# Rapid diagnosis
# MALDI-TOF and Fungal antigen assays

Ariya Chindamporn
Department of Microbiology, Faculty of Medicine,
Chulalongkorn University
Bangkok, Thailand

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## Outline

- Principle of MALTI-TOF
- Yeast identification from pure colony
- Yeast identification directly from blood cultures
- Influence of sample preparation method
- Mould identification from pure colony
- Prediction of antifungal drug resistance
- Cost effectiveness

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## Principle of MALDI-TOF

Robin P, Clin Chem January 2015
Bader O. Proteomics 2013

**MALDI plate preparation**

Matrix-Assisted Laser Desorption Ionization (MALDI) Time of Flight

Streak cells on the MALDI plate

Agar of choice *(Goyer M. J Clin Microbiol. 2012)*
- Sabouraud dextrose agar
- Columbia blood agar
- Chromagar

Overlay with the matrix dissolved in lysis reagents

Lysis reagent:
- 25–70% formic acid

*At this step cell components are released : cation (+ve)*

Let the mixture become crystallized matrix

Crystallized matrix serve as the chemical ionizing agent for energy transfer from the laser to the analyte.

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## Principle of MALDI-TOF TOF: Time of Fight

Robin P, Clin Chem January 2015
Bader O. Proteomics 2013

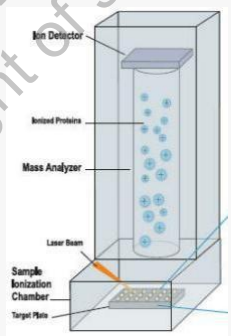
**MALDI plate preparation**

Streak cells on the MALDI plate

Overlay with the matrix disolved in lysis reagents

Let the mixture become crystallized matrix

**Protein analysis**

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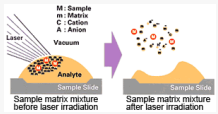
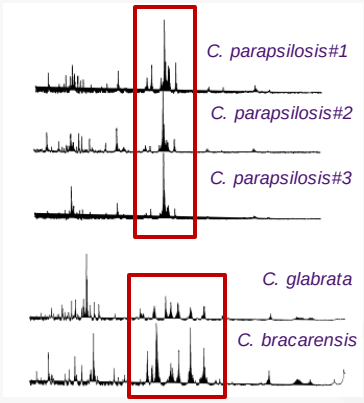



- By laser beam, the analyte is desorbed from the target plate resulting ions acceleration in an electric field.
- Mass and charge (M/Z) of the analyte are measured.

**Result analysis**




- M/Z ratio is generated the peak of spectra.
- These spectra can determine the precise species
  - highly reproducible within a species
  - sufficiently different even between highly related species

## Yeast identification from pure colony

- To prepare yeast sample is easier than to prepare mold
- The accuracy of identification depends on instrument type (database)

| MALDI-TOF | Accuracy | Study | Ref. |
|---|---|---|---|
| **MALDI Biotyper** | 95.7% | 4059/4247 isolates;16 studies | *Bader O. 2013* |
| **SARAMIS system** | 98.4% | 1463/1487 isolates;4 studies | *Bader O .2011*<br>*Rosenvinge F et al. 2012*<br>*Martinez-Lamas L et al. 2011*<br>*Santos C. 2011* |
| **VITEK-MS** | 98.4% | 183/184 isolates;1 study | *Iriart, X et al. 2012* |
| **Andromas databases** | 100% | 160/160 isolates;1 study | *Bille E et al. 2012* |



## Yeast identification from pure colony

Advantage of MALDI-TOF: Overcome the limitation of

- Undiscriminate with common biochemical methods
  - *Candida ortho-/meta-/parapsilosis*
    (Quiles-Melero I, *J Clin Microbiol Infect Dis*, 2012 , Martinez-Lamas L, *Infec Microbiol Clin*, 2011, Santos C, *Diagn, Microbiol Infect Dis*, 2011, Hendrickx M, *Diagn Microbiol Infect Dis*, 2011. Kubesova A, *Analyst*, 2012)
  - *Candida glabrata/bracarensis/nivariensis*
    *(Santos C, Diagn Microbiol Infect Dis, 2011)*
  - *C. albicans/dubliniensis*
    *(Santos C, Diagn Microbiol Infect Dis, 2011)*
  - *Candida haemulonii* group I and II complexes
    *(Cendejas-Bueno E, J Clin Microbiol, 2012)*
- Unidentify the similar Phenotypically species
  *(Jensen RH, J. Clin. Microbiol, 2011, Castanheira M, J Clin Microbiol, 2013, Desnos-Olivier M, J Clin Microbiol, 2008)*
  - *C. palmioleophila*
  - *C. famata*
  - *C. guilliermondii*

## Can MALDI-TOF identify yeast directly from blood cultures ?

**Factor 1** **Highly abundant substances disturb the identification process**:

- culture media (e.g. charcoal and cations other than H+) *(Szabados F, Clin Microbiol Infect, 2011)*
- human blood (e.g. hemoglobin or albumin) *(Marinach-Patrice C, PLoS One, 2010)*

- These components can generate mass peaks that partially overlap with spectra from yeasts. *(Marinach-Patrice C, PLoS One, 2010)*
- Challenge: purification procedure before extraction were developed from manufacturers:
  - Gel matrices *(Loonen A, Eur J Clin Microbiol Infect Dis, 2012, Sparbier K, J Clin Microbiol, 2012)*
  - Filtration devices *(Fothergill A, J. Clin. Microbiol, 2012)*
  - Saponification *(Ferroni A, J Clin Microbiol, 2010)*
  - Differential centrifugation *(Spanu T, J Clin Microbiol, 2012, Ferreira L, Clin.Microbiol, Infect. 2011)*
  - Washing steps: distilled water / low conc. of detergents ie. SDS or Tween80. *(Marinach-Patrice C, PLoS One, 2010, Spanu T, J Clin Microbiol, 2012, Ferreira L, Clin Microbiol, Infect. 2011)*

**Factor 2** **Amount of yeast cells in blood culture** (<4% of total sample)

- Extra step: centrifugation *(Bader O, Proteomics, 2013)*

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## The accuracy of yeast identification directly from blood culture

| Hemoculture | Sample prep. | Database (library version) | *C. albicans* | *C. glabrata* | *C. parapsilosis* | *C. tropicalis* | *C. krusei* | *C. guilliermondii* | Reference |
|---|---|---|---|---|---|---|---|---|---|
| BacT/Alert w/o charcoal | Extraction with TFA* only | Andromas (unknown) | 20/20 | - | - | - | - | - | *Ferroni A et al. 2010* |
| BACTEC | Differential centrifugation | Bioty per (V2.0.4.0) | 0/8 | 0/9 | - | 0/1 | - | - | *Ferreira L et al. 2011* |
| Mycosis IC/F | SD wash step | Other | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | - | *Marinach-Patrice C et al. 2010* |
| Bactec FX | Sepsity per | Bioty per (V3.1.1.0) | 28/28 | - | 8/8 | 5/5 | - | - | *Yan Y et al. 2011* |
| 10 Aerobic/anaerobic/F | Sepsity per | Bioty per (V3.1.1.0) | 4/5 | 2/7 | 1/2 | - | - | 1/1 | *Schubert S et al, 2011* |
| Mycosis IC/F | Tween 80 wash | Bioty per (unknown) | 187/195 | 22/26 | 65/69 | 28/32 | 6/8 | 6/10 | *Spanu T et al, 2012* |

**TFA: Trifluoroacetic acid*

## Yeast identification directly from blood cultures

**In summary**

- Direct identification of yeasts from positive blood culture is possible.
- The purity of the analyte have major effect to the result.
- However, even commercial kit *(Sepsityper, Bruker Daltonics)* for sample purification is available, protocols for purification used in the literature are not yet standardized.
- Research used only is now recommended.

Yeast identification directly from **urine** samples *(Sobel J D, Clin Infect Dis, 2011)*

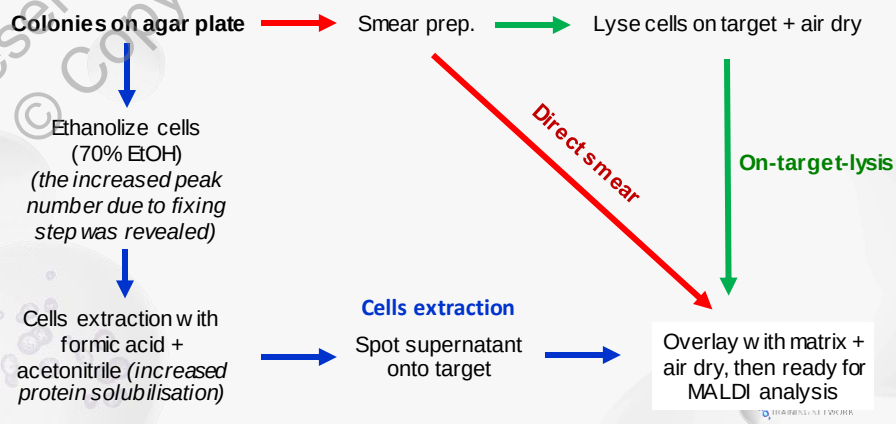
- Yeasts are also frequently recovered from urinary samples
  - catheter colonizers
- Technically, yeast identification directly from urine is possible.
- However, so far the study of yeast identification directly from urine samples by MALDI-TOF is very rare and all are in research work.

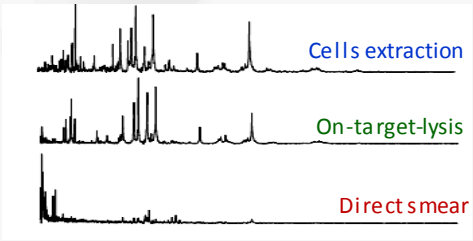


## Influence of sample preparation method

*(Kemptner J, Rapid Commun Mass Spectrom, 2009)*

- Standard protocol of sample prep. is appropriate for most yeasts.
- However, due to the stronger cell walls of some fungal cells resulting not sufficiently release their intracellular contents under these conditions.
- Modified procedures was developed.






***Candida glabrata*** spectra derived from each method of cells prep.

- Different method give different pattern of spectra.
- For cells that still do not lyse efficiently, other extraction methods ie. mechanical disruption in a bead-beater can be applied

Another factor need to be concern is **"Database"**

- Best preparation method is the same method with which the spectra of the database have been made.
- It will lead to the highest concordance between acquired test spectra and reference spectra in the identification database.
- Currently, available yeast database are
  - **MALDI Biotyper** provided both "On-target-lysis" and "cells extraction".
  - **SARAMIS**, **VITEK-MS** and **Andromas systems** provided only "On-target-lysis".

*(Bader O, Proteomics, 2013, Amiri-Eliasi B, Anal Chem, 2001, Cassagne C, PLoS One, 2011, Hettick J, Mass Spec-trom, 2008)*

## "On-target-lysis" faster & requires less hands-on-time than "cells extraction"

- Application of "On-target-lysis" have been developed for use with the MALDI Biotyper system *(Bader O, Proteomics, 2013)*
- To overcome the fewer peaks generated from this method, the analysis criteria was modified.
  - In general, M/Z ratio (log score) of MALDI Biotyper reference spectra
    - ≥2.000 "species level" identifications
    - 1.700 - 1.999 "genus level only."
  - Current V3.0 MALDI Biotyper, decreasing criteria of log score
    - Lower log-scores starting from 1.500 can be accepted as species-specific for yeasts if certain criteria are met *(Steensels D, Acta Clin Belg, 2011, Goyer M, J. Clin Microbiol, 2012, Sparbier K, J Clin Microbiol, 2012)*
    - The 3 additional criteria :
      *(Steensels D, Acta Clin Belg, 2011, Goyer M, J Clin Microbiol, 2012, Sparbier K, J Clin Microbiol, 2012)*
      (i) encompasses a certain number of database hits at least n = 2 or n = 3 of a single species at the top
      (ii) have no other species intermingled
      (iii) significantly difference of log-score to the next species (ie. 0.200).
    - Using these criteria, accuracy ~94% (1000/1067 isolates tested across 6 studies).
    - Only if the criteria are not met, the sample should be repeated the extraction step.

## Mould identification (pure colony)

- More difficult to analyze than yeast.
  *(Alanio A, Clin Microbiol Infect, 2011, Buskirk, A D, Anal Biochem, 2011)*
  - More complicated morphology + stronger cell walls
  - Presence/absence of conidia
  - Degree of agar invasion (amounts of contaminating agar in the analyte)
  - Degree of maturation of the colony
  - Presence of melanin (may inhibit ionization)
- To overcome those limitations
  - Mechanical disruption / bead-beating protocols / acid-containing matrix solution
  - Liquid cultures suppressing pigment formation *(Buskirk, A D, Anal Biochem, 2011)* / pre-analytical washing steps *(Dong H, Anal Bioanal Chem, 2009)*

**Mould identification is now recommended for research use only !!**

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## Prediction of antifungal drug resistance

- The study of drug resistant mechanism in Fungi is very rare.
- Mostly are "intrinsic drug resistance" (species-dependent distribution of drug susceptibility) *(Pfaller M, Antimicrob Agents Chemother, 2002, Alastruey A, Antimicrob Agents Chemother, 2010)*
- So antifungal drug resistance prediction by MALDI-TOF = Identification of the fungal species

**Under prolonged therapy or prophylaxis isolates** *(Pfalle M A, Am J Med, 2012)*

- Azoles, mainly by mutations leading to increased drug efflux or ergosterol biosynthesis pathway.
  - Echinocandins, mutations occur targeting glucan synthase
- All of these proteins have molecular weights
  - ~60 kDa Erg11 and Mdr1 proteins
  - ~110–125 kDa CaTac1/CgPdr1 transcription factor
  - ~170 kDa Cdr-type efflux pumps
  - >200 kDa Fks1 (glucan synthase)

*****out of the detection range of MALDI-TOF (2–20 kDa)*****

- **Modified method** : CLSI broth dilution + MALDI analysis of cells recovered from the wells in azoles *(Marinach C, Proteomics, 2009)* and echinocandins *(De Carolis E, J Clin Microbiol, 2012)*
- 15 h (modified MALDI-TOF) VS 24 h (the 1st MIC reading)
- "Trailing growth" from each isolate still effect to the true resistance.

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## Cost effectiveness (Thailand based)

*(modified from Galar A., Eur. J. Clin, Microbiol Infect Dis, 2012)*

| Method | Hand-on time/sample (min) | Turnaround time/sample (h) | Cost of reagents/samples (USD) |
|---|---|---|---|
| Conventional test | | | |
| • Biochemical test | 15 | 48-72 | 3 |
| • API 20C Aux yeast identification system | 15 | 48-72 | 7 |
| Molecular test | | | |
| • PCR & sequencing | 60 | 72 | 20 |
| MALDI-Biotyper | 5 | 0.5 | 0.5 |

**MALDI-TOF**

- Reagents: lower than conventional / molecular test
- Machine + Maintenance : High (USD 150,000 approx./year)

However, two of the strongest cost-driving factors in clinics are

- Prolonged hospitalization
- Application of expensive drugs within empirical therapy



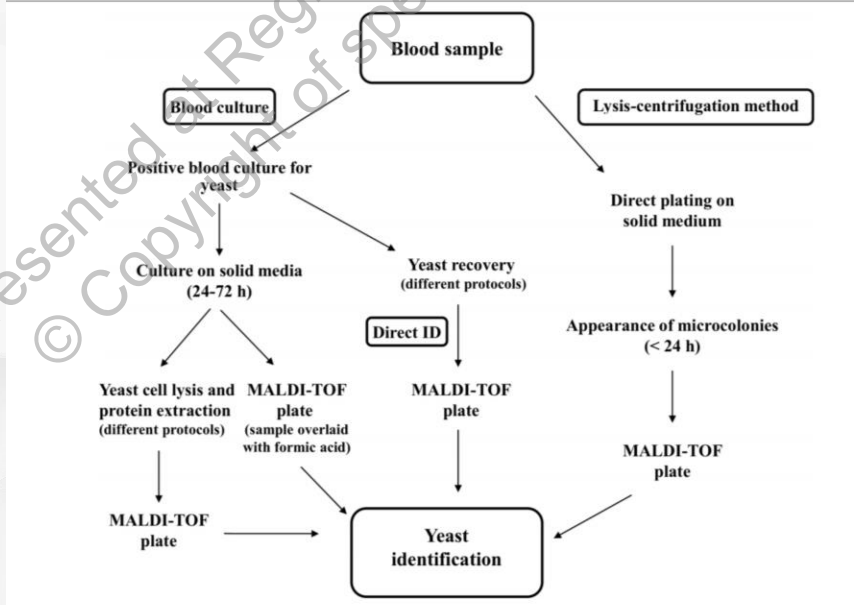


**FIGURE 1** | Schematic representation of the different procedures described for identification of yeasts in blood samples by MALDI-TOF MS.

Florio W, et al. Frontiers in Microbiol 2018 (9)

# Fungal Antigen Assay



## Invasive fungal infections show high mortality rates

| Disease (most common species) | Location | Estimated life-threatening infections/ year at that location* | Mortality rates (% in infected populations)* |
|---|---|---|---|
| Opportunistic invasive mycoses | | | |
| Aspergillosis (*Aspergillus fumigatus*) | Worldwide | >200,000 | 30–95 |
| Candidiasis (*Candida albicans*) | Worldwide | >400,000 | 46–75 |
| Cryptococcosis (*Cryptococcus neoformans*) | Worldwide | >1,000,000 | 20–70 |
| Mucormycosis (*Rhizopus oryzae*) | Worldwide | >10,000 | 30–90 |
| Pneumocystis (*Pneumocystis jirovecii*) | Worldwide | >400,000 | 20–80 |
| Endemic dimorphic mycoses*† | | | |
| Blastomycosis (*Blastomyces dermatitidis*) | Midwestern and Atlantic United States | ~3,000 | <2–68 |
| Coccidioidomycosis (*Coccidioides immitis*) | Southwestern United States | ~25,000 | <1–70 |
| Histoplasmosis (*Histoplasma capsulatum*) | Midwestern United States | ~25,000 | 28–50 |
| Paracoccidioidomycosis (*Paracoccidioides brasiliensis*) | Brazil | ~4,000 | 5–27 |
| Penicilliosis (*Penicillium marneffei*) | Southeast Asia | >8,000 | 2–75 |

*Most of these figures are estimates based on available data, and the logic behind these estimates can be found in the text and in the Supplementary Materials. †Endemic dimorphic mycoses can occur at many locations throughout the world. However, data for most of those locations are severely limited. For these mycoses, we have estimated the infections per year and the mortality at a specific location, where the most data are available.

Brown GD, et al. Sci Transl Med. 2012

It has been used since the 1950s

- Immunodiffusion (ID)
- Complement fixation (CF)
- Enzyme immuneassay (EIA)
- Lateral flow assay
- Etc.

**Advantages**

- It can be positive when culture results are negative or samples are difficult to obtain.
  - 50% of sero-positive sputum from patients with chronic aspergillosis are culture-negative.
- Less time consuming compare to cultivation
- Minimallize the invasive sample

**Disadvantages**

- Limited for immunocompromised patients
- Inability to distinguish between current or previous infection
- Sensitivity is dependent on the type of disease and the timing of testing relative to the disease process.

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## Candidiasis:

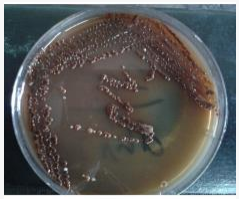
**The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia**

Małgorzata Mikulska,[1] Thierry Calandra,[2] Maurizio Sanguinetti,[3] Daniel Poulain,[4] and Claudio Viscoli[5], the Third European Conference on Infections in Leukemia Group

- Diagnosis of IC is difficult in high-risk patients, thus noninvasive tests that detect *Candida* components in the serum of patients with IC have been developed.
- Performance of Mn and A-Mn antibody tests was analysed and reviewed on behalf of ECIL-3.
- Investigated in 14 studies that comprised 453 patients and 767 controls
  - 7 studies: hematological & cancer cases & another 7: ICU & surgery cases) / 13 studies: retrospective
- Moderate sensitivity and good specificity of Mn and A-Mn were found (Mn, 58% and 93%; A-Mn, 59% and 83%, respectively).
- Combined Mn/A-Mn testing was better than each test alone (sensitivity 83% and specificity 86%).
  - *C. albicans* (80-100%)
  - *C. tropicalis* & *C. glabrata* (60-75%)
  - *C. parapsilosis* & *C. krusei* (40–50%)

Critical care 2010; 14(6): R222.

## Cryptococcosis



[Figure 1] Brown pigment of *Cryptococcus neoformans* on apple leaves agar

Journal of Biology, Agriculture and Healthcare
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Vol.4, No.5, 2014

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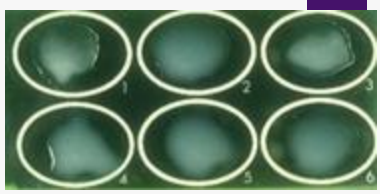

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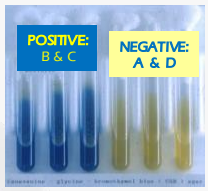


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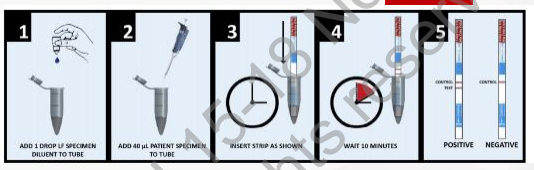
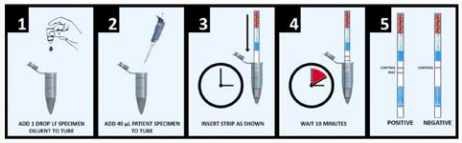





## Cryptococcosis

- Lateral Flow assay
  - Capsular antigen assay
  - Point of care test: qualitative or semiquantitative
  - US-FDA-approved for serum & CSF
  - Rapid TAT and easy to perform




http://www.immy.com/products/lateral-flow-assays/crag-lfa/

| Specimen Type | n | Sensitivity | 95% CI | Specificity | 95% CI | PPV | 95% CI | NPV | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| Serum | 693 | 100% | 98-100% | 99% | 97-99% | 97% | 94-99% | 100% | 99-100% |
| Plasma | 135 | 100% | 96-100% | 100% | 93-100% | 100% | 96-100% | 100% | 97-100% |
| CSF | 261 | 100% | 97-100% | 97% | 92-99% | 97% | 93-99% | 100% | 97-100% |
| Urine | 674 | 98.4% | 95-99.5% | 99% | 98-99.8% | 97% | 95-99.7% | 99% | 98-99.9% |
| Total | 1763 | 99.5% | 98-99.8% | 99% | 98.0-99% | 97% | 96-99% | 99.7% | 99.2-99.9% |

*From PELFREY & BAUMAN, 2012.

- Latex agglutination
  - Beads with polyclonal antibody to detect Cryptococcal capsular antigen

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Tanner DC, et al. 1994

## GM: Galactomannan

# Aspergillosis

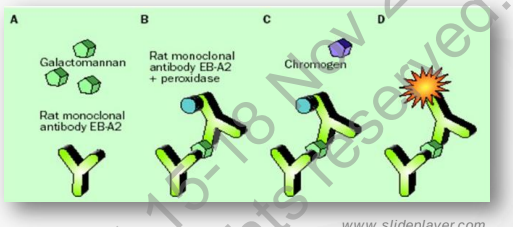
- Major component of fungal cell wall especially *Aspergillus* spp. and also found in
  - Family of Trichocomaceae ie. *Penicillium* spp., *Paecilomyces* spp.
  - *Fusarium* spp.
  - *Histoplasma capsulatum*
  - *Pneumocystis jirovecii*

*Tortorano et al. J Clin Microbiol 2012, Huang et al. AIDS 2007, Wheat et al. Clin Vaccine Immunol 2007*

- Platelia Aspergillus EIA (Bio-Rad, France)
  - Sandwich ELISA
  - Anti-GM monoclonal antibody : mAb EB-A2

    *(Marisa et al. Semin Res and Critical care Med 2015)*
  - FDA approved as a diagnostic aids for invasive aspergillosis (IA)

    *(FDA. May 2003)*




*www.slideplayer.com*

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- Clinical samples interpretation

| | Recommended cut-off | Collected samples | Sens. | Spec. | Reference |
|---|---|---|---|---|---|
| **Serum** | Index 0.5 | • 2 aliquots: same positive sample + 1 sample collected at a different time point | 60-80% | 80-95% | *Marisa 2015 Lamoth 2016* |
| | Index 1.0 | • 1 single sample | | | |
| **BAL** | Index 0.5-1.0 | • 2 aliquots of a single BAL fluid sample | 85-90% | 90-95% | *Guo Y L et al. 2010 Zou M et al. 2012* |
| **CSF*** | Index 0.5-2.0 | • 1 single sample | 85-90% | 95-100% | *Chong GM et al. 2016* |

- *CSF is validated only for cerebral aspergillosis diagnosis by EORTC-MSG and ECIL so using under expertise consultation is recommended* *(De Pauw B et al. 2008, Marchetti O et al. 2012)*
- *CSF has not been approved by FDA yet.*

- Two main strategies of use for serum sample:
  - Serial collection of samples (2-3 times/week) in high risk patients
  - Intensive testing in symptomatic patients

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*Marisa et al. Semin Res and Critical care Med 2015, Lamoth et al. J of fungi 2016*

# Aspergillosis

Invasive pulmonary aspergillosis

- Specific anti-aspergillus IgG for
  - Diagnosis
  - Prediction of IPA in patients undergoing allo-HSCT

Chronic pulmonary aspergillosis

- Galactomannan: Positive in only 25% of CPA patients
- Anti-Galactomannan: Positive in 90% of CPA patients
- Anti-Mannan: Positive in 77% of CPA patients
- Aspergillus specific IgG ELISA; Spec. >99%
  - Immunulite (Germany) Sens. 96%
  - ImmunoCAP (ThermoFisher) Sens. 85%
  - Serion (Germany) Sens. 86%
  - Genesis (UK) Sens. 93%
  - etc.
  - ➢ It might have a role in monitoring treatment response in CPA (On process of study)



# Lateral flow assay – Invasive Aspergillosis

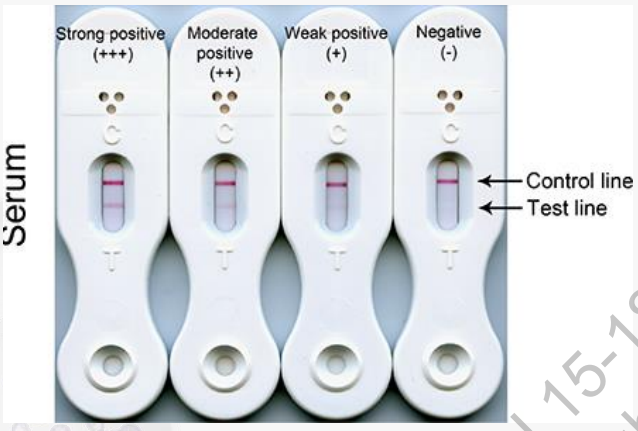
- Recently shown to be more accurate than the standard serologic markers.
- Detect
  - Glucoprotein antigen in the sera and BAL fluid of patients with invasive aspergillosis in 15 min (Thornton CR. 2008)
  - Secreted during active growth of *A. fumigatus*, binds to a monoclonal antibody (JF5)
- Useful for the confirmation or exclusion of invasive aspergillosis in combination with other tests, such as PCR
- Better clinical performance than that of GM assay when used as a screening test rather than a confirmatory test (Held J, et al. 2013)

**Table 1** Per BALF sample performance of the BALF *Aspergillus* LFD for probable/proven invasive pulmonary aspergillosis versus no evidence for invasive pulmonary aspergillosis in different patient cohorts (percentage and absolute numbers)[a]

| Patient group | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| Overall[b] | 73% (83/113) | 90% (498/552) | 61% (83/137) | 94% (498/528) |
| Solid organ transplantation | 94% (15/16) | 92% (89/97) | 65% (15/23) | 99% (89/90) |
| Intensive care unit | 79% (26/33) | 85% (176/206) | 46% (26/56) | 96% (176/183) |
| Respiratory diseases | 78% (25/32) | 91% (196/215) | 57% (25/44) | 97% (196/203) |
| Hematological malignancies | 67% (36/54) | 91% (126/139) | 73% (36/49) | 88% (126/144) |

Heldt S & Hoenigl M. 2017

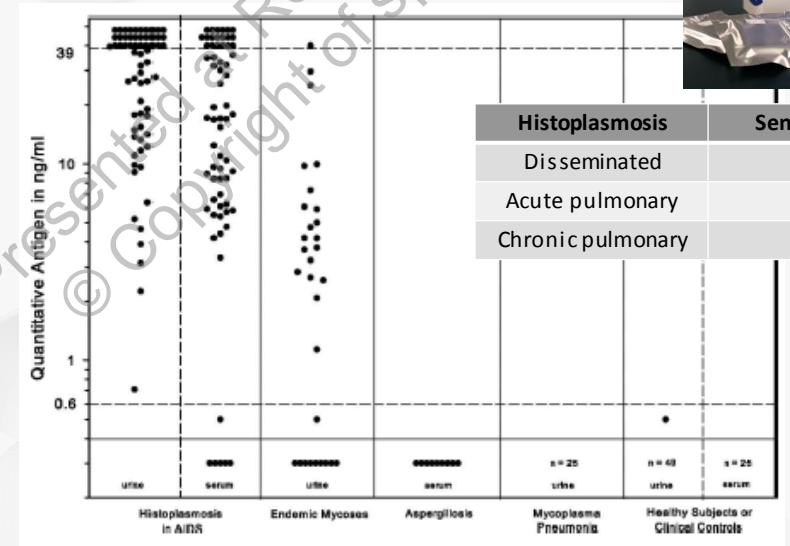
# Lateral flow assay – Invasive Aspergillosis




http://www.life-worldwide.org/media-centre/article/new-device-to-detect-invasive-aspergillosis

MMTN MEDICAL MYCOLOGY TRAINING NETWORK

# Histoplasmosis: Histoplasma antigen EIA


| Histoplasmosis | Sensitivity (%) |
|---|---|
| Disseminated | 90-95 |
| Acute pulmonary | 80 |
| Chronic pulmonary | 20 |

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Connolly *et al*. Clin Vaccine Immunology 2007

## BD: 1,3-β-D-glucan

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

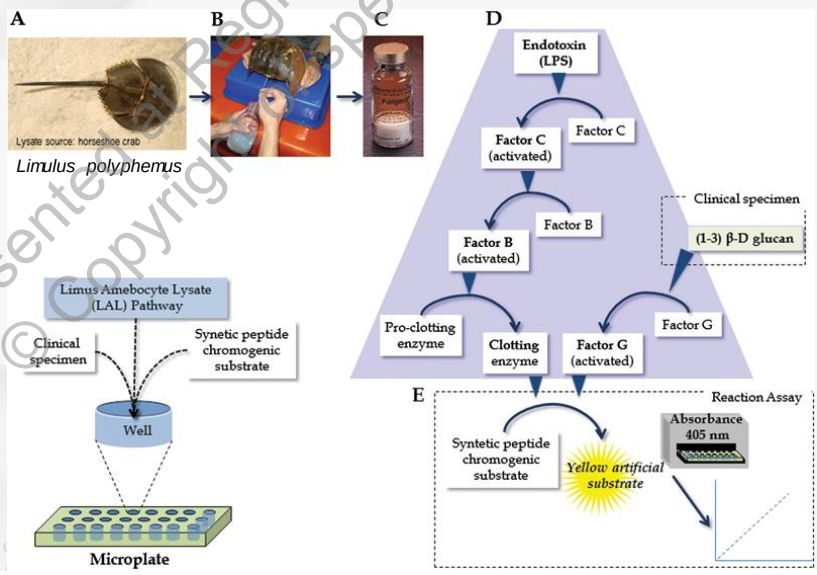
| Commercial assay | Manufacturer | Horseshoe crab substrate | Detection system | Manufacturer cut-off value | Available |
|---|---|---|---|---|---|
| Fungitell assay (Glucatell) | Associated of Cape Cod Inc., East Falmouth, MA, USA | *Limulus polyphemus* | Colorimetric | 60-80 pg/ml | US (FDA approved 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)*



*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**

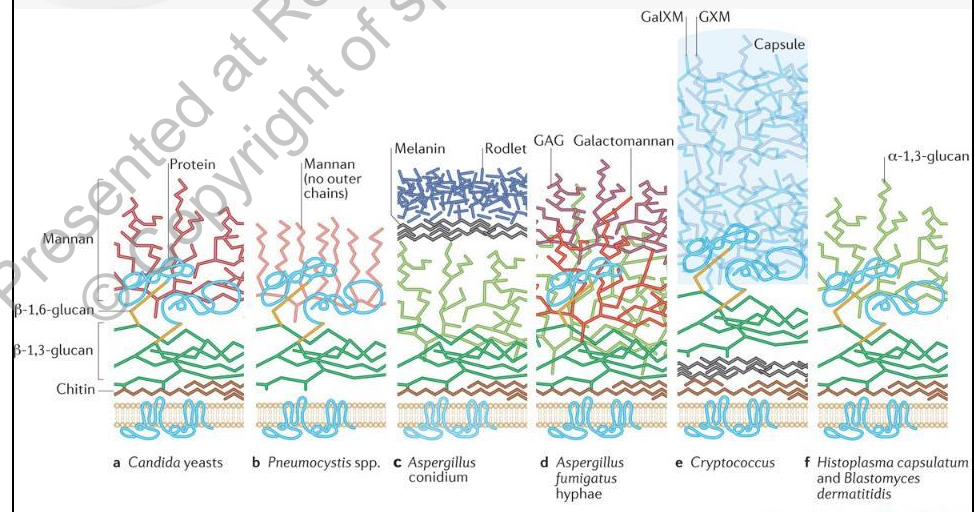
- Clinical specimen *(Lamoth et al. J of fungi 2016)*

| | 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)*

**Different fungi possess different cell wall components**




Erwig & Gow. 2016