Cryptococcal infections in HIV-infected patients: what is new in 2014?

Epidemiology and microbiology of Cryptococcus spp. in HIV-infected patients

Manuel Cuenca Estrella

24th ECCMID May, 2014

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Conflict of interest disclosure

- In the past 5 years, M.C.E. has received grant support from **Astellas Pharma, bioMerieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering Plough, Soria Melguizo SA**, **Ferrer International**
- He has been an advisor/consultant to the **Panamerican Health Organization, Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer, and Schering Plough**.
- He has been paid for talks on behalf of **Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough**.

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Cryptococcosis in HIV. 2014

- Epidemiology:
  - Global burden
  - Regional variations
- Microbiology
  - Diagnosis. New methods?
  - Antifungal susceptibility testing
  - Serotypes and taxonomy
  - Regional distribution

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Epidemiology 2014

- 80-90% of cryptococcosis are associated to HIV infection
- Antiretroviral therapy decreased prevalence of infection in Western countries
- Common in sub-Saharan Africa and South-East Asia regions
- *C. neoformans/grubii* are the etiological agent in HIV+
- *C. gattii* prevalence is not well-defined in HIV-infected (Kaocharoen. PLoS Negl Trop Dis. 2013;7:e2297. Chen CID. 2012)

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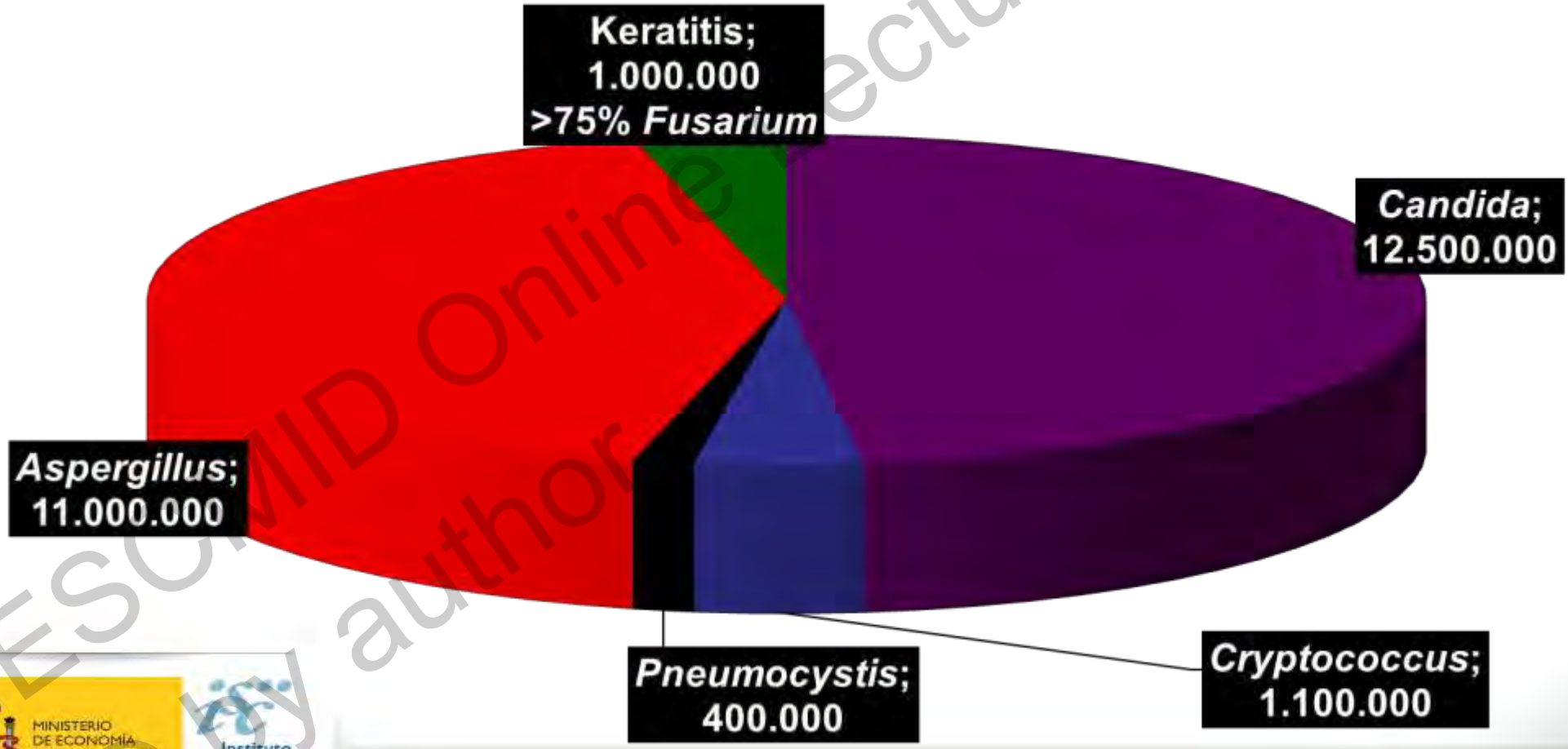
Relevant Mycoses. Global burden

According to LIFE and GAFFI (www.life-worldwide.org)



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Epidemiology 2014
(and several posters 24th ECCMID)

Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS

AIDS 2009, 23:525–530

Benjamin J. Park[a], Kathleen A. Wannemuehler[b], Barbara J. Marston[c], Nelesh Govender[d], Peter G. Pappas[e] and Tom M. Chiller[a]

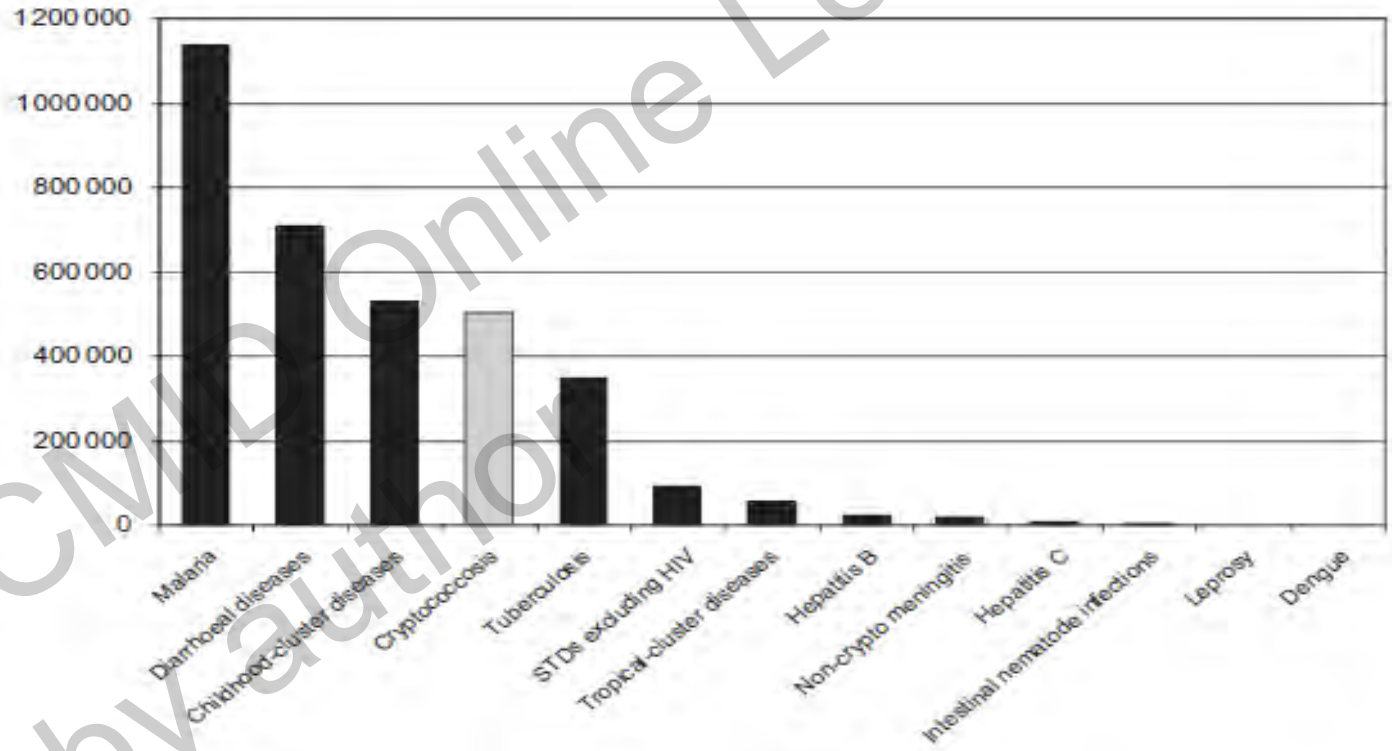

Fig. 1. Comparison of deaths in sub-Saharan Africa due to HIV-related cryptococcosis, as estimated in present study, and common infectious diseases excluding HIV, as estimated by World Health Organization. STD, sexually transmitted disease.

Cryptococcosis. Regional Variations Estimated number of cases

Park AIDS 2009; Rodriguez-Tudela CMI 2014 in press and 24th ECCMID posters

Region	Crypto meningitis	HIV prevalence	Deaths
Sub-Saharan Africa	720,000	22,500,000	500,000
South-East Asia	120,000	4,000,000	66,000
South-America	54,000	1,600,000	30,000
Eastern-Europe	27,000	1,600,000	15,000
Eastern-Asia	14,000 *	800,000 *	12,000 *
North-America	8,000	1,300,000	700
Western-Europe	1,000	1,100,000	90
Spain	15	120,000	1

Rate of Mortality
Estimated number of cases

Park AIDS 2009; Rodriguez-Tudela CMI 2014 in press and 24th ECCMID posters

Region	Crypto meningitis	Deaths	Rate %
Sub-Saharan Africa	720,000	500,000	70%
South-East Asia	120,000	66,000	55%
South-America	54,000	30,000	55%
Eastern-Europe	27,000	15,000	55%
Eastern-Asia	14,000 *	12,000 *	**
North-America	8,000	700	9%
Western-Europa	1,000	90	9%
Spain	15	1	7%

Diagnostic improvements in fungal diagnosis in last 20 years. Survey (www.life-worldwide.org)

- Non-culture based methods
  - *Aspergillus* antigen testing
  - *Histoplasma* antigen testing
  - Real Time PCR for many species
  - **Rapid antigen test for cryptococcal meningitis**
- Susceptibility testing of *Candida* and *Aspergillus*
- CT scanning of the chest
- Molecular identification of fungi and discovery of numerous cryptic species
- Direct identification from blood culture or agar plates

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Guidelines recommendations for Cryptococcal Ag test 2014

ESCMID AND ECMM PUBLICATIONS

Clinical Microbiology and Infection, Volume 20 Supplement 3, April 2014

ESCMID† and ECMM‡ joint clinical guidelines for the diagnosis and management of rare invasive yeast infections

M. C. Arendrup[1], T. Boekhout[2,3,4], M. Akova[5], J. F.Meis[6,7], O. A. Cornely[8], O. Lortholary[9,10] and on behalf of the ESCMID EFISG study group and ECMM*

Clinical Practice Guidelines for the Management of Cryptococcal Disease: 2010 Update by the Infectious Diseases Society of America

John R. Perfect,[1] William E. Dismukes,[2] Francoise Dromer,[11] David L. Goldman,[3] John R. Graybill,[4] Richard J. Hamill,[5] Thomas S. Harrison,[14] Robert A. Larsen,[6,7] Olivier Lortholary,[11,12] Minh-Hong Nguyen,[8] Peter G. Pappas,[2] William G. Powderly,[13] Nina Singh,[10] Jack D. Sobel,[10] and Tania C. Sorrell[15]

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Guidelines recommendations for Cryptococcal Ag test

Intervention	Recommendation
Ag detection in CSF or serum in HIV+	AII
Ag detection in non-HIV+	BII
Ag detection for therapeutic response evaluation in HIV+	BII
Ag detection in focal disease	BII
PCR-based methods	No recommendation so far
Other non-culture methods	Useless

Guidelines recommendations for Cryptococcal Ag test

Intervention	Recommendation
Ag detection in CSF or serum in HIV+	AII
A	
A	
e	
A	
PCR-based methods	No recommendation so far
Other non-culture methods	Useless

A II; Strongly recommended.
Evidence from at least 1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1 centre); from multiple time series; or from dramatic results of uncontrolled experiments

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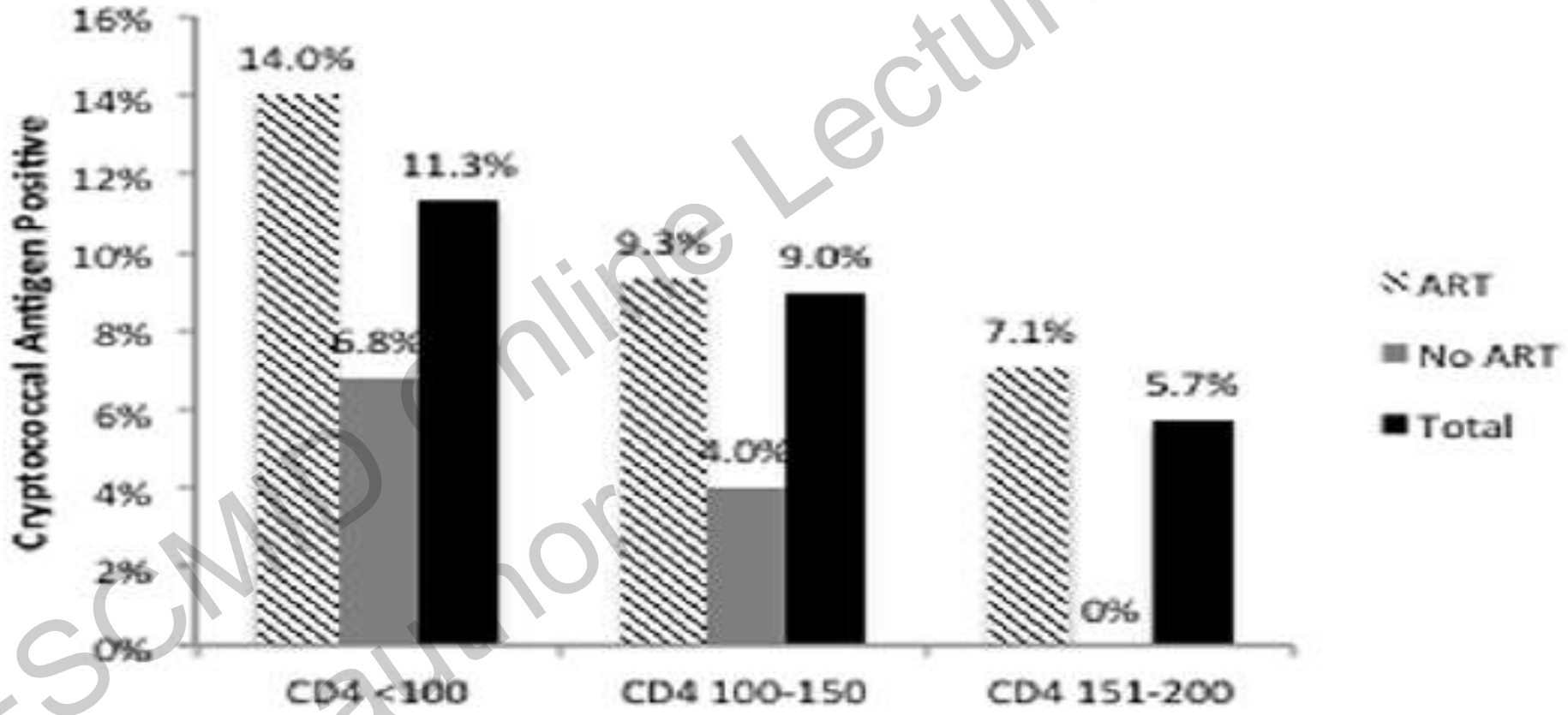
Diagnostic methods in Cryptococcosis Sensitivity

(Antinori ISRN AIDS, 2013)

Method	HIV+	African HIV+	Non-HIV	HIV+ Children
Ag detection in CSF in HIV+	92%	97%	96%	61%
Ag detection in serum in HIV+	96%	??	85%	29%
CSF culture	94%	90%	82%	88%
Blood-culture	47%	71%	27%	51%
India-ink	78%	91%	59%	88%

Is screening for asymptomatic antigenaemia possible for early diagnosis?
Additional use

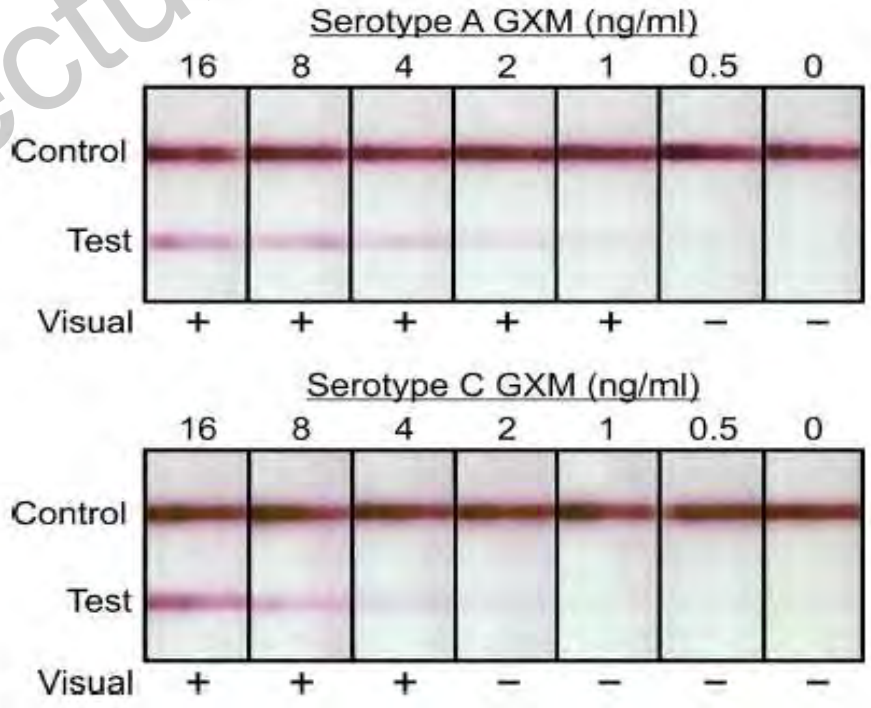


Alemu et al, PLoS One 2013;8:e58377

Lateral flow immunoassay for cryptococcal antigen – detects all serotypes

- Point of care testing being rolled out across S. Africa
- Screening of urine, serum or CSF
- 10 mins.
- $2
- Highly sensitive

Testing for cryptococcal antigens with a Lateral Flow assay

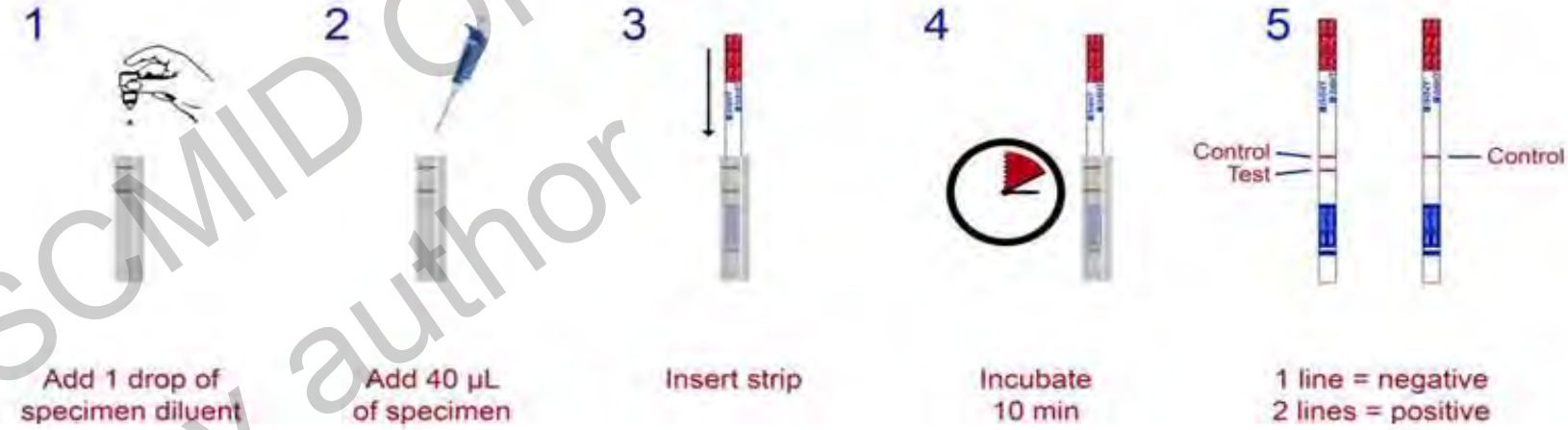


Gates-Hollinsworth & Kozel, Clin Vaccine Immunol 2013;20:634

Lateral-Flow device. Point-of-care (POC)
(www.imy.com)

The IMMY CrAg® LFA (Cryptococcal Antigen Lateral Flow Assay) is the first and only immunochromatographic dipstick assay for the qualitative and semiquanitative detection of cryptococcal antigen. This lateral flow assay is revolutionizing cryptococcal antigen testing, by delivering analytical sensitivity that is up to 200x more sensitive than other commercial assays. The CrAg® LFA is empowering health care providers in all clinical settings with rapid, reliable, and robust diagnostic results.

Procedure: 5-Easy Steps With No Specimen Pretreatment



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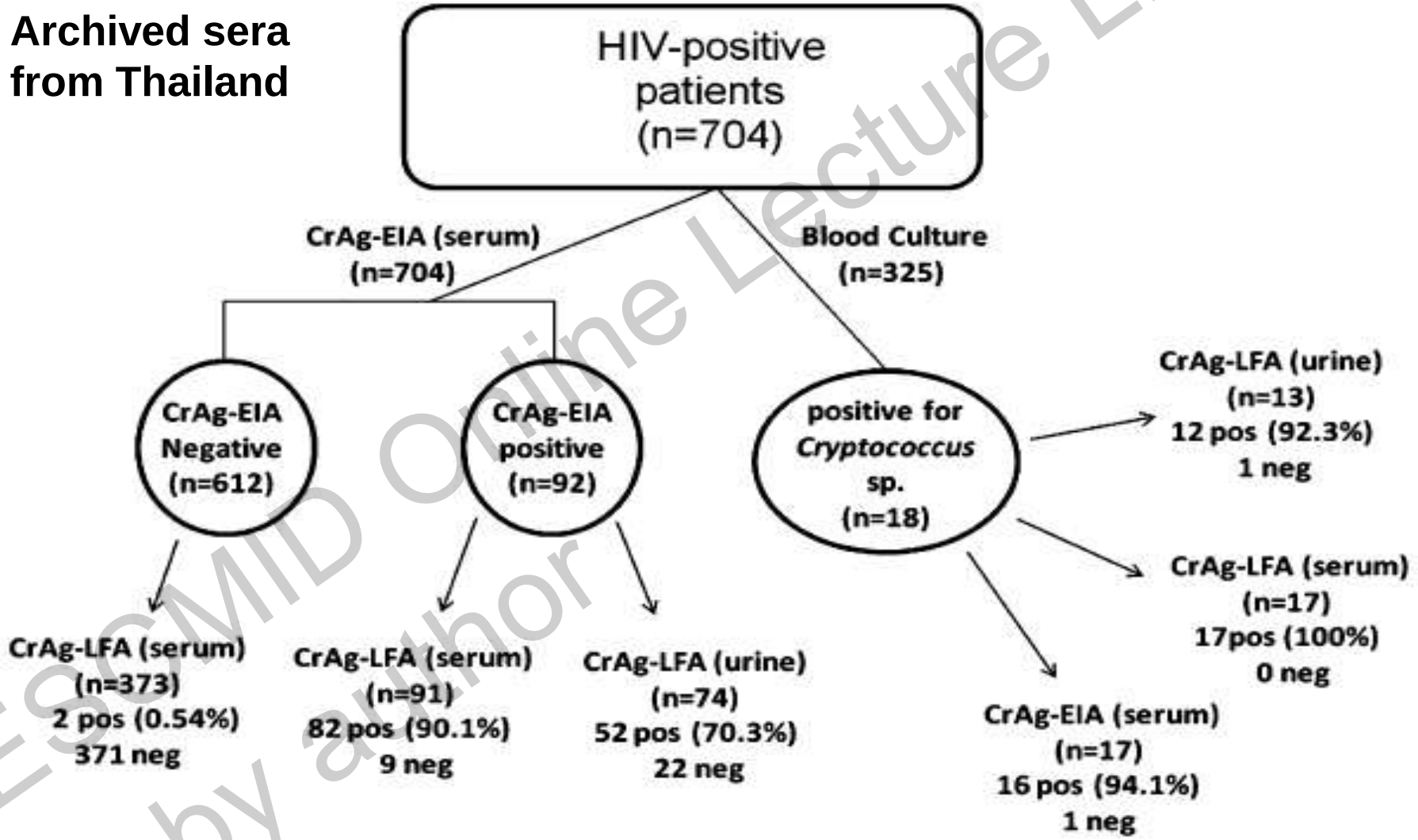
Lateral flow immunoassay for cryptococcal antigen

Archived sera from Thailand



Lindsley et al, Clin Infect Dis 2012;53:321

Guidelines recommendations for Cryptococcal PCR-based methods

Intervention	Recommendation
Ag detection in CSF or serum in HIV+	AII
Ag detection in non-HIV+	BII
Ag detection for therapeutic response evaluation in HIV+	BII
Ag detection in focal disease	BII
PCR-based methods	**No recommendation so far**
Other non-culture methods	Useless

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PCR-based methods
Diagnosis at Spanish Reference Lab

JCM
Journals.ASM.org

A Multiplex Real-Time PCR Assay for Identification of *Pneumocystis jirovecii*, *Histoplasma capsulatum*, and *Cryptococcus neoformans/Cryptococcus gattii* in Samples from AIDS Patients with Opportunistic Pneumonia

Sara Gago,[a] Cristina Esteban,[b] Clara Valero,[a] Óscar Zaragoza,[a] Jorge Puig de la Bellacasa,[b] María José Buitrago[a]
Mycology Department, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain[a]; Department of Clinical Microbiology, Hospital Clínic de Barcelona, Barcelona, Spain[b]

ORIGINAL ARTICLE MYCOLOGY

Efficacy of DNA amplification in tissue biopsy samples to improve the detection of invasive fungal disease

M. J. Buitrago[1], J. M. Aguado[2], A. Ballen[2], L. Bernal-Martinez[1], M. Prieto[3], A. Garcia-Reyne[2], J. Garcia-Rodriguez[3], J. L. Rodriguez-Tudela[1] and M. Cuenca-Estrella[1]
1) *Instituto de Salud Carlos III, Majadahonda,* 2) *Hospital Universitario '12 de Octubre', Instituto de Investigación Hospital '12 de Octubre' (i + 12), School of Medicine, Universidad Complutense, and* 3) *Hospital Universitario La Paz, Madrid, Spain*

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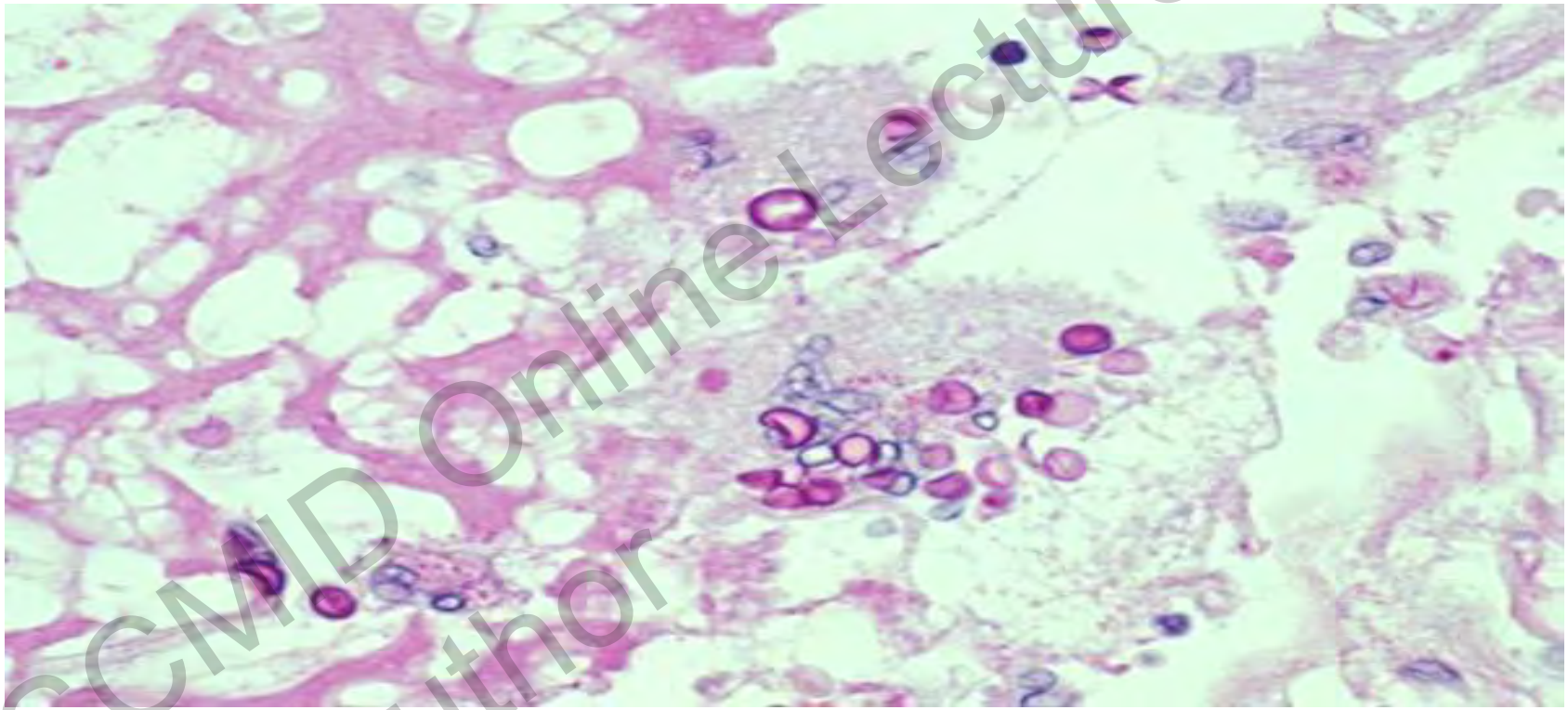
PCR in tissues or sterile clinical samples. Proven IFI

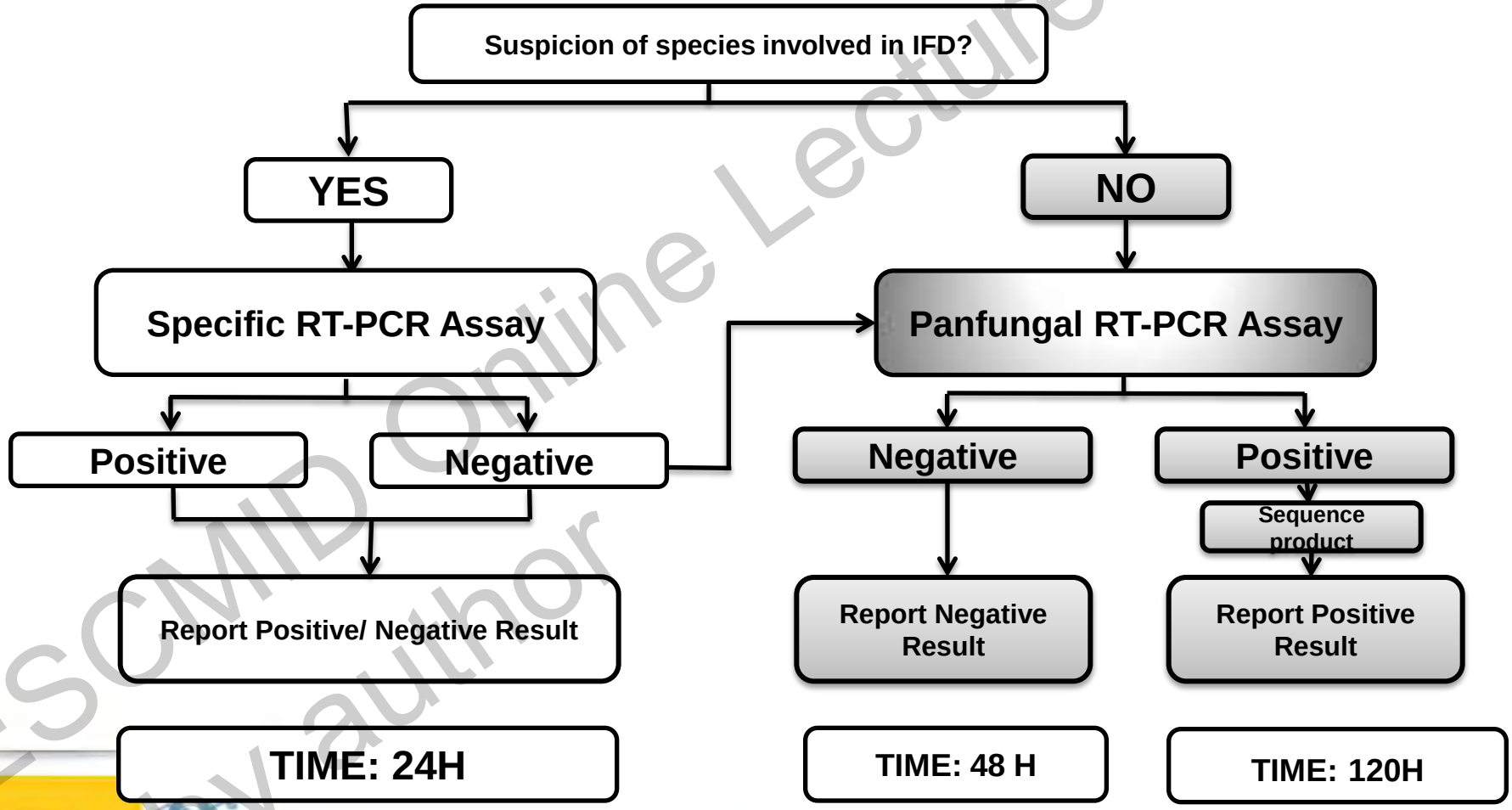


PAS stain of cryptococcal meningitis

http://info.fujita-hu.ac.jp/~tsutsumi/case/case104.htm

Scheme of procedures performed on biopsy samples
(Buitrago et al JCM 2014;52:1737)

Alternatively, when specific assays were negatives, a panfungal assay was performed



AST of *Cryptococcus* 2014

- Guidelines recommends AST for organisms from deep samples
- Reference procedures by EUCAST and CLSI
- No BPs available
- Low incidence in Western countries decreases its relevance

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Fungemia due to *Cryptococcus* in population-based surveys

(Arendrup, CMI 2014 and others)

Survey	Country	% Yeasts other than *Candida*	% *C. neoformans* among rare yeasts
National Survey	Denmark	1.0%	30%
Yeast Network Paris	France	5.1%	73%
US Cancer Center	USA	2.8%	40%
Sao Paulo Hospitals	Brazil	14.5%	45%
CANDIPOP study	Spain	5.5%	25%

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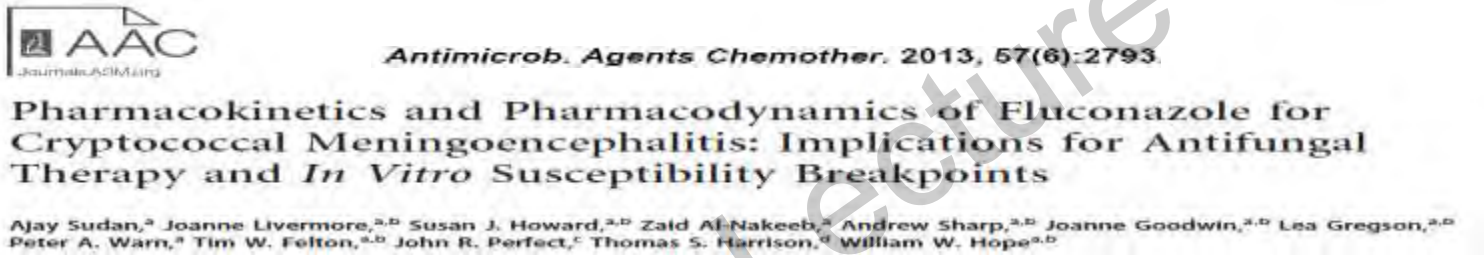
Tentative BPs of fluconazole for *Cryptococcus*



AAC Journals.ASM.org

Antimicrob. Agents Chemother. 2013, 57(6):2793

Pharmacokinetics and Pharmacodynamics of Fluconazole for Cryptococcal Meningoencephalitis: Implications for Antifungal Therapy and *In Vitro* Susceptibility Breakpoints

Ajay Sudan,[a] Joanne Livermore,[a,b] Susan J. Howard,[a,b] Zaid Al-Nakeeb,[a] Andrew Sharp,[a,b] Joanne Goodwin,[a,b] Lea Gregson,[a,b] Peter A. Warn,[a] Tim W. Felton,[a,b] John R. Perfect,[c] Thomas S. Harrison,[d] William W. Hope[a,b]

- Mice animal model
- *C. neoformans* with several FLC MIC (by CLSI) values and different dosage regimens
- AUC/MIC related to stasis: 389
- **≤ 2 mg/L, susceptible. > 2mg/L, resistant**

Is *C. neoformans* resistant to fluconazole? CLSI

ELSEVIER Diagnostic Microbiology and Infectious Disease 71 (2011) 252–259
www.elsevier.com/locate/diagmicrobio

Mycology

Wild-type MIC distributions and epidemiologic cutoff values for fluconazole, posaconazole, and voriconazole when testing *Cryptococcus neoformans* as determined by the CLSI broth microdilution method ☆

Michael A. Pfaller[a,b,*], Mariana Castanheira[b], Daniel J. Diekema[a], Shawn A. Messer[b], Ronald N. Jones[b,c]

Table 1
Wild-type MIC distributions of fluconazole, posaconazole, and voriconazole for 285 *Cryptococcus neoformans* strains obtained using CLSI reference broth microdilution methods[a]

Antifungal agent	No. of isolates with MIC (mg/L)												
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Fluconazole						3	2	24	62	140	45	8	1
Posaconazole			12	100	102	61	10						
Voriconazole	1	15	48	163	44	13	1						

[a] MIC values determined according to CLSI document M27-A3 (CLSI, 2008a) with an incubation time of 72 h.

Only 32% of isolates are susceptible in vitro to FLC

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Is *C. neoformans* resistant to fluconazole? EUCAST

Journal of Antimicrobial Chemotherapy (2005) **56**, 1144–1147
doi:10.1093/jac/dki393
Advance Access publication 9 November 2005

JAC

Rates of antifungal resistance among Spanish clinical isolates of *Cryptococcus neoformans* var. *neoformans*

Alexander Perkins, Alicia Gomez-Lopez, Emilia Mellado, Juan L. Rodriguez-Tudela and Manuel Cuenca-Estrella*

Table 1. Susceptibility data (in mg/L) of 317 clinical isolates of *C. neoformans* var. *neoformans*

Antifungal agent	MIC_{50}	MIC_{90}	Range	Mode	Geometric mean	Percentage of strains with antifungal resistance[a]
Amphotericin B	0.25	1.00	0.031–2	0.50	0.26	5.3
Flucytosine	4	16	0.125–128	8	4.16	46
Fluconazole	4	16	0.125–32	16	7.33	46.6
Itraconazole	0.25	1	0.015–2	0.25	0.26	15.8
Voriconazole	0.12	0.50	0.015–4	0.25	0.12	0.94
Ravuconazole	0.12	1	0.015–4	0.25	0.16	3.1

MIC_{50}, MIC value causing inhibition of 50% of isolates; MIC_{90}, MIC value causing inhibition of 90% of isolates.
[a]Antifungal resistance was defined as: MICs of amphotericin B ≥2 mg/L; MICs of flucytosine ≥8 mg/L; MICs of fluconazole ≥16 mg/L; MICs of itraconazole ≥1 mg/L; MICs of voriconazole ≥2 mg/L; MICs of ravuconazole ≥2 mg/L.[16–24]

Cryptococcal taxonomy and types 2014

- *Cryptococcus* genus under re-classification (>30 species now)
- *C. neoformans* complex with different sub-species OR two species *C. neoformans* and *C. gattii*
- Classic serotyping is still widely used:
  - Serotype A: *C. neoformans* var. *grubii*
  - Serotype B and C: *C. gattii*
  - Serotype D: *C. neoformans* var. *neoformans*
  - Serotype hybrid AD

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Cryptococcal genotypes 2014

- *C. neoformans* var. *grubii*:
  - VNI/AFLP1, VNII/AFLP1A
- AD hybrid:
  - VNIII/AFLP3
- *C. neoformans* var. *neoformans:*
  - VNIV/AFLP2
- *C. gattii*:
  - VGI/AFLP4,VGII/AFLP6, VGIII/AFLP5, VGIV/AFLP7

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Cryptococcal genotypes 2014

OPEN ACCESS Freely available online

PLOS | ONE

PLoS ONE 9(4): e94648. doi:10.1371/journal.pone.0094648

Identification of the Major Molecular Types of *Cryptococcus neoformans* and *C. gattii* by Hyperbranched Rolling Circle Amplification

Luciana Trilles[1,2*], Bin Wang[3], Carolina Firacative[1], Márcia dos Santos Lazéra[2], Bodo Wanke[2], Wieland Meyer[1]

ISHAM Study Group has recommended a MLST scheme and genotypes number is increasing

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Hindawi Publishing Corporation
ISRN AIDS
Volume 2013, Article ID 471363, 22 pages
http://dx.doi.org/10.1155/2013/471363

Review Article

New Insights into HIV/AIDS-Associated Cryptococcosis

Spinello Antinori

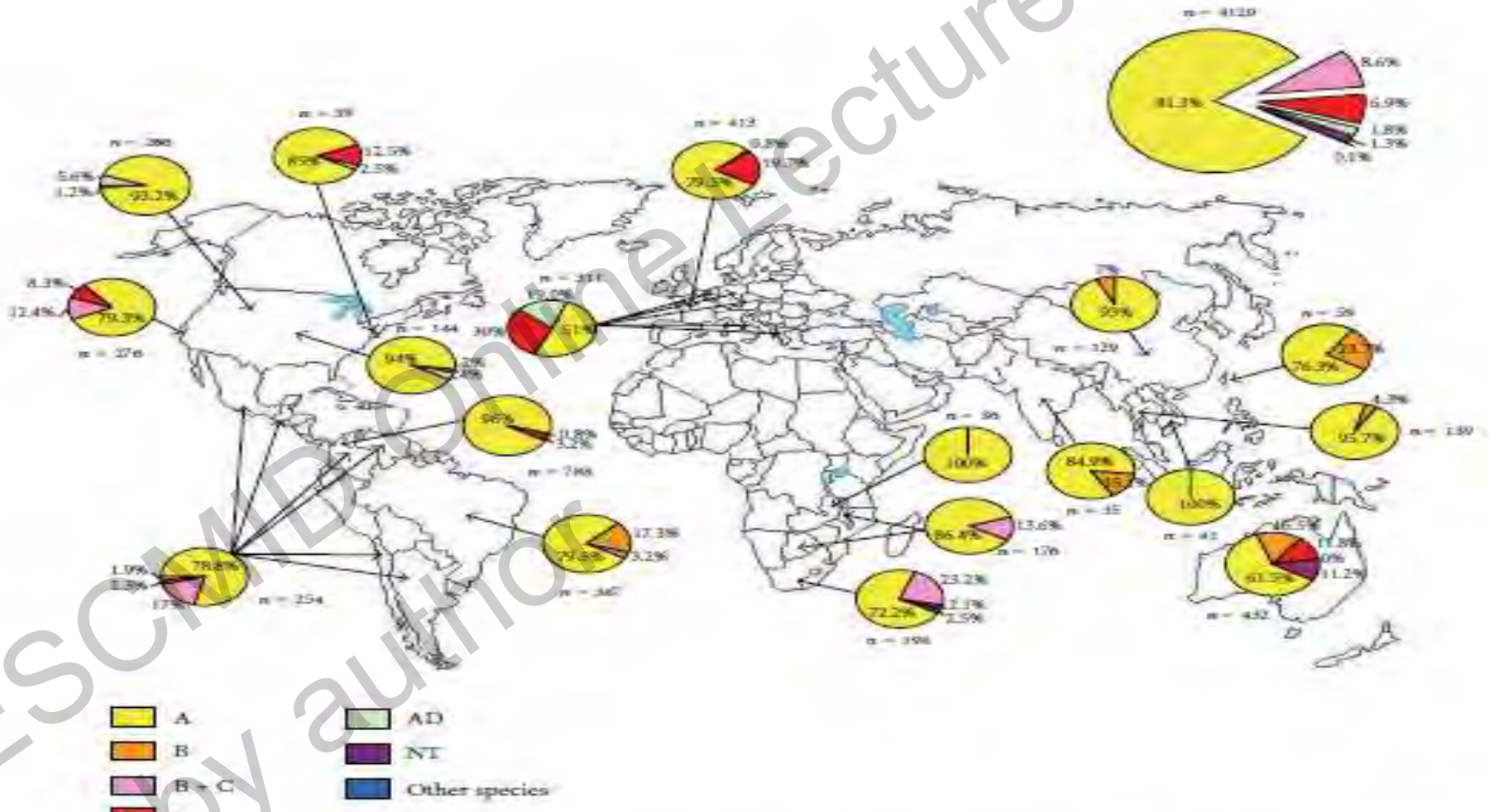

Figure 1: Worldwide geographic diffusion of different serotypes of *Cryptococcus neoformans* complex (based on [25–48]).

Cryptococcal genotypes 2014 Practical relevance

- Distinction between *neoformans/grubii* and *C. gattii* (Gomez-Lopez et al CMI 2008;14:716)

Table 1. Geometric means (GMs) of MIC values (mg/L), MIC ranges and MICs inhibiting 90% (MIC_{90}) and 50% (MIC_{50}), respectively, of the isolates included in this study

	Cryptococcus gattii (n = 23)				Cryptococcus neoformans (n = 340)			
Antifungal agent	GM	Range	MIC_{90}	MIC_{50}	GM	Range	MIC_{90}	MIC_{50}
Amphotericin B	0.09	0.03–0.25	0.125	0.125	0.24	0.03–4	1	0.25
Flucytosine	1.52	0.25–32	16	1	4.47	0.12 to >64	16	4
Fluconazole	15.52	4 to >64	25.6	16	7.57	0.12 to >64	16	8
Itraconazole	0.44	0.12–2	1	0.5	0.25	0.015–2	1	0.5
Voriconazole	0.47	0.03–1	1	0.5	0.12	0.015–4	0.5	0.125
Ravuconazole	0.41	0.03–2	2	0.5	0.15	0.015–4	1	0.125
Posaconazole	0.26	0.03–0.5	0.5	0.25	0.15	0.015–2	0.5	0.125
Caspofungin	17.51	8 to >16	>16	16	18.37	8 to >16	>16	16

Cryptococcal genotypes 2014 Practical relevance

- Distinction between *neoformans/grubii* and *C. gattii*
- There are some controversial results about AST of *grubii/neoformans*:
  - VNII and VNI (*grubii*) seems to be more resistant to azoles than other genotypes (*neoformans*)
  - Hybrid strains?
  - Policlonal infections?

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Future perspectives

- MLST and ultra-sequencing will change the genotyping. Hybrids and co-infection (Van Wyk, JCM 2014)
- Transcriptome description. Initiation and progression (Chen, MBio. 2014;5(1):e01087)
- *Titan* cells in *C. neoformans.* Tissue forms? (Zaragoza, Curr Opin Microbiol. 2013;16:409)
- Resistance to antifungal agents and most appropriate treatments

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Interested readers….

Drugs. 2013 May;73(6):495-504

Drugs
DOI 10.1007/s40265-013-0037-z

CURRENT OPINION

Cryptococcal Infections: Changing Epidemiology and Implications for Therapy

Ricardo M. La Hoz · Peter G. Pappas

Conclusions and controversial points

- *C. neoformans/grubii* is still one of the most common cause of death due to infection in the HIV-infected population (Africa and Asia). *C. gattii*???
- It is the third most common serious IFD
- Ag detection by LFD is already useful. Pre-emptive treatment
- PCR-based methods are available as well (molecular types)
- Fluconazole is not the best option for induction therapy (65-70% resistance in vitro)

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