Regional MMTN Conference

Advances in Fungal Diagnostics and Application in Asian Laboratories

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Bangkok, Thailand

November 17th, 2018. 11:00-11:30 a.m.

Current situation of fungal diseases

Fungal infection diseases

- kill more than 1.5 million
- affect over a billion people
- are still a neglected topic by public health authorities

Serious fungal infections occur as a consequence of other health issues:

- Asthma
- AIDS
- Cancer
- organ transplantation
- corticosteroid therapies
- etc.

Recent global estimates per year :

- ~3,000,000 cases of chronic pulmonary aspergillosis
- ~223,100 cases of cryptococcal meningitis complicating HIV/AIDS
- ~700,000 cases of invasive candidiasis
- ~500,000 cases of *Pneumocystis jirovecii* pneumonia
- ~250,000 cases of invasive aspergillosis
- ~100,000 cases of disseminated histoplasmosis
- over 10,000,000 cases of fungal asthma
- ~1,000,000 cases of fungal keratitis

Bongomin F et al. 2017

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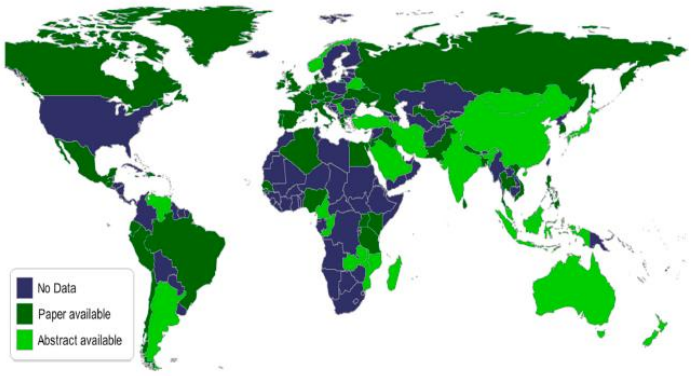
Burden of serious fungal infections country by country for over 5.7 billion people (>80% of the world's population) during 2014-2017

By the Leading International Fungal Education (LIFE) initiative: 43 published papers



The main fungal pathogens responsible for the majority cases of serious fungal disease

- Endemic dimorphic fungi: *H. capsulatum*
- Mucormycosis
- *C. albicans* is the main agent responsible for mucosal disease
- *A. fumigatus* for most allergic fungal disease
- *Cryptococcus* species, *P. jirovecii*
- *Trichophyton* spp., especially *T. rubrum*, for skin infections.

Bongomin F et al. 2017

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Diagnostic of Fungal Infections before 2014 *Malhotra et al. 2014*

Methods	Classical approach	Advance approach
Direct examination	- KOH preparation - KOH preparation plus calcofluor white - India ink preparation - Gram stain	- New technology of microscope (Differential Interference Contrast) - DNA array hybridization
Histopathology	- PAS, H&E, GMS,	- Fluorescence in situ - hybridization (FISH)
Cultural - based	- Morphology - Biochemical : classical, automated	- PCR, Real time PCR, - T2
Serology-based	- Antigen : GM, Mannan, Crypto. antigen - Antibody : Anti-mannan, ID, LA, ELISA, WB	- Antigen : 1,3-b-D-Glucan
Nucleic acid-based	- PCR, Reversed hybridization	- Array, Next generation sequencing
Protein - based	- ----	- MALDI-TOF

Purpose: Detect pathogens after symptom presentation

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Advances in fungal diagnostics after 2014 – present

1. Combination of established techniques

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Combination technique

Clin Infect Dis. 2015 Oct 15;61(8):1263-72. doi: 10.1093/cid/civ555. Epub 2015 Jul 8.

Galactomannan and Polymerase Chain Reaction-Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-analysis.

Arvanitis M[1], Anagnostou T[2], Mylonakis E[2].

Screening high-risk patients for IA:
- Meta-analysis: 13 studies (1670 cases)
- Keyword: : "Aspergil*" and "PCR" and "GM"
- According to EORTC/MSG criteria

GM	PCR	Pre-treatment with antifungal agents	Predictive value
-ve	-ve	No	100% NPV
-ve	+ve	Yes	
+ve	-ve	Yes	88% PPV
+ve	+ve	Yes	

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Advances in fungal diagnostics after 2014 - present

1. **Combination of established techniques**
2. **New techniques**
   - **POC: point of care testing**
   - **Not POC: performing in laboratory**

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POC: point of care testing

Field applications
- Remote and resource-depleted regions
- Lack of diagnostic facilities, equipment and trained manpower

Ideal POC; ASSURED criteria (WHO)
- Affordable
- Sensitive
- Specific
- User-friendly
- Rapid
- Robust
- Equipment-free
- Deliverable to end-users

Peeling et al. 2006

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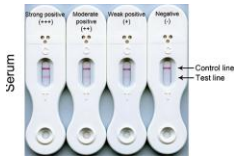
Aspergillus LFD performance in several group of patients



JF5 antibody (Ab), detects an extracellular glycoprotein antigen secreted during active growth of *Aspergillus* spp.

Patient group	Sens.	Spec.	PPV	NPV
SOT	94 % (15/16)	92 % (89/97)	65 % (15/23)	99 % (89/90)
ICU	79 % (26/33)	85 % (176/206)	57 % (26/46)	96 % (176/183)
Respiratory diseases	77 % (24/31)	92 % (195/211)	60 % (24/40)	97 % (195/202)
HM	65 % (30/47)	89 % (88/99)	73 % (30/41)	84 % (88/105)

HM hematological patients, *SOT* solid organ transplant recipients, *ICU* intensive care unit, *HSCT* hematological stem cell transplantation recipients, *BALF* bronchoalveolar lavage fluid
[a]Single testing = a minimum of one positive LFD results is required for diagnosis
[b] Multiple testing = a minimum of two or more positive LFD results are required for diagnosis

Juergen P et al. 2016

Aspergillus LFD performance in BAL and serum in several studies *Juergen P et al. 2016*

Risk group (n of patients)	Specimens	Sens.	Spec.	Ref
HM (29)	BAL	100	81.8	Hoenigl 2012
SOT (10)	BAL	100	80	Hoenigl 2012
HSCT (101)	Serum	40[a]	86.8[a]	Held 2013
		20[b]	97.8[b]	
HM (103)	Serum	81.8[a]	84.8[a]	White 2013
		59.1[b]	98[b]	
SOT (47)	BAL	91	83	Willinger 2014
Respiratory Disease (221)	BAL	77	92	Prattes 2014
HM (72)	BAL	71	76	Prattes 2015
HM and non-HM (32)	BAL	100	80	Johnson 2015
ICU (133)	BAL	80	81	Eigl 2015

HM hematological patients, *SOT* solid organ transplant recipients, *ICU* intensive care unit, *HSCT* hematological stem cell transplantation recipients, *BALF* bronchoalveolar lavage fluid [a]Single testing = a minimum of one positive LFD results is required for diagnosis
[b] Multiple testing = a minimum of two or more positive LFD results are required for diagnosis

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Advances in fungal diagnostics after 2014 - present

1. Combination of established techniques
2. New techniques
   - POC: point of care testing
   - Not POC

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Proximity ligation assays (PLA) for the early detection antigen in invasive aspergillosis

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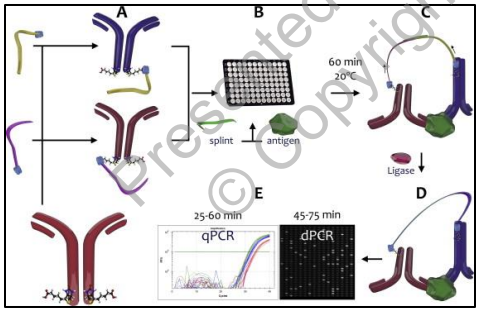
Duolink®

The new standard in protein interaction studies

Proximity ligation assays (PLA) for the early detection of invasive aspergillosis



PLA® detection of target antigen

- Sensitivity 1000x greater than the LFD, which utilises the same MAb.
- Sensitivity 10x -100x greater than the GM assay.
- Highly specific, with no cross-reactivity demonstrated against soluble antigens from *Candida, Mucor, Fusarium, Rhizopus, Lichtheimia or Cryptococcus* species.

Christina G et al. 2015

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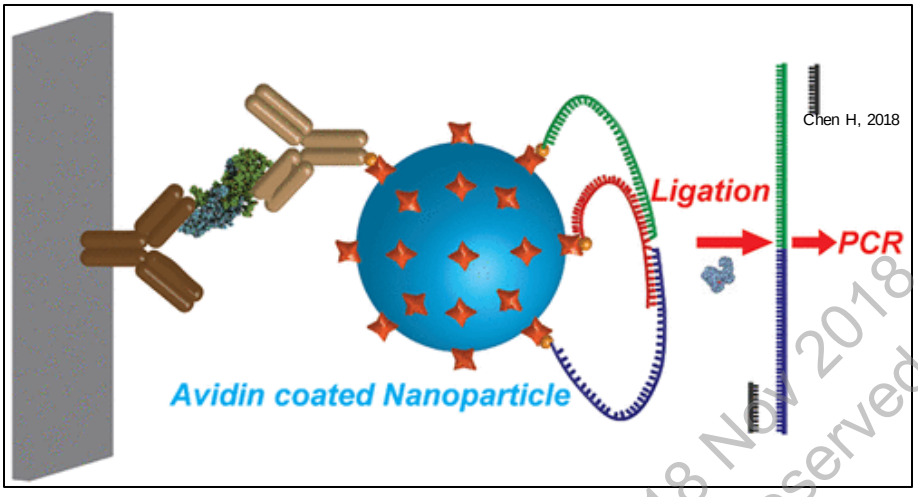
PLA technique with nanoparticle (NP-PLA)



- Test for human chorionic gonadotropin (hCG) levels
- Nearly 1000x more sensitive than the normal PLA
- On testing

Chen H et al. 2018

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Siderophore detection for Invasive Aspergillosis

Principle

- *In vivo*
  - To obtain host iron, *A. fumigatus* secretes the siderophore, *N*,*N*',*N*''-triacetylfusarinine C (TAFC)
  - TAFC: essential for virulence of *A. fumigatus* in a mouse model of invasive aspergillosis
- *In vitro*
  - TAFC is secreted soon after conidia germination in iron-limited media.

Cassandra S et al. 2016

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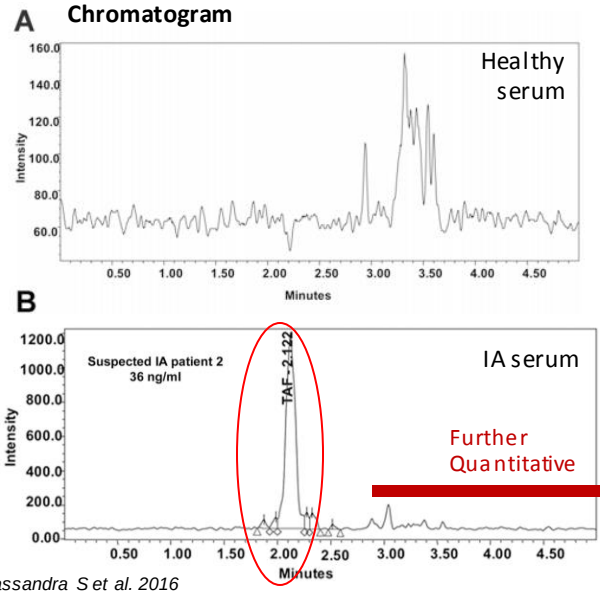
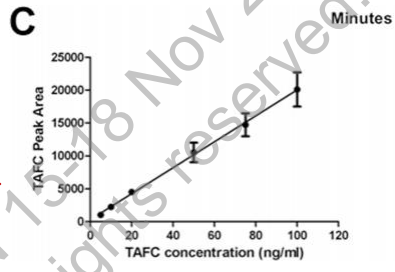
Ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for TAFC analysis



- 14 patients with proven/probable aspergillosis
  - 28% TAFC-positive (n=4).
- 36 GM-positive samples
  - 69% TAFC-positive (n=25).
- 40 GM-negative samples
  - 70% TAFC-positive (n=28)



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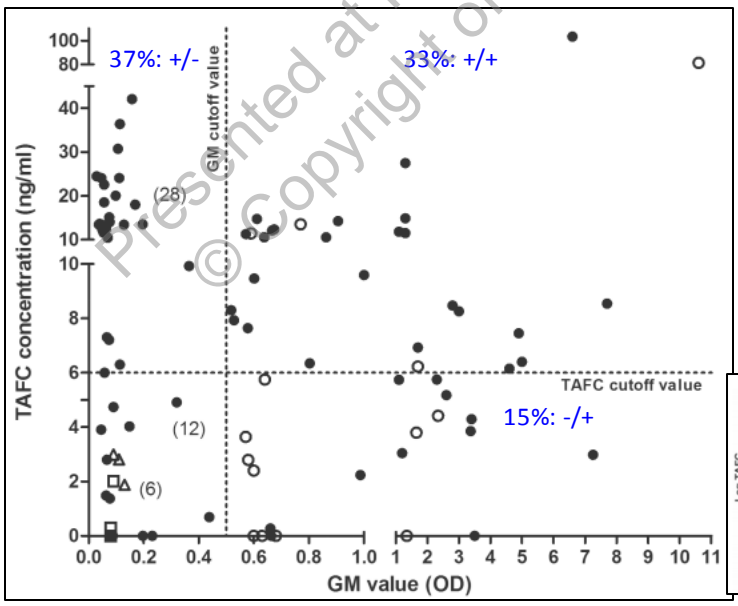
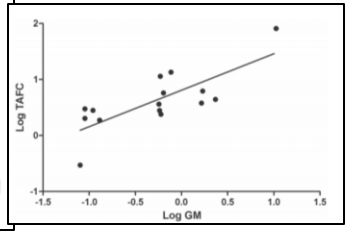
Cassandra S et al. 2016

Correlation of the amounts of TAFC and GM values based on EORTC/MSG



- Suspected IA samples (n=76; •)
  - 25 TAFC positive & GM positive
  - 28 TAFC positive & GM negative
  - 11 TAFC negative & GM positive
  - 12 TAFC negative & GM negative
- Healthy sera (n=3; □)
  - 3 TAFC negative & GM negative
- Pooled SLE sera (n=3; Δ)
  - 3 TAFC negative & GM negative
- Proven/probable IA samples (n= 14; O)
  - 4 TAFC positive & GM positive



Significant correlation with a Pearson r value of 0.77.

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Cassandra S et al. 2016

Electronic nose (eNose) for fungal infection

- Analyze human breath profiles or breath-print which presents specific volatile metabolites or volatile organic compounds (VOCs) to disease.
- Cheap
- Noninvasive
- Yields results within minutes

Wilson AD et al. 2016

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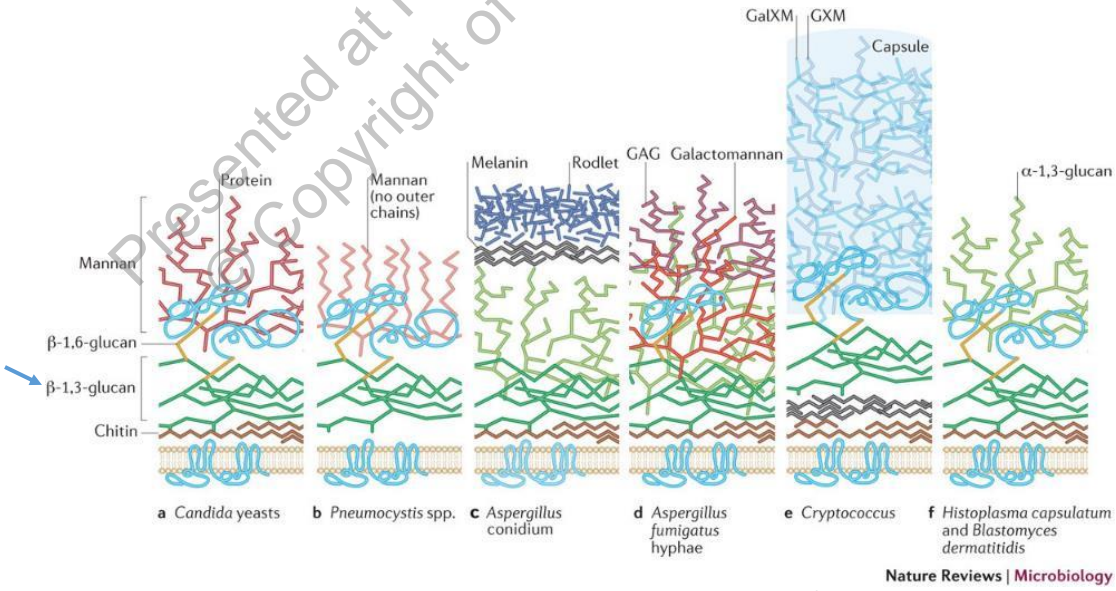
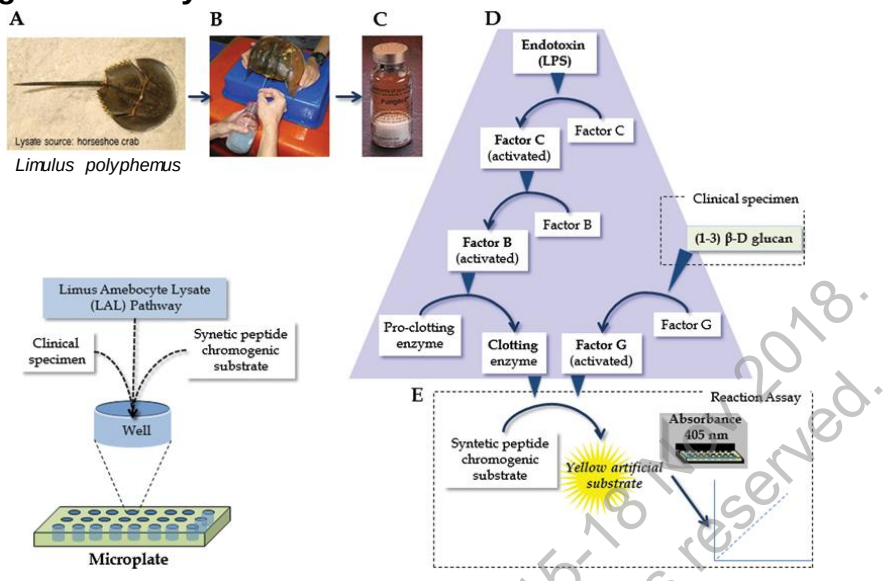
Different fungi possess different cell wall components



Erwig & Gow. 2016

BD: 1,3-β-D-glucan assay

Marisa et al. Sem in Res and Critical care Med 2015



Biological cascade-based assay

Measuring activation of Factor G through horseshoe crab substrates

BD: 1,3-β-D-glucan assay

- Major component of fungal cell wall but less in
  - Mucorales ie. *Mucor* spp., *Rhizopus* spp.
  - *Cryptococcus* spp. and some other Basidiomycota ie. *Malassezia* spp.

Commercial assay	Manufacturer	Horseshoe crab substrate	Detection system	Manufacturer cut-off value	Available
Fungitell assay (Glucatell)	Associated of Cape Cod Inc., East Falmouth, MA, USA	*Limulus polyphemus*	Colorimetric	60-80 pg/ml	US (FDA approved in 2003) and Europe
Fungitec-G test MK (G-MK)	Seikagaku Corporation. Tokyo, Japan)	*Tachypleus tridentatus*	Colorimetric	20 pg/ml	Japan
Beta-glucan test Wako	Wako Pure Chemicals Industries Ltd., Osaka, Japan	*Tachypleus tridentatus*	Turbidimetric	11 pg/ml	Japan
BGSTAR beta glucan test Maruha	Maruha Nichiro Foods Inc.Tokyo, Japan	*Tachypleus tridentatus*	Colorimetric	11 pg/ml	Japan

(Marisa et al. Sem in Res and Critical care Med 2015)

- Fungitell assay (Cape Cod Inc., USA)
  - FDA approved as an aid to diagnose deep-seated mycoses and fungimia.
  - European medical center : presumptive diagnosis of invasive fungal disease
    (Marisa et al. Sem in Res and Critical care Med 2015)
  - The EORTC-MSG panel : included a positive BD test as a microbiological criterion of IFI
    (Lamoth et al. J of fungi 2016)

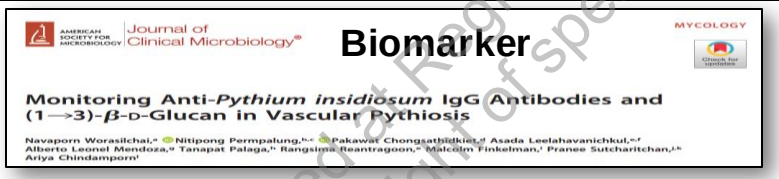
BD: 1,3-β-D-glucan

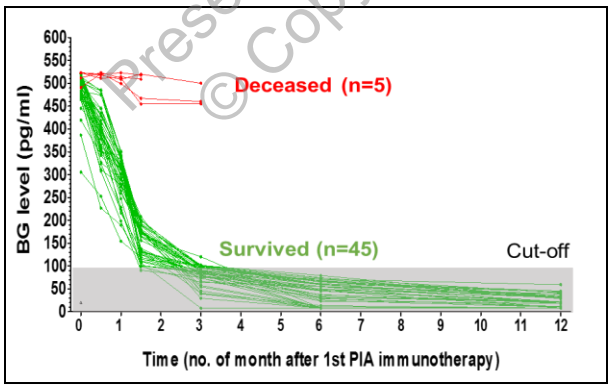
- Clinical specimen

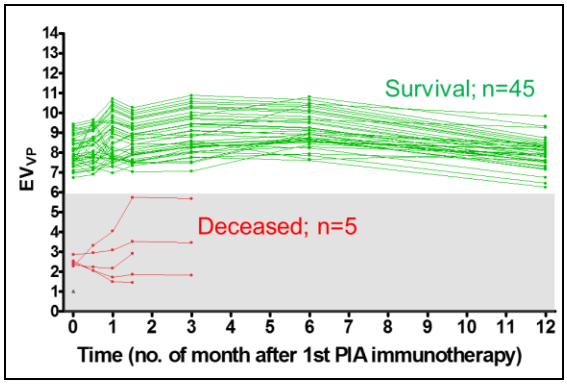
	Sensitivity	Specificity	Note
Serum	60-80%	80-95%	FDA approved
BAL	85-89%	86-95%	
CSF	85-90%	95-100%	

- Unfortunately, BD is not pathogen specific and cannot differentiate fungal species.
- Major limitations : its low specificity and frequent occurrence of false-positive reaction
  - Non-fungal infection
    - *Pseudomonas* spp.
    - *Streptococcus* spp.
  - Non-infectious disease conditions
    - Hemodialysis with cellulose membranes
    - Albumin transfusion
    - Intravenous immunoglobulin
    - Gauze packing of serosal surfaces
    - Use of cellulose filters for intravenous administration
    - Intravenous amoxicillin-clavulanate

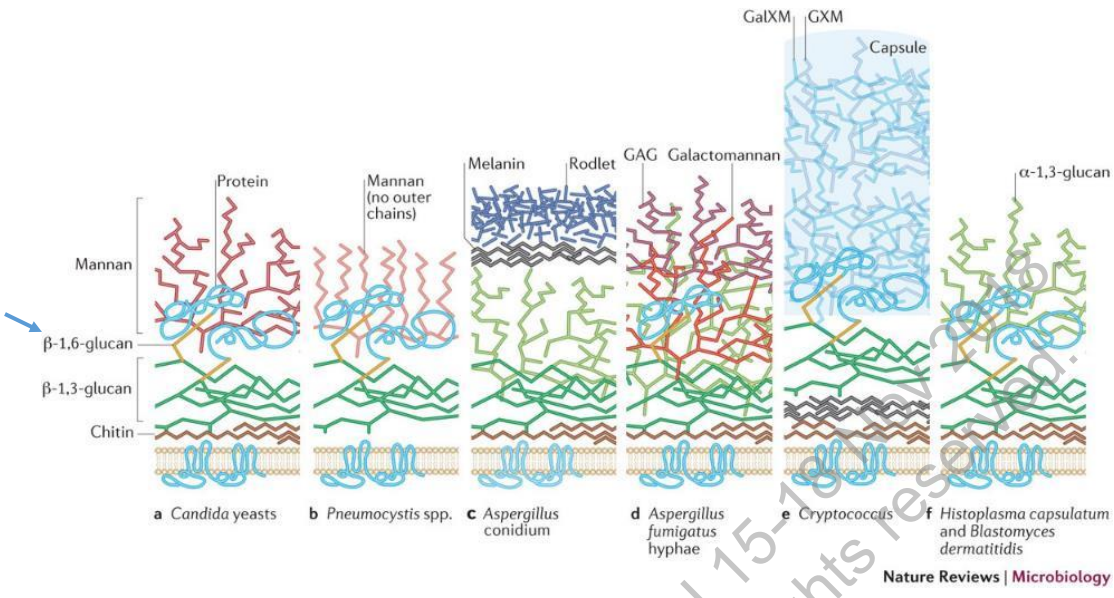
(Marty et al. Med Mycol 2009, Mennink-Kersten MA et al. N Eng J Med 2006, Mennink-Kersten MA et al. Clin Infect Dis 2008) *(Lamoth et al. J of fungi 2016)*







Different fungi possess different cell wall components



Erwig & Gow. 2016

Conservation of Mannan Synthesis in Fungi of the Zygomycota and Ascomycota Reveals a Broad Diagnostic Target

Amanda R. Burnham-Marusich,[a] Breeana Hubbard,[a] Alexander J. Kvam,[a] Marcellene Gates-Hollingsworth,[a] Heather R. Green,[a] Eric Soukup,[a] Andrew H. Limper,[b] Thomas R. Kozel[a]

PAN FUNGAL MANNAN : fungal immune assay as diagnostic tool in all fungal infections

- Identification of a MAb with broad reactivity across fungal mannans
- 1,6 mannan backbone contains an epitope that is shared across the Ascomycota and Zygomycota

TABLE 1 IgG subclass and spectrum of mannan reactivity of MAbs produced in response to immunization with *A. fumigatus* cellular antigen

Mannan source	Reactivity of hybridoma cell line (IgG subclass)										
	4EE9 (IgG1)	1AG7 (IgG2b)	1AC1 (IgG1)	1CD6 (IgG2b)	3AE6 (IgG2b)	2BG2 (IgG2b)	2AG9 (IgG3)	4AF11 (IgG1)	3ED9 (IgG2b)	1AD7 (IgG1)	2DA6 (IgG1)
A. fumigatus	+	+	+	+	+	+	+	+	+	+	+
Mucor spp.	–	–	–	–	+	–	+	+	+	+	+
Fusarium spp.	–	–	+	+	–	+	+	+	–	+	+
C. albicans	–	–	–	–	–	–	–	–	+	+	+

-1,6-linked mannose in the mannan backbone is required for MAb 2DA6 binding.

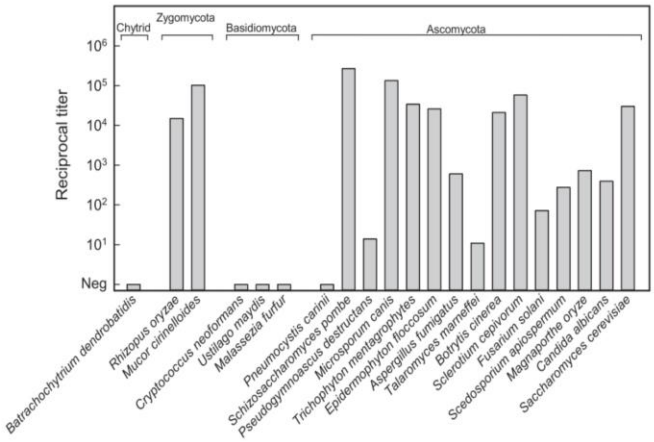


FIG 4 Reactivity of hot citrate extracts from various fungi in a sandwich ELISA constructed from MAb 2DA6 *Pneumocystis carinii* isolated from infected rat lung was used for that fungus. In all other cases, extracts were prepared from mycelia or yeasts from culture.

May/June 2018 Volume 3 Issue 3 e00094-18

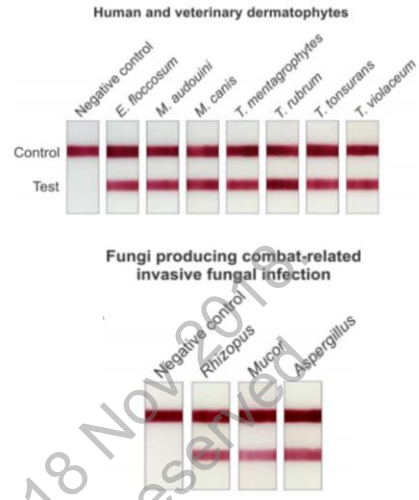


FIG 5 Detection of mannan in hot citrate extracts from cultures of medically relevant fungi in a lateral flow immunoassay constructed from MAb 2DA6. (Left) Extracts from cultures of *Epidermophyton* spp., *Microsporum* spp., and *Trichophyton* spp. that cause dermatophyte infection in humans and animals. (Right) Extracts from cultures of fungi that produce combat-related invasive fungal infection. Negative control, citrate buffer.

msphere.asm.org, May/June 2018: 3 (3) 27

Current situation in Asian laboratories

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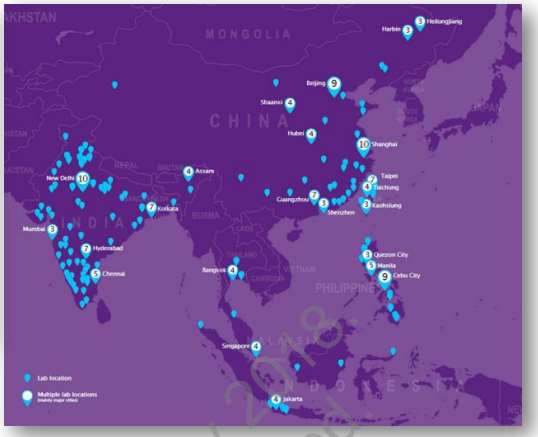
ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2018, 56, 416–425
doi: 10.1093/mmy/myx066
Advance Access Publication Date: 20 September 2017
Original Article

Original Article

Survey of laboratory practices for diagnosis of fungal infection in seven Asian countries: An Asia Fungal Working Group (AFWG) initiative

Ariya Chindamporn[1], Arunaloke Chakrabarti[2,*], Ruoyu Li[3], Pei-Lun Sun[4], Ban-Hock Tan[5], Mitzi Chua[6], Retno Wahyuningsih[7], Atul Patel[8], Zhengyin Liu[9], Yee-Chun Chen[10] and Methee Chayakulkeeree[11]



Recent Survey of 241 laboratory practices for fungal diagnosis in 7 Asian countries by an Asia Fungal Working Group (AFWG)

- Initiative and poor assessment of biomarker tests such as galactomannan, β-D-glucan and PCR in Indonesia, the Philippines and Thailand.
- This information indicates that the training of advanced non–culture-based diagnostic tests is needed for Asian laboratories to improve the process of fungal diagnostics and promote the high quality of life in Asian population.

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