Rodrigo Almeida-Paes

Diagnosis of histoplasmosis

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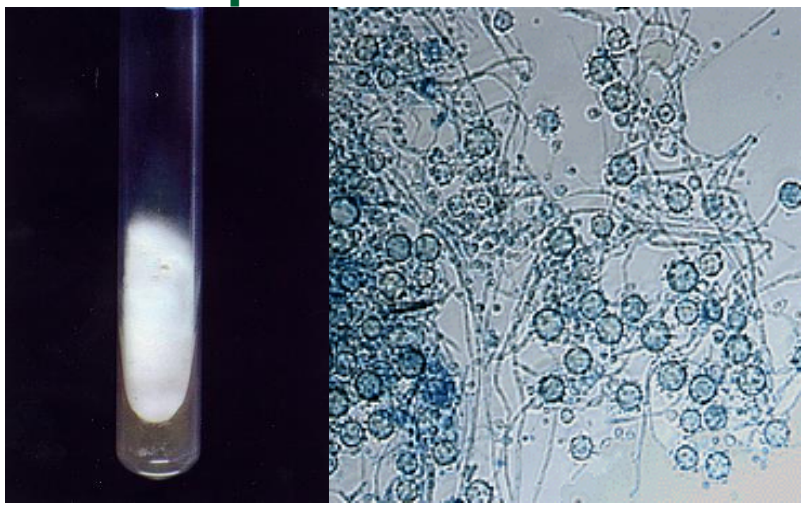
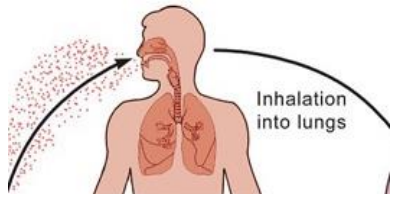
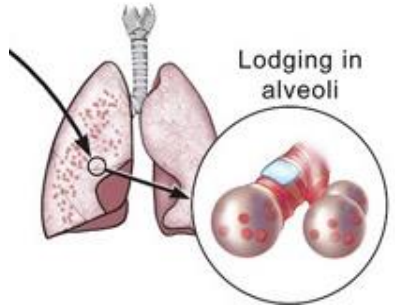
Histoplasma capsulatum and histoplasmosis

Filamentous phase - soil or *in vitro* - 25°C

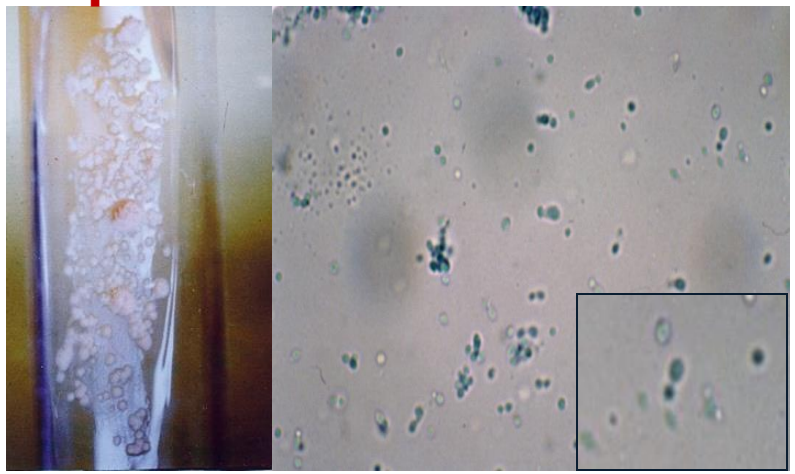


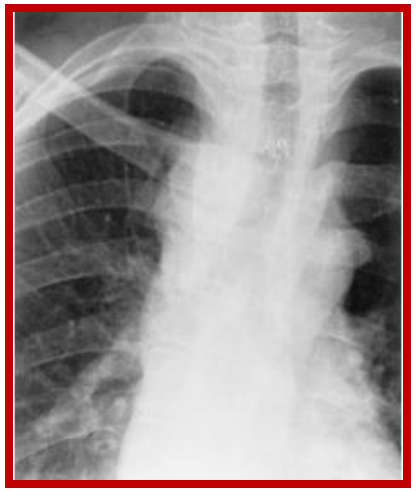




Dimorphism

Yeast phase – Tissue or *in vitro* - 37°C





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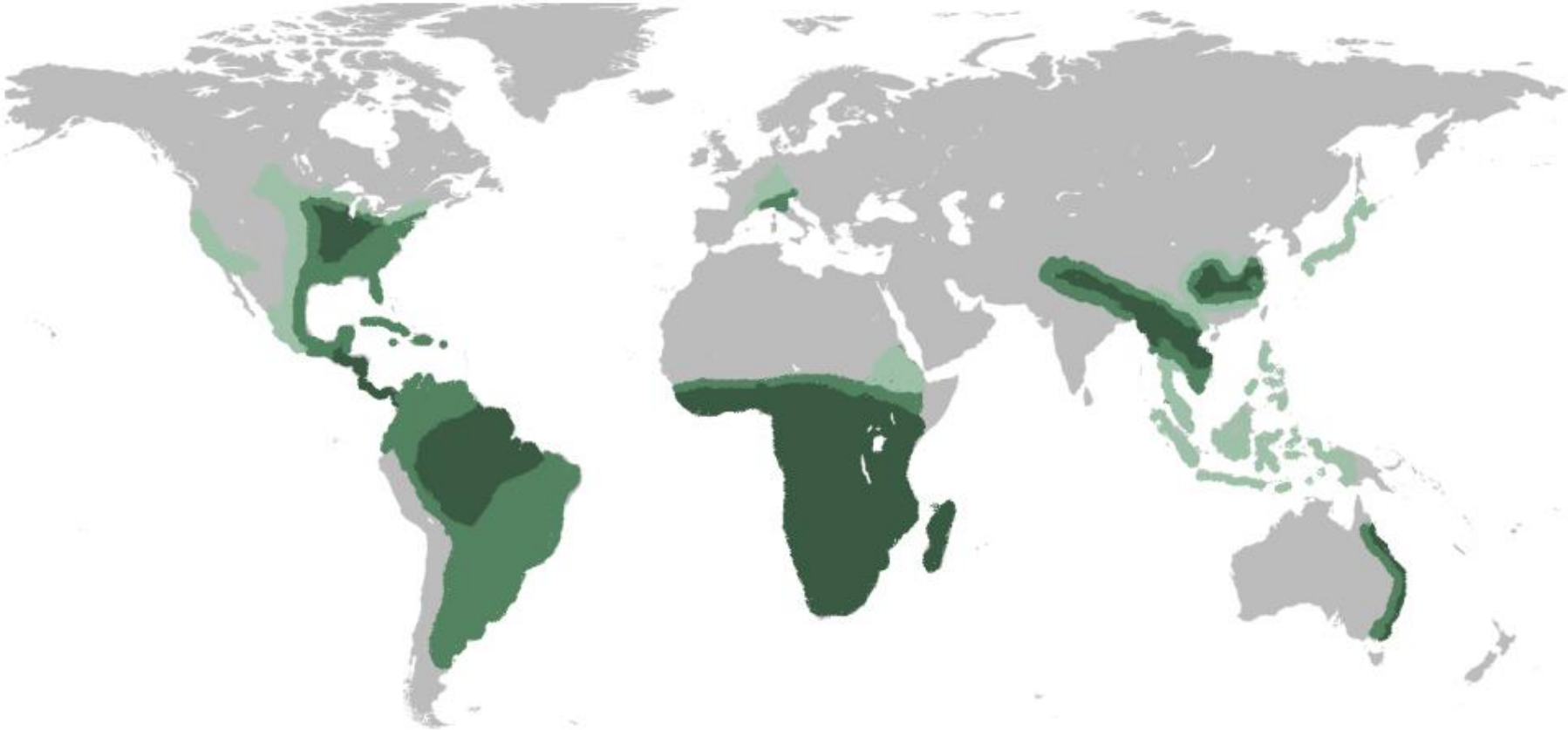
Worldwide distribution



Areas likely to be hyperendemics

Areas where infection occurs regularly

Areas where local infection have been reported

J. Fungi 2023, 9, 236. https://doi.org/10.3390/jof9020236

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Histoplasmosis in Latin America

In Latin America the real incidence of the histoplasmosis is unknown ;

Histoplasmosis is not a reportable disease;

The cases of histoplasmosis are underestimated. Probably, there are more extensive endemic areas;

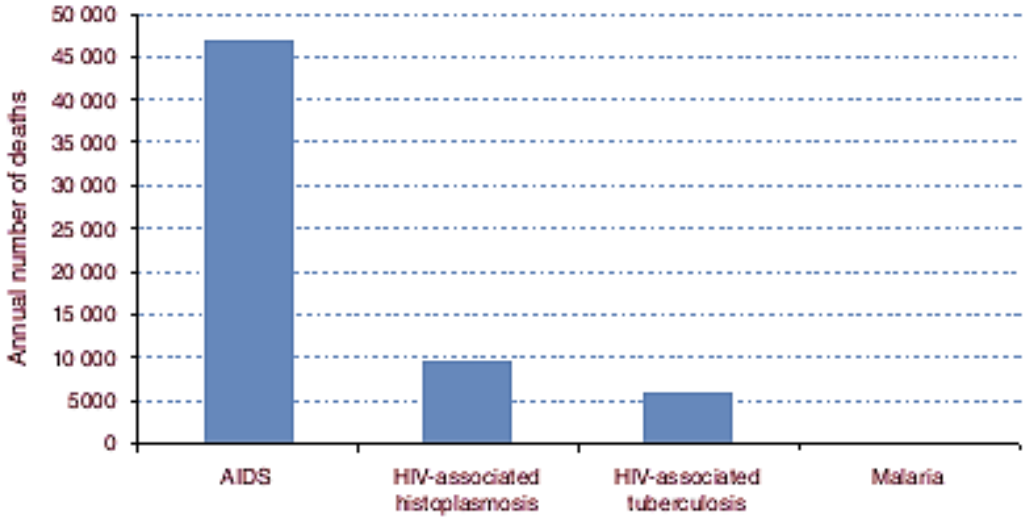

Fig. 1. Estimated number of deaths per year for different major infectious diseases in Latin America.

More frequent: Argentina, Brazil, Colômbia, Venezuela, Guiana Shield, Guatemala, Mexico

Annual rate of death from histoplasmosis in HIV-positive people with AIDS in Latin America equals 70 air crashes

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THE DIAGNOSIS OF HISTOPLASMOSIS

↓

Challenge

↓

Requires multifactorial approach

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Diagnosis of histoplasmosis

HISTOPLASMOSIS

- Clinical data
- Epidemiological data
- Laboratorial data
- Radiological data

DIRECT EXAMINATION/ CULTURE

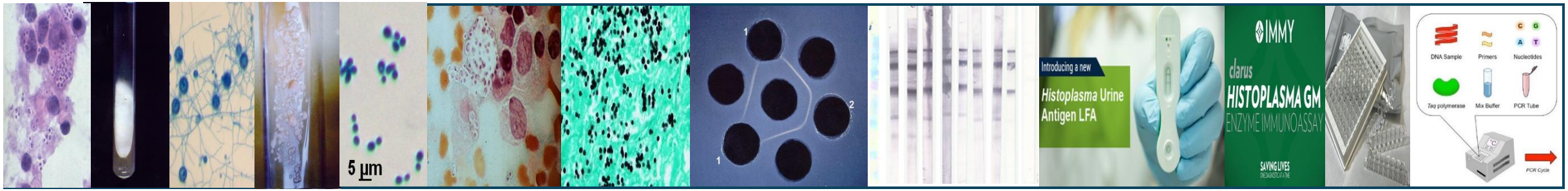
- ↓ Sensitivity
- Fastidious
- Dimorphism M-Y
- Location of lesions

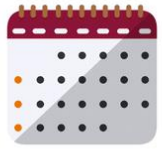
ANTIBODIES / ANTIGENS

- Sensitivity
- Rapid diagnosis and prognostic makers
- Biosafety
- Less invasive procedures

Diagnostic Methods

Culture | **Histopathology** | **Serology** | **Antigen Detection**





4-6 weeks

3 -10 days

2-5 days

30 min-4 h

Methodologies → Minimal laboratory structure

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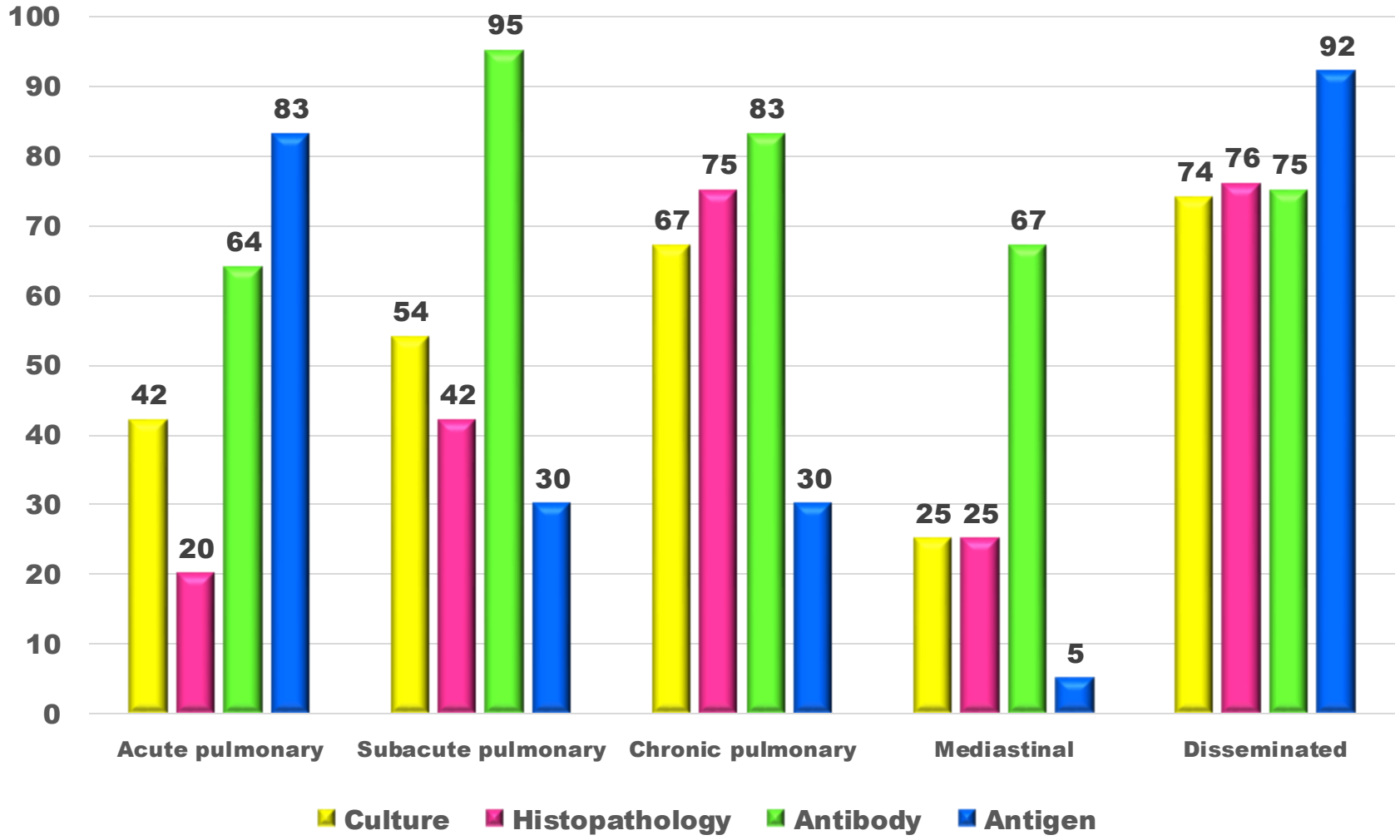
Sensitivity in different clinical forms



J. Fungi 2023, 9, 236. https://doi.org/10.3390/jof9020236

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Non culture methods

- **Antibody detection**
- **Antigen detection**
- **Detection of nucleic acids**

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Antibody detection

Table 2. Summary of sensitivity and specificity values of immunological tests used for diagnosis of endemic mycoses by antibody detection.

Disease	Test	Sensitivity	Specificity	References
Histoplasmosis	ID	75–95	100	[58]
	CF	72–95	70–80	[58]
	EIA	66–97	54–100	[65,67–70]
	Western blot	95	94	[72,73]

ID: immunodiffusion; CF: complement fixation; EIA: enzyme immunoassay; CIE: counterimmunoelectrophoresis.

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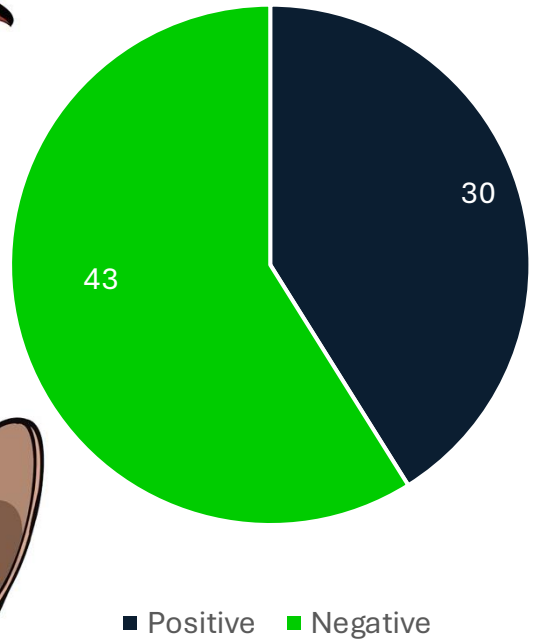
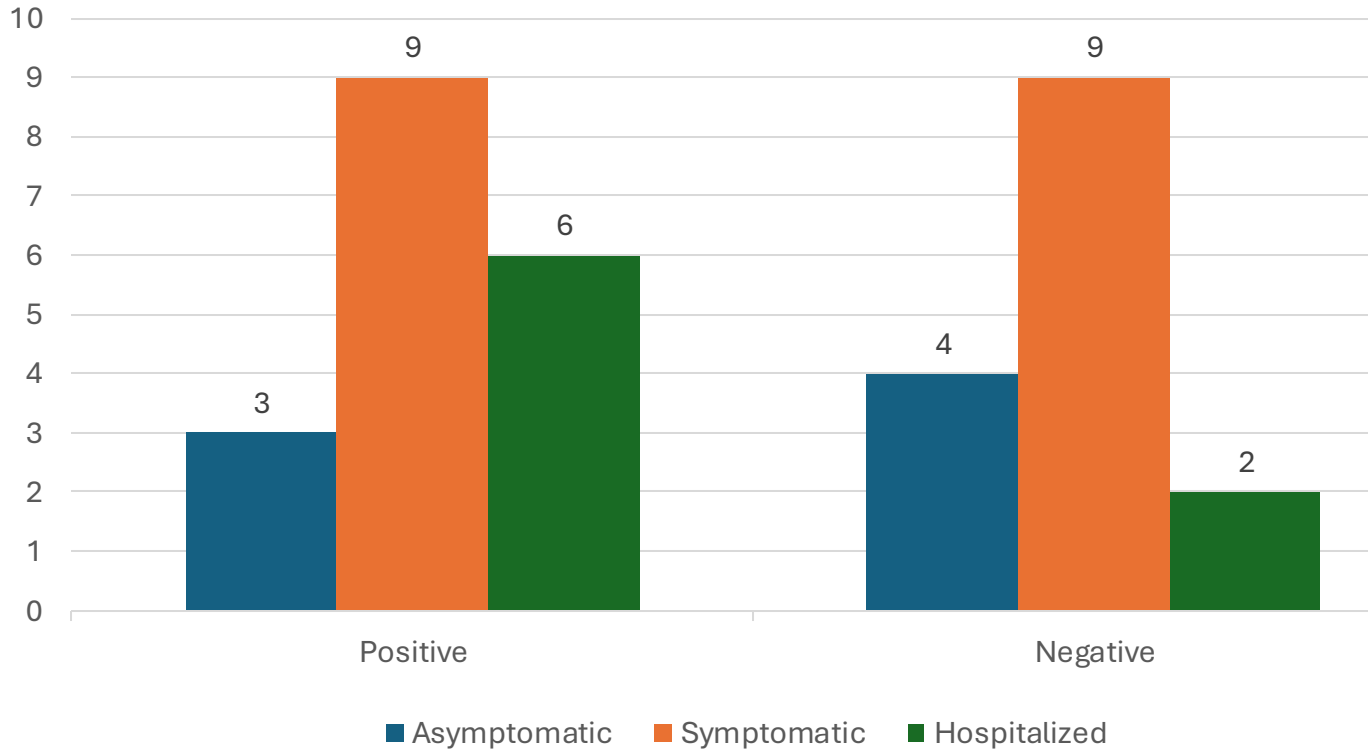
Outbreak of histoplasmosis

- **Population with history of participation in cleaning, in September 2022, of a building uninhabitated since 1994 with dirty, bat feces, bird feathers, peeling and mold walls, with plants and without lighting**
- **11/10/2022: Reported the occurrence of a probable outbreak of histoplasmosis**
- **Up to 13/10/2022: 33 suspected cases, with 05 hospitalized patients and 03 referred for outpatient follow-up in the municipal primary care network**

Western blot





33 suspected cases : 22 WB positive (66%)

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Antigen detection

Table 3. Summary of sensitivity and specificity values of immunological tests used for diagnosis of endemic mycoses by antigen detection.

Disease	Test	Target	Specimen	Sensitivity	Specificity	References
Histoplasmosis	RIA	100 kDa (HPA)	Urine	96.7	100	[76]
			Serum	78.7	100	
	EIA	69–70 kDa	Serum	71.4	85.4	[80]
	EIA	Galactomannan	Urine	61.9–100	32–99.8	[82–87]
			Serum	92.3	99	[83]
			BAL	93.5	97.8	[84]
	EIA	Cell wall antigen	Serum	81	95	[79]
	EIA	100 kDa (HPA)	Urine	86	94	[81]
	LFA	Galactomannan	Urine	96	96	[88,89]
			Serum	92	94	

RIA: radioimmunoassay; EIA: enzyme immunoassay; LFA: lateral flow assay; LA: latex agglutination; ID: immunodiffusion; CSF: cerebrospinal fluid.

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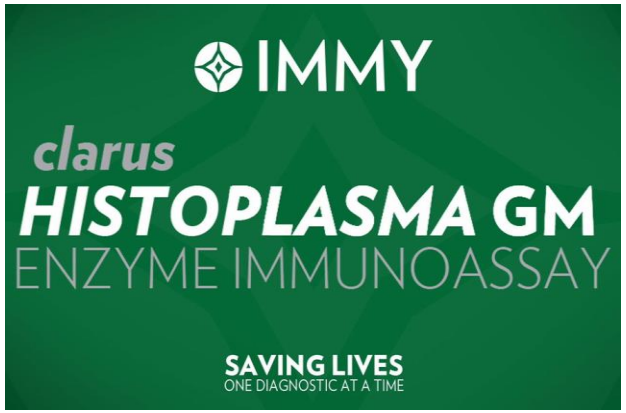
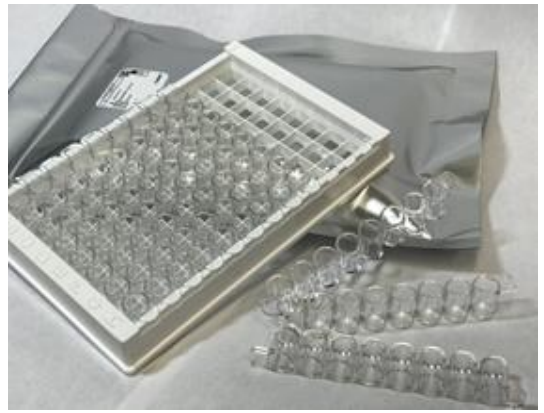
Our experience at INI/Fiocruz





ANTIGEN DETECTION FOR HISTOPLASMOSIS

JANUARY to DECEMBER 2021: 385 PACIENTS – 5 POSITIVES (1.3%)

JANUARY to SETEMBER 2022: 356 PATIENTS – 11 POSITIVES (3.0%)

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Detection of nucleic acids

Environmental samples
Clinical samples
→ Extraction → **Detecting *Histoplasma capsulatum* DNA and/or RNA specifically**

H.c.f. has been adapted for the detection of *H. capsulatum* var. *farciminosum*

conventional PCR

Frías de Leòn et al.[67]
SCAR220

nested and semi-nested PCR

Bialek et al.[71]
18S rDNA
Hcp100

Scantlebury et al.[44]
ITS
H.c.f.

Zatti et al.[59]; Ueda et al.[58]; Reid et al.[57]
ITS

Ohno et al.[61];
M antigen

Bracca et al. [42]
H antigen

Muraosa et al.[46]
NAALADase

realtime PCR

Simon et al.[49]; Gago et al.†[43]; Buitrago et al.[29]; Amorim-Conselheiro et al.[36]; Martagon-Villamil et al.[55]
ITS
Gómez et al.[65]; Koepsell et al.[63]
Hcp100
Gallo et al.[66]
CFP4
PPK
López et al.[62]
Hcp100
H antigen
M antigen
Muraosa et al.[46]
NAALADase
Babady et al.[48]
GAPDH
Highland et al.[64]
M antigen

others

Reverse transcriptase qPCR
Alanio et al.[28]
mtSSU

Loop-mediated isothermal amplification
Zatti et al.[59]
ITS1
Scheel et al.[50]
Hcp100

Fluorescence in-situ hybridization
da Silva et al.[72]
Hcp100

PCR-EIA
Tang et al.[73]
Hcp100
Lindsley et al.[54]
5.8S rDNA

Rolling circle amplification
Furuie et al.[53]
ITS

Mycoses. 2023;00:1–13.

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Our experience at INI/Fiocruz

Nested Hc 100	Culture	Sample
Negative	Negative	Sputum
Negative	NR	Blood
Negative	NR	Induced sputum
Negative	NR	BAL
Negative	NR	Blood
Negative	NR	Blood
Negative	NR	Sputum
Negative	NR	Blood
Negative	Negative	Blood
Negative	Negative	Blood
Positive	Positive	Bone marrow
Positive	Negative	Induced sputum
Positive	Positive	BAL
Positive	Positive	BAL
Positive	Negative	BAL
Positive	Positive	Bone marrow
Positive	Negative	Sputum

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Combination of methods for histoplasmosis diagnosis

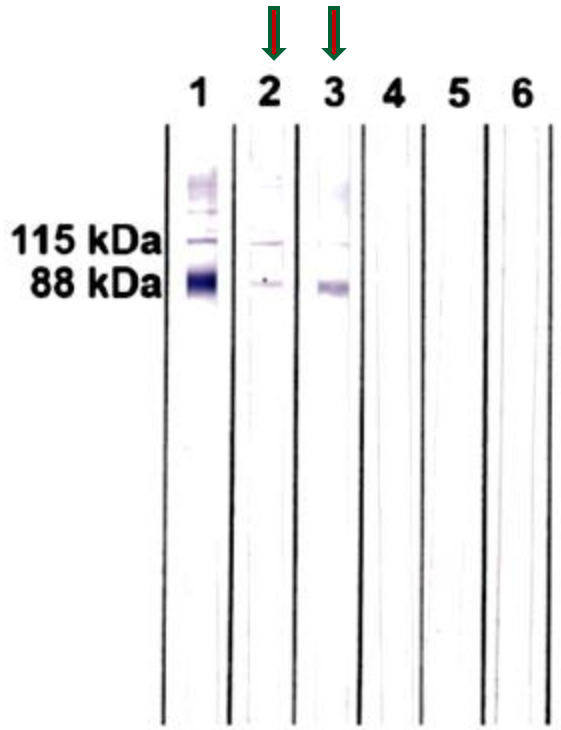

Figure 4. Western blot assay for anti-*Histoplasma* antibody detection: Line 1 = positive control (patient with proven histoplasmosis), line 2 = case 1, line 3 = case 2, line 4 = negative control (patient with COVID-19), line 5 = negative control (normal human serum), line 6 = secondary antibody control. Molecular weights of H and M antigens of *H. capsulatum* are indicated at the left.

Table 1. Galactomannan results of the two patients with acute pulmonary histoplasmosis following COVID-19.

Case	Urine (clarus, IMMY [1])	Serum (clarus, IMMY)	Serum (Platelia, Bio-Rad [2])
1	0.46	0.39	0.72
→ 2	1.38	0.33	0.56
PC [3]	37.64	35.98	1.21
NC [4]	0.13	0.28	0.25

[1] Results present as EIA units. Samples with EIA units ≥1.00 are considered positive; [2] Results present as index. Samples with an index ≥0.50 are considered positive; [3] PC: Positive control (patient with proven histoplasmosis); [4] NC: Negative control (patient with COVID-19).

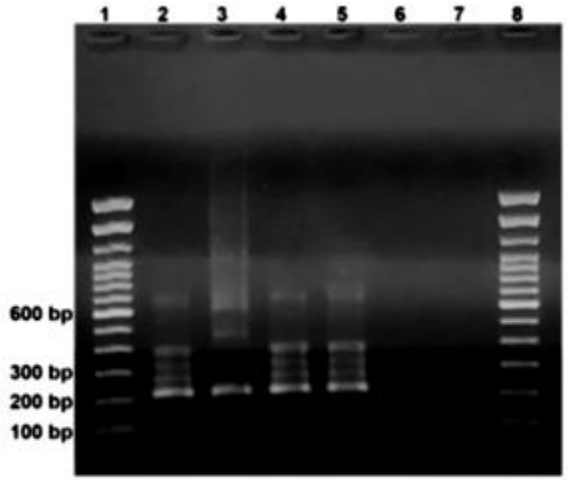

Figure 3. Nested Polymerase chain reaction for *H. capsulatum*: Slot 1 and 8 = molecular weight (100 bp DNA ladder— ThermoFisher Scientific, Inc.), slot 2 = case 1, slot 3 = case 2, slot 4 = positive control (patient with proven histoplasmosis), slot 5 = positive control (G217B DNA), slot 6 = negative control (patient with COVID-19), slot 7 = negative (water) control. The base pairs (bp) of representative bands are indicated at the left.

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Take home messages

- Serology is useful tool for rapid diagnosis of histoplasmosis
- Results may be obtianed several days before the clinical symptoms develop
- Continued screening allows to follow the progress of the disease
- Major disadvantage is cross reaction between various pathogens
- Sensitivity of test (different among methods)
- Association of serology, antigen detection increase the sensitivity of tests
- No serological test is confirmatory for the diagnosis of histoplasmosis

Obrigado
Merci

INI – EVANDRO CHAGAS

New Treatment strategies for histoplasmosis including oral Amphotericin B

Andréa d'Avila Freitas
MD and PhD –Infectious Diseases
Technologist in Public Health – Instituto Nacional de Infectologia Fundação Oswaldo Cruz – RJ - Brazil

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ANTIFUNGAL DRUGS FOR HISTOPLAMOSIS TREATMENT

-AMPHOTERICIN B IV

-ITRACONAZOLE (ORAL)

-POSACONAZOLE (ORAL)

- ISAVUCONAZOLE (IV AND ORAL)

IZAVUCONAZOLE

NEW GENERATION TRIAZOLE

FDA-APPROVED OF INVASIVE ASPERGILLOSIS AND MUCORMYCOSIS

IV AND ORAL FORMULATION

YEAST: *CANDIDA* spp, *CRYPTOCOCCUS* spp

HYALINE MOLDS: *ASPERGILLUS, *MUCORALES***

***In Vitro* ACTIVITY FOR ENDEMIC AND DIMORPHIC FUNGI:**
***BLASTOMYCES* , *COCCIDIODES* AND *HISTOPLASMA* (VITAL study)**

Thompson et al. – Clin Infect Dis 2016; 63: 356-362
Lewis II et al.- Antimicrob Agents Chemother 2022; 66:1-12

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IZAVUCONAZOLE

POTENTIAL ADVANTAGES:

-LACK OF QTc INTERVAL PROLONGATION

-MORE PREDICTABLE PHARMACOKINETICS (Therapeutic Drug Monitoring only for pediatric population and obese patients)

-LESS DRUG INTERACTION PROFILE

- IMPROVED TOLERABILITY

-LONGER HALF-LIFE (allowing once-daily dosing after initial loading dose)

-GOOD PENETRATION INTO CEREBRAL SPINAL FLUID AND CNS

DISADVANTAGE:

- VERY EXPENSIVE

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POSACONAZOLE

-SECOND-GENERATION TRIAZOLE

-FDA APPROVED AS ORAL SUSPENSION IN 2006, DELAYED-RELEASE TABLET IN 2013, AND IV IN 2014

- INDICATION: ASPERGILLOSIS, FUSARIOSIS, MUCORALES, SPOROTRICHOSIS, HISTOPLASMOSIS *

- SALVAGE TREATMENT OF HISTOPLAMOSIS

Ramos-Ospina N. et al. – Medical Mycology 2024; 62(7): myae 058
Restrepo A. et al- J Infect 2007; 54 (4): 319-17
Clark B. et al. - J Infect 2005;51 (3):e177-80

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POSACONAZOLE

- RETROSPECTIVE OBSERVATIONAL STUDY IN COLOMBIA (2016 to 2022)

-31 ADULT PATIENTS WITH DISSEMINATED HISTOPLASMOSIS

-HIV (38.7%), SOT (29%), AND ONCOLOGIC DISEASES (12.9%)

Induction therapy with L-AMB:

- 3mg/kg for 2 weeks

Consolidation:

-Itraconazole (9) – 300mg/day

-Posaconazole (22) – 300 mg/day

Ramos-Ospina N. et al. – Medical Mycology 2024; 62(7): myae 058

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POSACONAZOLE

FACTORS THAT INFLUENCED THE CHOICE BETWEEN ITRACONAZOL & POSACONAZOL :

- USE OF TRACOLIMUS AND CALCINEURIM INHIBITORS
- RISK OF HEPATOXICITY
- NARROWER SPECTRUM OF DRUG INTERACTIONS COMPARED TO ITRACONAZOLE

RESULTS:

-No relapses occurred

-Three deaths unrelated to histoplasmosis

Ramos-Ospina N. et al. – Medical Mycology 2024; 62(7): myae 058

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POSACONAZOLE

CONCLUSION:

POSACONAZOLE IS AN EFFECTIVE AND WELL-TOLERATED ALTERNATIVE FOR CONSOLIDATION TREATMENT

LIMITATIONS OF THE STUDY:

- ITS RETROSPECTIVE NATURE
- USE OF MEDICAL RECORDS AS A SECONDARY SOURCE
- LACK OF URINARY ANTIGEN
- LACK OF ITRACONAZOLE AND POSACONAZOLE TDM

Ramos-Ospina N. et al. – Medical Mycology 2024; 62(7): myae 058

L AMPHOTERICIN B

PROSPECTIVE RANDOMIZED MULTICENTER OPEN-LABEL TRIAL OF 1-or-2 DOSE INDUCTION THERAPY WITH L AMB X CONTROL FOR DISSEMINATED HISTOPLAMOSIS IN AIDS

Induction therapy with L-AMB:

- 1st arm: Single dose: 10 mg/kg IV
- 2nd arm: 2 doses: 10 mg/kg D1 and 5 mg/kg IV
- 3rd arm: CONTROL: 3mg/kg IV for 2 weeks

Consolidation:

- Itraconazole (Oral)

Pasqualotto, A.C. et al. - Clin Infect Dis 2023;77(8):1126-32

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L AMPHOTERICIN B

TOTAL : 118 PARTICIPANTS

PRIMARY ENDPOINT:

- Clinical Resolution on D14 (resolution of fever and signs/symptoms)

SECONDARY ENDPOINT:

- Overwall survival on D14
- Infusion-related, Renal, Kidney toxicity, Anemia, and Electrolytes abnormalities

Pasqualotto, A.C. et al. - Clin Infect Dis 2023;77(8):1126-32

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L AMPHOTERICIN B

RESULTS

- NO DIFFERENCES IN PRIMARY AND SECONDARY ENDPOINTS ON D14

ENDPOINTS	1 DOSE	2 DOSE	CONTROL	P -VALUE
CLINICAL RESPONSE	84%	69.0%	74.0%	
SURVIVAL	89.0%	78.0%	89.7%	0.440
INFUSION TOXICITY	18.9%	14.3%	10.3%	
SERUM K (< 3.5)	9%	19%	33.0%	0.40
SERUM K(<2.5)	----	----	6%	0.09
SERUM Mg (<1.8)	57%	41%	42%	0.405

- KIDNEY AND LIVER TOXICITIES WERE SIMILAR BETWEEN GROUPS
- SURVIVAL DID NOT DIFFER AT 1 YEAR AMONG GROUPS

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L AMPHOTERICIN B

CONCLUSION

A ONE-DAY HIGH DOSE OF L AMB FOLLOWED BY ITRACONAZOLE WAS SAFE AND EFFICACIOUS AS INDUCTION THERAPY OF DH IN HIV PEOPLE.

LIMITATIONS OF THE STUDY:

-SMALL NUMBER OF PATIENTS (PHASE II STUDY)
-ALL PATIENTS HAD "PROBABLE" HISTOPLASMOSIS RATHER THAN "PROVEN"
-A CONFIRMATORY PHASE-THREE CLINICAL TRIAL IS NEEDED

Pasqualotto, A.C. et al. - Clin Infect Dis 2023;77(8):1126-32

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ORAL LIPID AMPHOTERICIN B

THE USE OF NANOSTRUCTURED SYSTEMS FOR ANTIFUNGAL THERAPY BEGAN IN THE 1990s

The challenge is the development of an oral formulation of AmB that could:

- **Be Easy to administer**
- **Be Cost-effective**
- **Be Nontoxic**
- **Have excellent pharmacological activity**
- **Have clinical application**

Zhong, X. et al. Drug Delivery -2023; 30(*1) 2161671
Dalton, L.M. et al. Open Forum Infect Dis- 2024 PMID:38989533

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ORAL LIPID NANOCRYSTAL AMPHOTERICIN B

- MAT2203 (MATINAS Biopharma) is an investigational AmB formulation that uses a Rolled Phosphatidylserine Lipid Nanocrystal (LNC) bilayer structure to deliver AmB

The LNC has 3 components: Amphotericin B, Calcium, and phosphatidylserine

When the LNC is administered, target cells (e.g. macrophages) engulf and transport LNCs to sites of infection.

Boulware D.R. et al. Clin Infect Dis 2023 ;77(12) 1659-67

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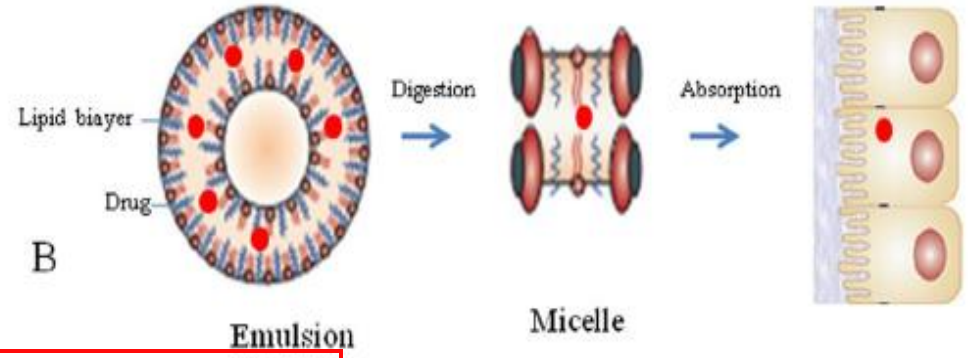
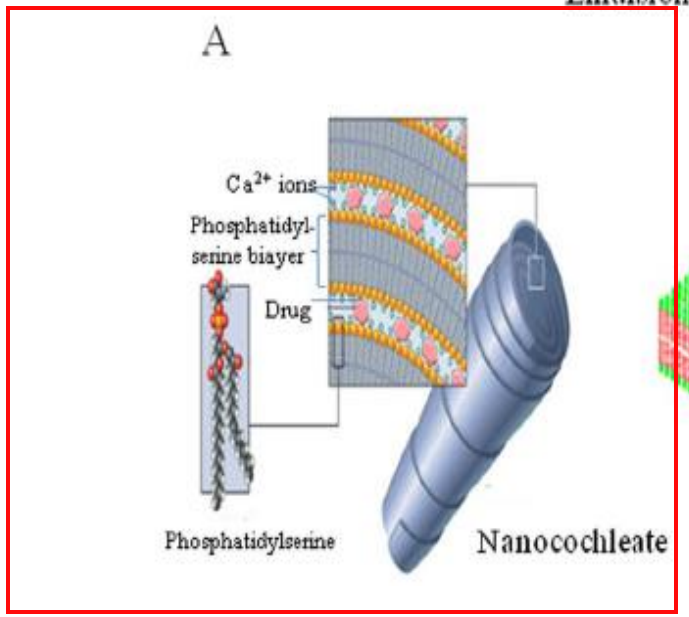
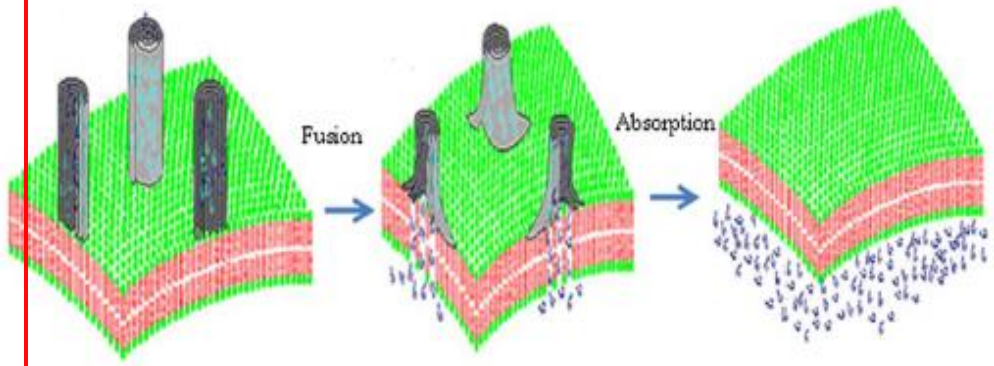
ORAL LIPID NANOCRYSTAL AMPHOTERICIN B







Zhong, X. et al. Drug Delivery -2023; 30(*1) 2161671

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Clinical Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Oral Lipid Nanocrystal Amphotericin B for Cryptococcal Meningitis: A Randomized Clinical Trial

David R. Boulware,[1,a] Mucunguzi Atukunda,[2,a] Enock Kagimu,[2] Abdu K. Musubire,[2] Andrew Akampurira,[2] Lillian Tugume,[2] Kenneth Ssebambulidde,[2,3] John Kasibante,[2] Laura Nsangi,[2] Timothy Mugabi,[2] Jane Gakuru,[2] Sarah Kimuda,[2] Derrick Kasozi,[2] Suzan Namombwe,[2] Isaac Turyasingura,[2] Morris K. Rutakingirwa,[2] Edward Mpoza,[2] Enos Kigozi,[4] Conrad Muzoora,[4] Jayne Ellis,[2] Caleb P. Skipper,[1] Theresa Matkovits,[5] Peter R. Williamson,[3] Darlisha A. Williams,[1] Ann Fieberg,[6] Kathy H. Hullsiek,[6] Mahsa Abassi,[1] Biyue Dai,[6] and David B. Meya[1,2]

[1]Department of Medicine, University of Minnesota, Minneapolis, Minnesota, USA; [2]Infectious Diseases Institute, Makerere University, Kampala, Uganda; [3]Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; [4]Department of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda; [5]Matinas Biopharma Nanotechnologies, Bedminster, New Jersey, USA; and [6]Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA

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NOVEL ID CASES

Oral Lipid Nanocrystal Amphotericin B (MAT2203) for the Treatment of Invasive Fungal Infections

Liam M. Dalton, Carol A. Kauffman, and Marisa H. Miceli

Division of Infectious Diseases, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA

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ORAL LIPID NANOCRYSTAL AMPHOTERICIN B

Table 1. Findings in of 5 Patients Treated With MAT2203

Patient	Age/ Sex	Organism	Infection Site	Prior Antifungal Therapy (Duration)	MAT2203 Duration	Adverse Effects	Response
1	38/F	*Rhodotorula mucilaginosa*	Bone	L-AmB (4 wk)	24 wk	None	Complete
2	61/M	*Candida krusei*	Bladder	AmB-d (4 d)	2 wk	Moderate diarrhea	Complete
3	40/F	*Fusarium* species	Burn wound	L-AmB (6 d)	17 d	None	Complete
4	48/F	*Fusarium falciforme*	Deep-tissue wound	Voriconazole (4 wk)	25 wk	Nausea, bloating "weird taste"	Complete
5	44/M	*Histoplasma capsulatum*	Disseminated with CNS involvement	L-AmB (6 wk); itraconazole (4 d); L-AmB (13 d)	Ongoing (>28 wk)	None	Improvement; ongoing therapy

Abbreviations: AmB-d, amphotericin B deoxycholate (given intravenously); CNS, central nervous system; F, female; L-AmB, liposomal amphotericin B (given intravenously); M, male.

The patient was asymptomatic, without adverse effects

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WHAT CAN WE CONSIDER AS NEW TREATMENT STRATEGIES FOR HISTOPLASMA?

- FUNGICIDAL EFFECT

-LESS TOXICITY, DRUG INTERACTION, AND ADVERSE EVENTS

-MORE PREDICTABLE PHARMACOKINETICS

-POSSIBILITY OF ORAL USE

-NO NEED FOR THERAPEUTIC DRUG MONITORING (TDM)

-ABILITY TO MARKEDLY SHORTEN AND SIMPLIFY TREATMENT

-LOWER COST

THANK YOU !

MERCI !