope to see you there.



THE UNIVERSITY OF THE WEST INDIES  
MONA, JAMAICA, WEST INDIES

The University of the West Indies (UWI) is a dynamic, international institution serving the countries of the Commonwealth Caribbean. Its faculties offer a wide range of undergraduate, masters and doctoral programmes in Humanities and Education, Pure and Applied Sciences, Science and Agriculture, Engineering, Law, Medical Sciences and Social Sciences. The Department of Microbiology provides teaching, research and service in the sub-specialties of medical bacteriology, virology, immunology, parasitology and mycology. It provides training and laboratory diagnostic services to the University Hospital of the West Indies and a number of private stakeholders. In furtherance of these objectives, the UWI, Mona Campus, is seeking to fill the positions (2) of:

## **LECTURER/ASSISTANT LECTURER IN MICROBIOLOGY**

### **Department of Microbiology**

#### **Qualifications and Experience:**

*The ideal candidate must possess:*

*A higher degree in Medical Microbiology and should be medically qualified;*

*Considerable experience in Diagnostic Microbiology;*

*Considerable experience in teaching at the University level.*

The Lecturer will report to the Head, Department of Microbiology and the duties of the post include:

- Teaching at both graduate and undergraduate levels
- Supervising graduate research

Developing a field of research in line with the many opportunities for studying infectious agents in a tropical developing country.

The successful candidate will be eligible for appointment to a Consultant post at the University Hospital of the West Indies.

Detailed application along with a copy of curriculum vitae giving full particulars of qualifications, experience, marital status, nationality, names and addresses of three referees and certified copies of degrees should be sent by electronic mail to: [hrmd.sed@uwimona.edu.jm](mailto:hrmd.sed@uwimona.edu.jm) or to the Human Resource Officer, Staffing, Human Resource Management Division, The University of the West Indies, Mona, Kingston 7. The final date for receipt of applications is **January 30, 2009**. **The University of the West Indies thanks all applicants, however, only short listed applicants will be contacted.**

## J. Fred Denton, PhD, MD Birder versus Blasto A Biography

James Frederick Denton, Jr. was a man for all seasons; parasitologist and physician by training and noted ornithologist, gardener, fisherman and naturalist by avocation. Dr. Denton's significant achievement was the first isolation, and several subsequent isolations, of *Blastomyces dermatitidis* from nature.

James Frederick Denton, Jr. went by the name J. Fred Denton to distinguish himself from his father. Fred, as he was known to friends, relatives and colleagues, was born in Americus, Georgia in 1914; his father - a traveling salesman and his mother - an elegant Southern lady

During the second half of his professional career Dr. Denton became a medical mycologist. His demeanor and speech were deceiving. Born and educated in middle Georgia, he was tall, lanky and spoke with a Southern drawl. Shortly after receiving his Ph.D. from Rice In-

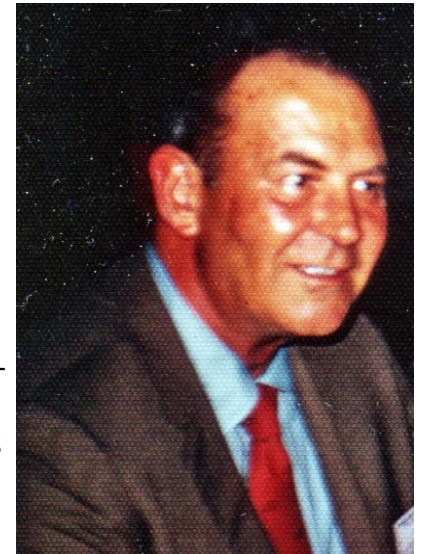
stitute he became a professor of microbiology at the Medical College of Georgia where he remained until his retirement in 1976. He attended medical school part time while maintaining his full teaching schedule, Parasitology and medical mycology, and received his MD in 1956. Dr Denton was a Markle Foundation Fellow, a China Medical Board Fellow and was a member of Sigma XI and the medical honorary society Alpha Omega Alpha.

In 1958 he received an NIH grant of \$10,000 (which included my annual salary of \$3600) to investigate the natural habitat of *B. dermatitidis*. The common wisdom for the isolation of mycotic pathogens at that time was the intraperitoneal inoculation of mice. Emmons used that method to isolate *Histoplasma capsulatum* and *Cryptococcus* from soil samples and likewise Ajello isolated various mycotic pathogens from nature. Dr.

Denton's theory was that an inoculum into the tail vein of a mouse would enter the vena

cava, thence to the heart for a journey down the aorta to the lungs, liver and spleen. In the capillaries of these organs, the infectious particles had three opportunities to be trapped and result in an infection. A saline suspension of the suspect material was allowed to settle to permit the large particles to precipitate and a sample of supernatant was inoculated into the mice. Streptomycin and penicillin were added to the saline solution to reduce the bacterial load. The subsequent isolation of the organisms would follow a period of incubation in the mice, their sacrifice and autopsy, and the *in vitro* culturing of these arresting organs.

Three avenues of investigation were being followed simultaneously. One was refining the recovery technique using various concentrations of *B. dermatitidis* yeast forms and varying the amount of inoculum.



# J. Fred Denton, PhD, MD

## Birder versus Blasto A Biography continued

Second, a variety of laboratory experimental devices were developed to evaluate a range of environmental conditions to establish the suitable habitat for the fungus. Thirdly we were sampling suspect loci that most resembled the ecologic conditions which appeared favorable for the survival of *B. dermatitidis*.

There were naysayers to this method at the 1967 ISHAM meeting in New Orleans. He was told in no uncertain terms that streptomycin could not be used in the saline to remove bacteria because streptomycin was lethal for mice. It was not. He was also warned that mice could not be injected intravenously with soil suspension because they would not tolerate all the particles which then would cause an embolism and kill the experimental animal. After many experimental processes with the IV inoculation of known suspensions of *B. dermatitidis* this protocol resulted in 100 percent recovery of *B. dermatitidis* from experimentally infected mice.

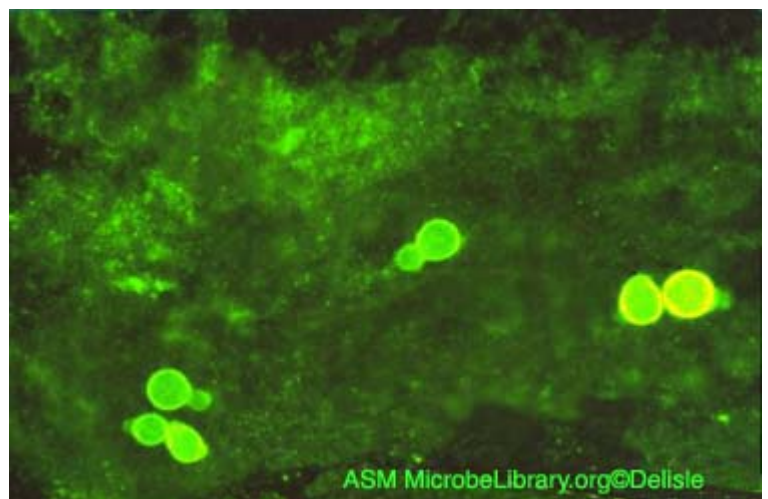
Dr. Denton was a member of several professional societies including the American Society For Microbiology, the Medical Mycological Society of The Americas, the American Society of Tropical Medicine and Hygiene, the International Society for Human and Animal Mycology, the American Ornithologists Union and others. His research interests were the trematode parasites and the ecology of pathogenic fungi. His microbiological publications were divided between these two fields. Some of his

outstanding accomplishments include the description of several new species of trematodes and his investigations into the ecology of *Blastomyces dermatitidis*, including the first isolation of this pathogen from the environment.

In addition to being a microbiologist, Dr Denton was a well-known ornithologist. Included among his numerous publications in ornithology are two editions of the "Annotated Checklist of Georgia Birds" published by the Georgia Ornithological Society and carried by most Georgia bird-watchers. He was the epitome of a true biologist with abundant knowledge of hymenoptera, reptiles, fish and flora which he always discussed by their common name and specific epithet. The majority of his 40 scientific publications were in Parasitology followed closely by ornithology and mycology.

Dr. Denton was my mentor, teacher and friend.

Arthur F. Di Salvo



## William Kaplan, DVM

Dr. William (Bill) Kaplan, a distinguished medical mycologist, passed away July 30 at the age of 86. Bill earned his DVM at Cornell University. After graduating, he took a position with a United Nations agency in Mexico where he worked on a variety of animal diseases. It was here that he became proficient in Spanish, a language which was to help him in future collaborative investigations in Central and South America. Subsequently, he joined Dr. Libero Ajello and Dr. Lucille Georg in the Division of Mycotic Diseases at the Centers for Disease Control where he became an authority on fungal histopathology, and the diagnosis of dermatophyte infections, protothecosis and multiple systemic mycotic infections.

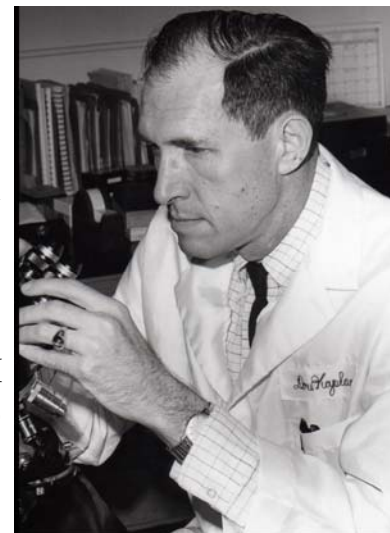
Bill was a meticulous researcher and made

significant and innovative contributions in the application of fluorescent antibodies to the immunoidentification of fungal pathogens both in culture and tissues. His extensive laboratory studies led to the authoring of numerous peer-reviewed scientific articles, manuals, and textbooks. These documents and his in-depth, authoritative and explicit lectures earned him many prestigious awards, and international and national recognition. He was a member of the American Academy of Microbiology, American Society for Microbiology, the International Society of Human and Animal Mycoses (ISHAM), and the Medical Mycological Society of the Americas and served with distinction on their editorial boards. He was a diplomat of the American Board of

Medical Microbiology in Medical Mycology and the recipient of the Lucille Georg Award of the ISHAM in 1988. Bill loved to teach and mentored many Doctorate and Post-Doctorate candidates. Students from the United States and from countries world-wide attended courses he developed and taught under the auspices of CDC and the World Health Organization.

Like his beloved wife Marge, who passed away earlier, he was an avid gardener, as well as a fisherman and passionate dog lover. His friends and colleagues will remember him for his warmth and scholarly contributions.

Leo Kaufman, Ph.D.



### In Memoriam

**2008-2009 MMSA Council e-mail** [council@mycologicalsociety.org](mailto:council@mycologicalsociety.org)

President	Arturo Casadevall	2008-09
Vice President	Glenn Roberts	2008-09
President elect	Kevin Hazen	2008-09
Secretary Treasurer	Annette Fothergill	2005-10

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	Joe Heitman	2007-10

West	Mike Saubolle	2006-09
	Paul Szanszlo	2007-10

Latin America	Rosa Puccia	2007-10
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Link to the Council is found on the MMSA WEBSITE:

<http://d2153591.u37.hosting-advantage.com/index.html>

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