ASIA FUNGAL WORKING GROUP
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INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

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# Update on serological tests to diagnose mould infections – 2023

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http://www.richardsonbio.cn

# Disclosures

- The speaker declares no conflict of interest.



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# Fungal serology: Asia 2023


| | Overall (*n*=235) | | <3000-US$ (*n*=89) | | 3000–20 000-US$ (*n*=102) | | >20 000-US$ (*n*=44) | |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % |
| **Serology** | 149 | 63.4 | 44 | 49.4 | 66 | 64.7 | 39 | 88.6 |
| *Aspergillus* spp. | 139 | 59.1 | 40 | 44.9 | 62 | 60.8 | 37 | 84.1 |
| *Onsite* | 93 | 39.6 | 21 | 23.6 | 49 | 48.0 | 23 | 52.3 |
| *Outsourced* | 46 | 19.6 | 19 | 21.3 | 13 | 12.7 | 14 | 31.8 |
| *Candida* spp. | 92 | 39.1 | 25 | 28.1 | 48 | 47.1 | 19 | 43.2 |
| *Onsite* | 55 | 23.4 | 9 | 10.1 | 36 | 35.3 | 10 | 22.7 |
| *Outsourced* | 37 | 15.7 | 16 | 18.0 | 12 | 11.8 | 9 | 20.5 |
| *Histoplasma* spp. | 63 | 26.8 | 25 | 28.1 | 18 | 17.6 | 20 | 45.5 |
| *Onsite* | 27 | 11.5 | 10 | 11.2 | 10 | 9.8 | 7 | 15.9 |
| *Outsourced* | 36 | 15.3 | 15 | 16.9 | 8 | 7.8 | 13 | 29.5 |



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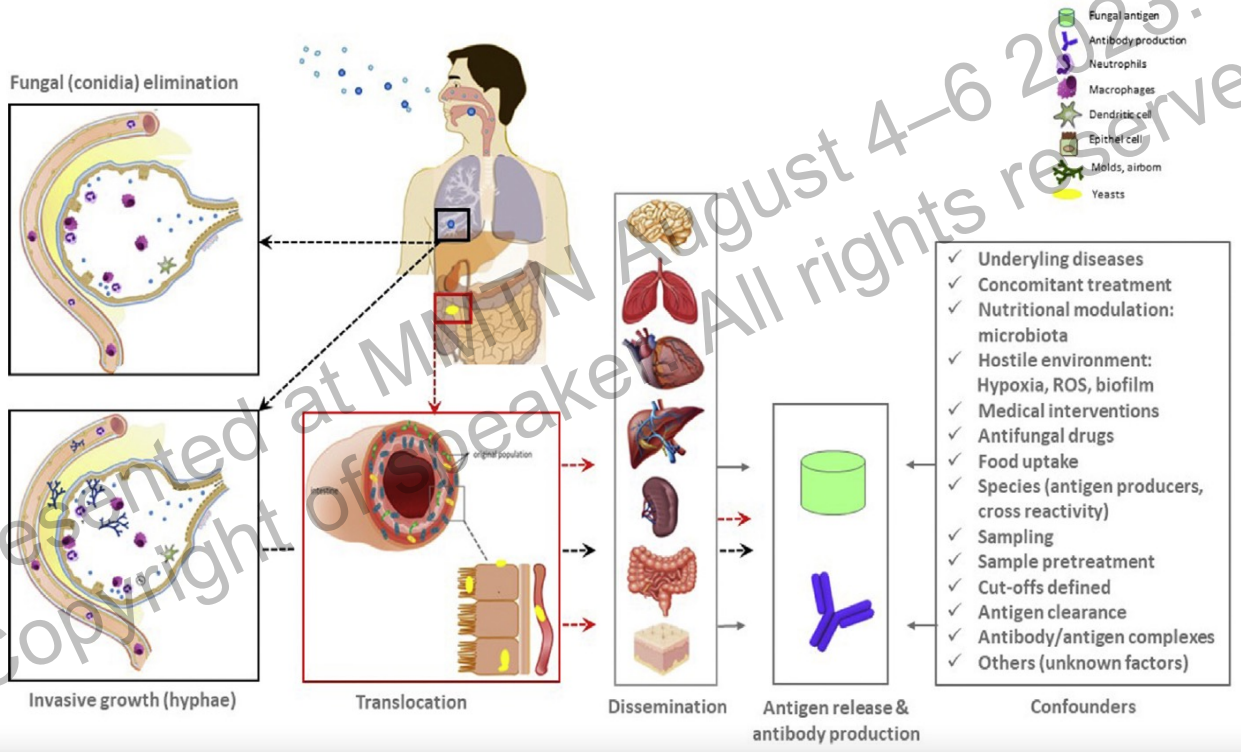
# Exposure pathways (1)



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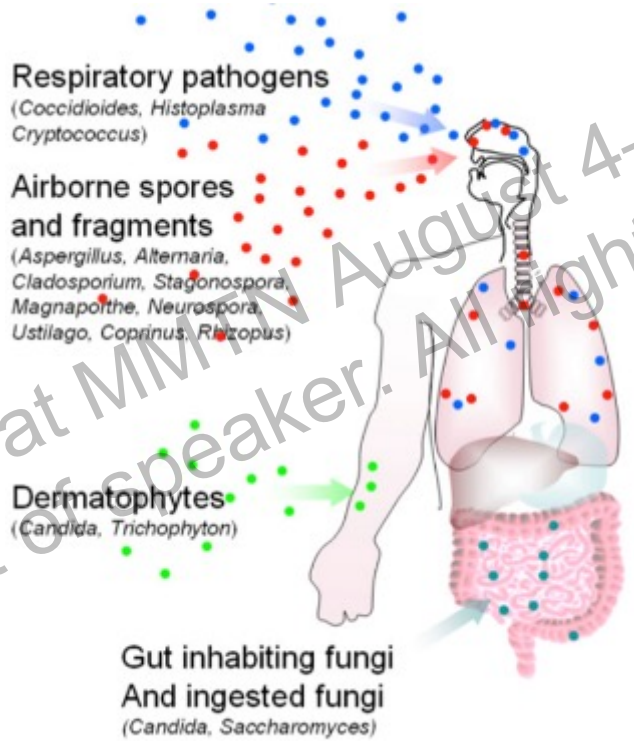
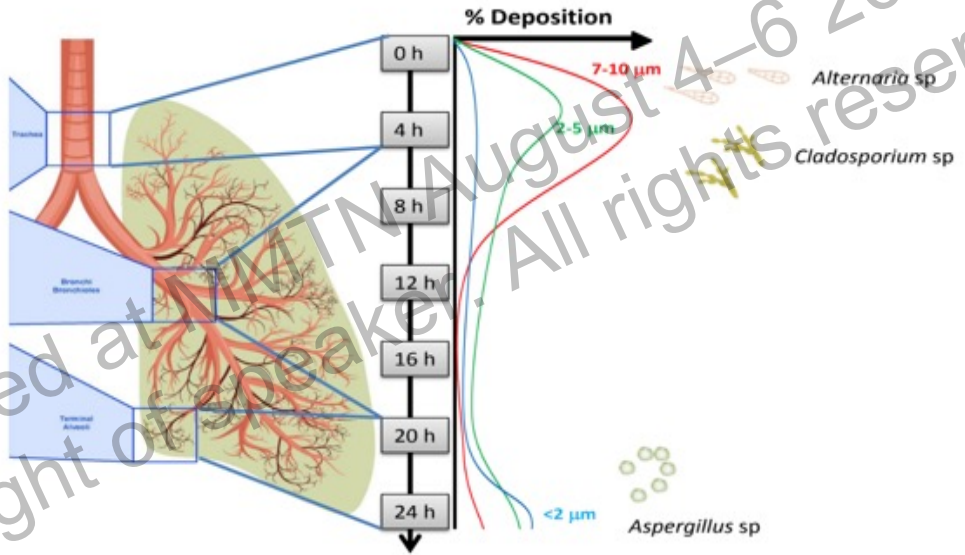
# Exposure pathways (2)



# DEPOSITION OF SPORES IN THE LUNG DEPENDS ON SPORE SIZE, BRANCHING AND TUBULE DIAMETER






Wiesner D, Klien B. *Curr Opin Microbiol* 2017;40:8–13.

# Serology – Definition

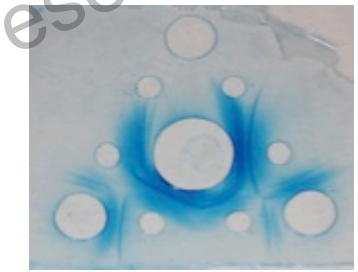
- Diagnostic identification of antibodies against a pathogen in serum
- 2013: "*Finally, the future of fungal serology is still to be seen. While future molecular technologies may supplant serologic methodologies, molecular methods for performance directly from patient specimens still need standardization. The current complexity of fungal serology testing relegates most antibody testing to only larger clinical and reference laboratories". (Lindsay, 2013, Current Fungal Infection Reports).*

# Serology – 1960s

Mycopathol Mycol Appl. 1963 Dec 30;21:272-8.

**THE PRECIPITIN TEST IN HUMAN SYSTEMIC ASPERGILLOSIS.**

STALLYBRASS FC.



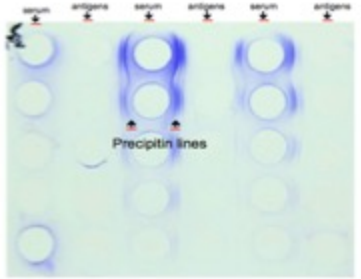
THE LANCET

Volume 283, Issue 7333, 14 March 1964, Pages 588–589
Originally published as Volume 1, Issue 7333

Preliminary Communications

DIAGNOSTIC PRECIPITIN TEST IN ASPERGILLUS PULMONARY MYCETOMA

JoanL Longbottom, M.SC., N.Z.[a], J Pepys, M.B. W'srand, M.R.C.P., M.R.C.P.E.[a], F.Temple Clive, M.B. Lond.[a, b]



# Serology – 1980s

*J Clin Pathol* 1982;**35**:1134–1137

## Rapid enzyme-linked immunosorbent assay (ELISA) for *Aspergillus fumigatus* antibodies

MD RICHARDSON, JUDITH M STUBBINS, DW WARNOCK

*From the Department of Microbiology, Bristol Royal Infirmary, Bristol BS2 8HW*



*Correlation between AGDD, long and rapid ELISA for IgG antibodies to A fumigatus*

| | | *Number and (%) of sera giving positive reactions* | | | |
|---|---|---|---|---|---|
| | | *Antigen 1* | | *Antigen 2* | |
| *AGDD reaction* | *No of sera* | *Long ELISA* | *Rapid ELISA* | *Long ELISA* | *Rapid ELISA* |
| Negative: antigens 1 and 2 | 13 | 0 | 0 | 0 | 0 |
| Positive: antigen 1, negative: antigen 2 | 2 | 1(50) | 0 | 0 | 0 |
| Negative: antigen 1, positive: antigen 2 | 2 | 2(100) | 2(100) | 2(100) | 1(50) |
| Positive: antigens 1 and 2 | 11 | 11(100) | 11(100) | 9(82) | 8(73) |

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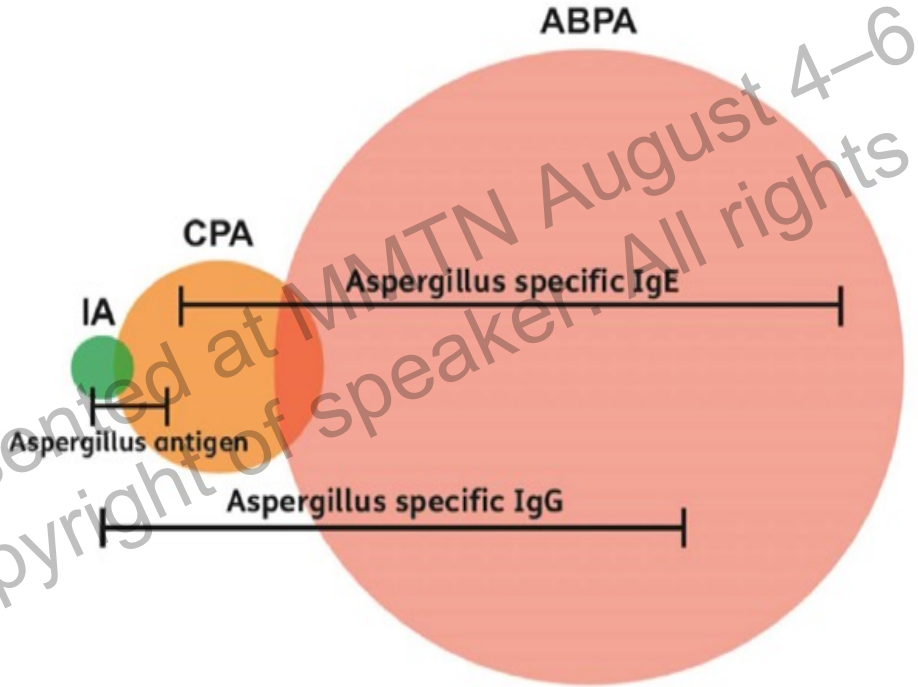
# Application of antibody tests in the diagnosis of pulmonary aspergillosis – 2023



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# *A. fumigatus*-specific IgG antibody assays

- Traditional immunodiffusion/counter-immunoelectrophoresis precipitins less sensitive than immunoassays
- In-house immunoassays unstandardized
- Standardized commercial immunoassays
  - ImmunoCAP: Cutoffs 27–90 mg/L in different populations (cases, controls)
  - Newer alternatives (Bio-Rad, Bordier, Virion/Serion)
    - Variety of antigens employed




Barton RC, et al. *Diagn Microbiol Infect Dis* 2008;62:287; Baxter CG, et al. *Clin Microbiol Inf* 2013;19:E197; Dumollard C, et al. *J Clin Microbiol* 2016;54:1236; Barrera C, et al. *Clin Vacc Immunol* 2016;23:196; Agarwal R, et al. *Mycoses* 2017;60:33

# Lateral Flow Devices – do they fulfil the criteria for POC Tests?

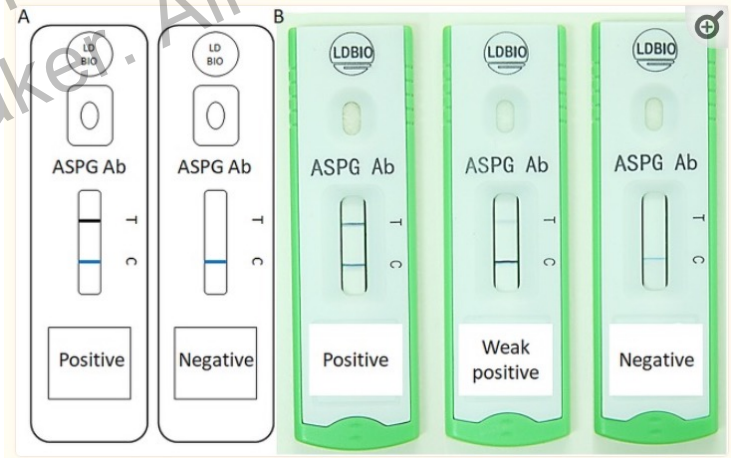
- Affordable,
- Sensitive,
- Specific,
- User-friendly,
- Rapid/robust,
- Equipment-free
- Deliverable to end users (ASSURED)

Journal of Clinical Microbiology

J Clin Microbiol. 2019 Sep; 57(9): e00538-19. PMCID: PMC6711894
Published online 2019 Aug 26. Prepublished online 2019 Jun 19. doi: 10.1128/JCM.00538-19 PMID: 31217272

Evaluation of LDBio *Aspergillus* ICT Lateral Flow Assay for IgG and IgM Antibody Detection in Chronic Pulmonary Aspergillosis

Elizabeth Stucky Hunter,[a] Malcolm D. Richardson,[a,c] and David W. Denning[a,b]



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# Invasive pulmonary aspergillosis (IPA)

Yu *et al. BMC Pulmonary Medicine* (2020) 20:89
https://doi.org/10.1186/s12890-020-1125-y

BMC Pulmonary Medicine

RESEARCH ARTICLE Open Access

## Potential value of serum *Aspergillus* IgG antibody detection in the diagnosis of invasive and chronic pulmonary aspergillosis in non-agranulocytic patients

Qihong Yu[1], Jingdong He[2], Bin Xing[1], Xin Li[3], Hongyu Qian[1], Hong Zhang[4], Meilin Xu[5] and Haiying Peng[1*]



- Detection of *Aspergillus* IgG distinguished IPA from community acquired bacterial pneumonia and health controls
- Conclusion: "Serum *Aspergillus* IgG antibody detection may have certain value in the diagnosis of IPA

Yu Q, et al. *BMC Pulmonary Medicine* 2020;20:89.

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# Talaromycosis

- Endemic in Southeast Asia
- Fatal disease in HIV-infected individuals
- Antibody tests: Specific but less sensitive than culture
  - Immunodiffusion
  - Indirect immunofluorescence
  - ELISA

Diagnostic Microbiology and Infectious Disease 96 (2020) 114959

Contents lists available at ScienceDirect

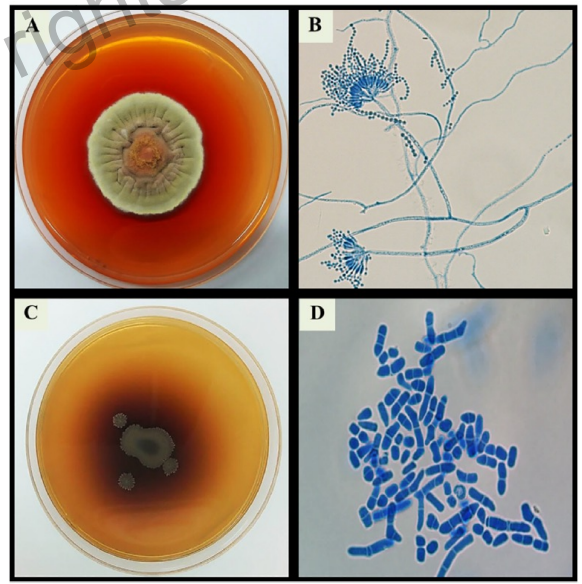
Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

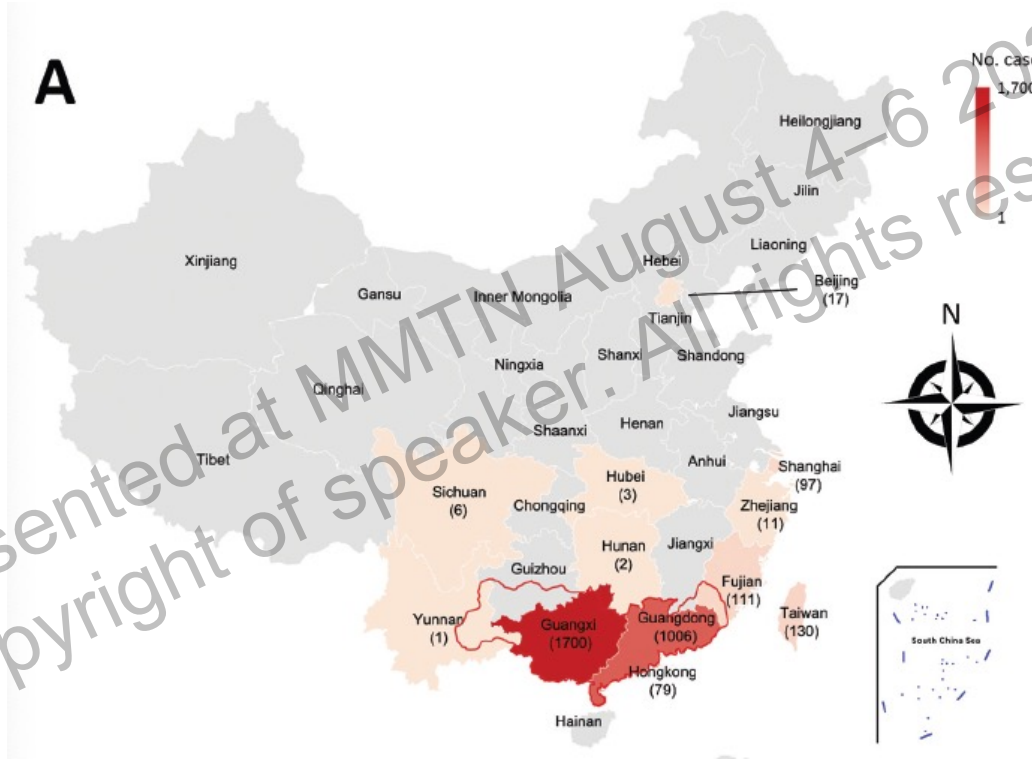
Review

## Diagnostic laboratory immunology for talaromycosis (penicilliosis): review from the bench-top techniques to the point-of-care testing

Kritsada Pruksaphon [a], Akarin Intaramat [b], Kavi Ratanabanangkoon [c], Joshua D. Nosanchuk [d], Nongnuch Vanittanakom [e], Sirida Youngchim [c,*]



# Talaromycosis – Endemic areas






Reports: January 1950 – October 2019
Zhou et al. *Emerging Infectious Disease* 2020.

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# Talaromycosis – Antibody detection (1994–2011)

| # | Methods | Diagnostic antigen | Diagnostic sensitivity[a] | Diagnostic specificity[b] |
|---|---|---|---|---|
| 1 | Immunodiffusion (microimmunodiffusion) | Exoantigen | 25% (2/8) | N/A |
| 2 | Immunodiffusion | Fission arthroconidia filtrate | 11.7% (2/17) | 100% (0/40) |
| 3 | Indirect immunofluorescent assay | Germinating conidia and yeast-hyphae cells | 100% (8/8)<br>IgG titer >160 | N/A |
| 4 | Immunoblotting | Secreted yeast early stationary phase exoantigen profiles | 200 kDa: 72.7% (24/33)<br>88 kDa: 94% (31/33)<br>54 kDa: 60.6% (20/33)<br>50 kDa: 57.6% (19/33) | 200 kDa: 79.3% (23/29)<br>88 kDa: 93.1% (27/29)<br>54 kDa: 13.8% (4/29)<br>50 kDa: 10.8% (3/29)<br>(for AIDS patients without talaromycosis) |
| 5 | Immunoblotting | 38 kDa of mycelial cell culture filtrate | 45% (23/51) | 28% (11/39) cross- reacted with cryptococcosis<br>21% (6/28) cross-reacted wit candidiasis |
| 6 | Immunoblotting | Cytoplasmic yeast antigen (TM CYA) profiles | 61 kDa: 48% (10/21)<br>54 kDa: 71% (15/21)<br>50 kDa: 48% (10/21)<br>86% (18/21)<br>Recognized at least 1 band of TM CYA | 100% (0/80) |
| 7 | Indirect ELISA | Recombinant fusion Mp1p (expressed in *Escherichia coli*) | 82% (14/17) | 100% (0/165) |
| 8 | Immunoblotting | Recombinant Mp1p6 | 95% (19/20) | 100% (0/35) |
| 9 | Immunoblotting | Recombinant Hsp30 fusion protein | 20% (2/10) | 100% (0/10) |
| 10 | Indirect Mp1p IgG ELISA | Recombinant Mp1p (expressed in *Pichia pastoris*) | 30% (6/20) | 98.5% (532/540) |



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# Mucormycosis


Journal of Fungi

MDPI

Review

**Epidemiology and Diagnosis of Mucormycosis: An Update**

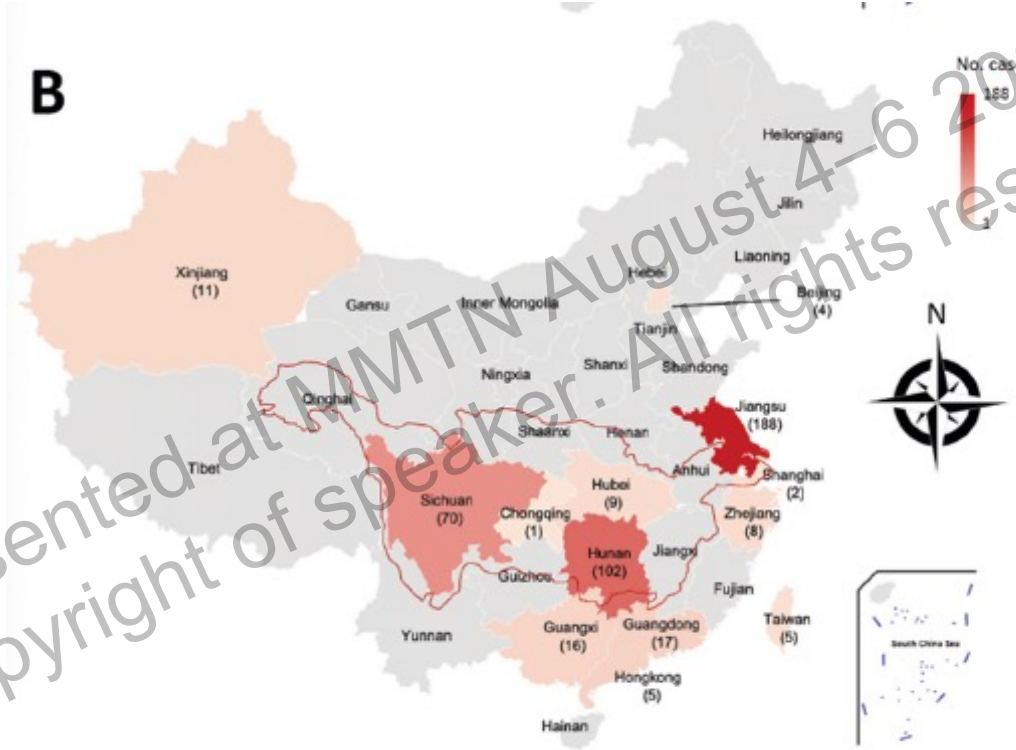
Anna Skiada [1,*], Ioannis Pavleas [2] and Maria Drogari-Apiranthitou [3]



- No commercially available serological tests
- New direction: monoclonal antibody 2DA6 against purified fucomannan of agents of mucormycosis used to develop an antigen lateral flow assay

Skiada A, et al. *J Fungi (Basel)* 2020;6:265.

# Histoplasmosis in China – High prevalence areas






Zhou et al. *Emerging Infectious Disease* 2020.

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# Histoplasmosis – Serological evidence of acute infection

Serologic evidence of acute infection per CSTE criteria[a]

| Criterion[b] | Specimen source[c] |
|---|---|
| ≥4-fold rise in *H. capsulatum* CF titers taken at least 2 wk apart | Serum |
| Detection of H band by *H. capsulatum* ID test | Serum |
| Detection of M band by *H. capsulatum* ID test after documented lack of M band on previous test | Serum |
| Detection of *H. capsulatum* antibodies by single CF titer of ≥1:32 | Serum or CSF |
| Detection of M band by *H. capsulatum* ID test without previous negative test | Serum or CSF |



CSTE = Council of State and Territorial Epidemiologists
CF = complement fixation
ID = immunodiffusion
CSF = cerebrospinal fluid

Azar MM, et al. *J Clin Microbiol* 2017;55:1612–1620.

# Histoplasmosis – Serology – Sensitivity

Applied Microbiology and Biotechnology (2021) 105:1837–1859
https://doi.org/10.1007/s00253-021-11170-9

MINI-REVIEW

## Diagnosis of histoplasmosis: current status and perspectives

María Agustina Toscanini[1] · Alejandro David Nusblat[1] · María Luján Cuestas[2]

| Acute pulmonary | Subacute pulmonary | Chronic pulmonary | Meningeal | Disseminated |
|---|---|---|---|---|
| 40–80% | 78–92% | 65–100% | 59–89% (in CSF) | 58–73% |



Toscanini M, et al. *Appl Microbiol Biotechnol* 2021;105:1837–1859.

# Commercially available serology tests – Dimorphics

| MVD Test | Specimen | Sensitivity | Specificity |
|---|---|---|---|
| ***Histoplasma* antigen**[1] | Urine-disseminated, AIDS | 97% | 99% |
| | Serum-disseminated, AIDS | 100% | 100% |
| | CSF-meningitis | 78% | 93% |
| | BAL-pulmonary | 94% | 98% |
| ***Histoplasma* IgG and IgM antibody** | Serum-pulmonary, acute | 89% | 95% |
| | CSF-meningitis | 82% | 93% |
| ***Coccidioides* antigen**[1] | Urine-disseminated[3] | 51% | 98% |
| | Serum-disseminated[3] | 72% | 100% |
| | Urine or serum, disseminated[3] | 80% | ND |
| | CSF-meningitis | 91% | 100% |
| | BAL-pulmonary | ND2 | ND |
| ***Coccidioides* IgG and IgM antibody** | Serum-pulmonary, | 88% | 90% |
| | disseminatedCSF-meningitis[3] | 73% | 88% |
| ***Blastomyces* antigen** | Urine-pulmonary or disseminated | 90% | 99% |
| | Serum-pulmonary or disseminated | 57% | ND[2] |
| | CSF-meningitis[3] | 86% | ND |
| | BAL | 80% | 98% |
| ***Blastomyces* IgG antibody (RUO)**[4] | Serum, pulmonary or disseminated | 88% | 99% |



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# Pythiosis

- *Pythium insidiosum* – pathogenic in mammals – horses/dogs
- Human infections: Primarily in Thailand
- Early diagnosis essential:
  - Clinical: Cutaneous and vascular
  - Direct microscopy and culture
  - Detection of anti-*P. insidiosum* antibody:
    - Immunodiffusion
    - ELISA
    - Western blotting
  - PCR and sequencing

Vascular pythiosis – Thalassemic patient

# New developments: Antibody tests

- *Schizophyllum commune* – Patients with ABPM/fungal ball
- *Rhizopus oryzae* – Aspartic protease allergen Rhi o 1
- Pneumocystosis
- Cryptococcosis
- Sporotrichochosis
- Scedosporiosis

# Biomarkers for mold infections

- Galactomannan – pan-fungal: ELISA, LFD, LFA
  - Bio-Rad
  - Virclia
  - Euroimmun
  - OLM
  - IMMY
- Gliotoxin

# Further reading

Current Fungal Infection Reports (2018) 12:127–136
https://doi.org/10.1007/s12281-018-0321-1

ADVANCES IN DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS (S CHEN, SECTION EDITOR)

## Role of Serological Tests in the Diagnosis of Mold Infections

Malcolm Richardson[1,2] · Iain Page[2,3]

Clinical Microbiology and Infection 27 (2021) 1230–1241

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Narrative review

## Serology anno 2021—fungal infections: from invasive to chronic

Cornelia Lass-Flörl*, Eldina Samardzic, Miriam Knoll

Curr Fungal Infect Rep (2016) 10:43–50
DOI 10.1007/s12281-016-0254-5

CLINICAL MYCOLOGY LAB ISSUES (K LAGROU, SECTION EDITOR)

## Point of Care Testing for the Diagnosis of Fungal Infections: Are We There Yet?

Juergen Prattes[1,2] · Sven Heldt[1] · Susanne Eigl[1] · Martin Hoenigl[1,2,3,4]

Current Fungal Infection Reports
https://doi.org/10.1007/s12281-023-00462-4

## Developments in Fungal Serology

2023

P. Lewis White[1]



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# Thank you