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MALDI-TOF Technique

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ASIA FUNGAL WORKING GROUP
an ISHAM working group

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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

Disclosure: no conflict of interest

Principle of MALDI-TOF

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

MALDI: Matrix Assisted Laser Desorption/Ionization

Agar of choice *(Goyer M. J Clin Microbiol. 2012)*

- Sabouraud dextrose agar
- Columbia blood agar
- Chromagar

Lysis reagent:

- 25–70% formic acid

At this step cell components are released

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

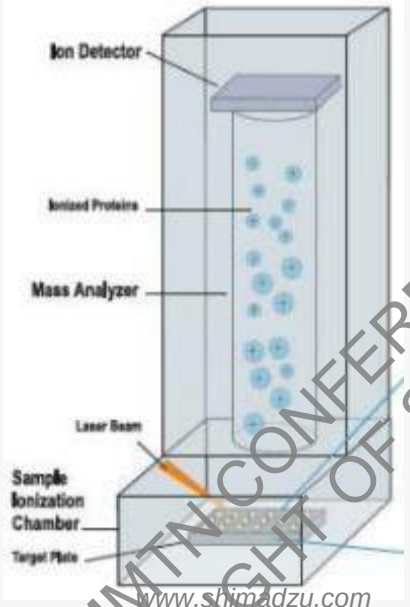
MALDI plate preparation

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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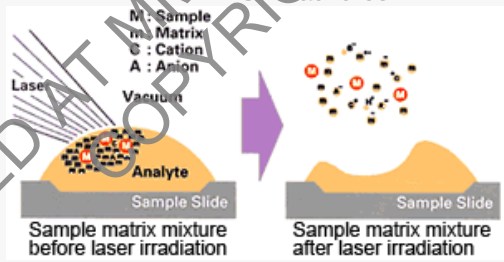
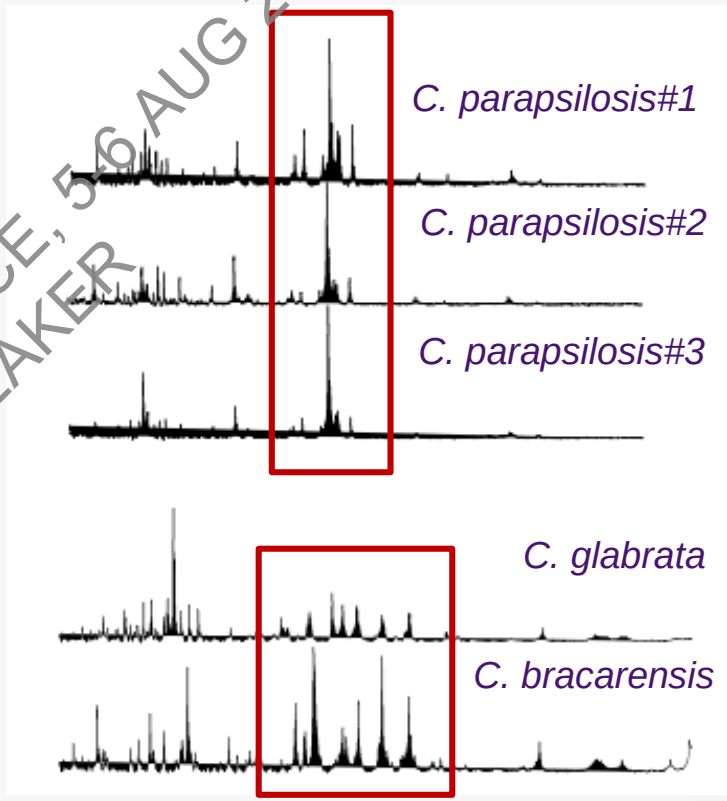


- By laser beam, the analyte is desorbed from the target plate resulting ions acceleration in an electric field.
- Mass and charge (M/Z) of the analyte are measured.

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

- Due to simple cell structure of yeast compared to mould, much easier of yeast identification is revealed.
- The accuracy of identification depended 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* *Rosenvinge F et al. 2012* *Martinez-Lamas L et al. 2011* *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

MALDI-TOF can also apply for

- Closely related yeast species which can not be clearly discriminated 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)
- Phenotypically similar species
  (Jensen RH, J. Clin. Microbiol, 2011, Castanheira M, J Clin Microbiol, 2013, Desnos-Ollivier 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)*
- To solve the problem, 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)*

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	Biotyper (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	Sepsityper	Biotyper (V3.1.1.0)	28/28	-	8/8	5/5	-	-	*Yan Y et al. 2011*
10 Aerobic/anaerobic/F	Sepsityper	Biotyper (V3.1.1.0)	4/5	2/7	1/2	-	-	1/1	*Schubert S et al, 2011*
Mycosis IC/F	Tween 80 wash	Biotyper (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.

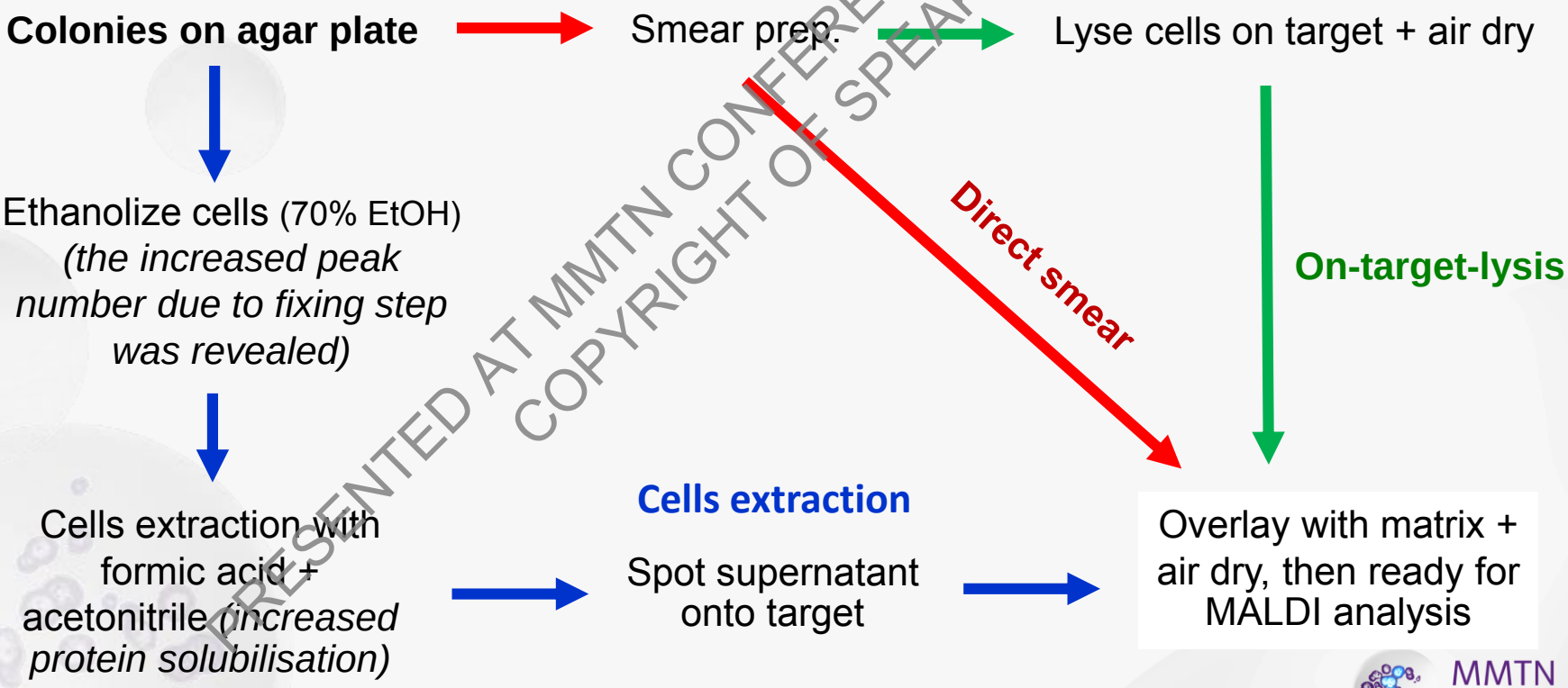
Yeast identification directly from **urine** samples *(Sobel J D, Clin Infect Dis, 2011)*

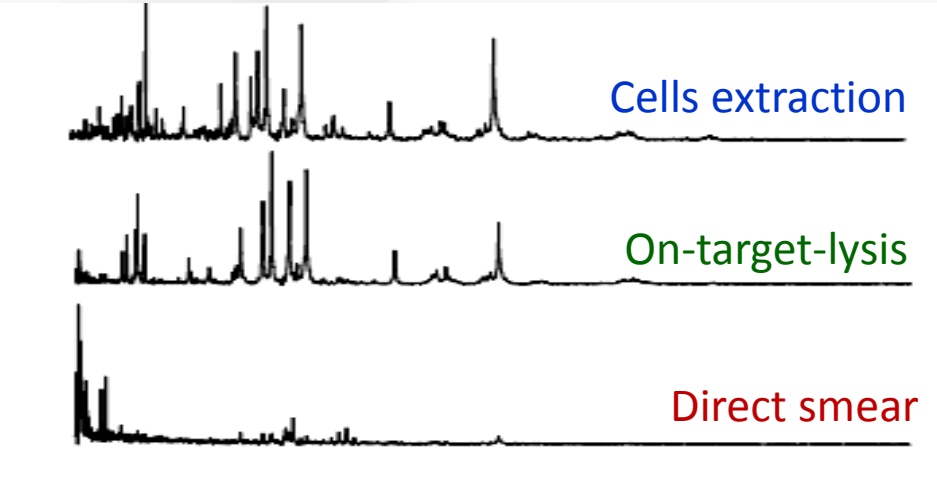
- Yeasts are also frequently recovered from urinary samples
  - catheter colonizers
- Technically, yeast identification directly from urine is possible.
- However, so far the study of yeast identification directly from urine samples by MALDI-TOF is very rare and all are in research work.

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".

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(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 n ≥ 2 or 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).

Rank (Qualtity)	Matched pattern	Score value (log score)
1 (-)	*Candida albicans* ATCC 24433	1.5
2 (-)	*Candida albicans* ATCC 10231	1.49
3 (-)	*Candida albicans* ATCC 90028	1.47
4 (-)	*Candida parapsilosis* ATCC 22019	1.23
5 (-)	*Candida parapsilosis* ATCC 90018	1.22

Answer:
C. albicans

- 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)
  - Growth rate is difference
  - 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 !!

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 *(Pfaller 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.

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: cheaper 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

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Summary

- To use MALDI-TOF in fungi, need to aware of how big and update of the database will be.
  - Limitation for the new species and genus identification. – the way to develop the databases
- How to prepare the sample related to the spectrum of results and effect the interpretation
- Whenever you start to use the new technic, please validate with the classical method you used to.

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Biomarker assays: GM & BD

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Outline

- Principle of GM & BD
- Guideline for using in clinical practice
- Clinical samples
- Other applications

Disclosure: no conflict of interest of both GM & BG

GM: Galactomannan

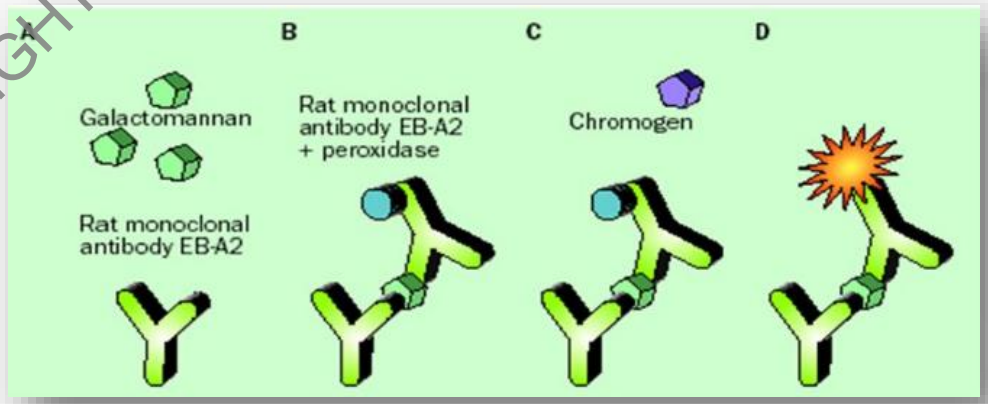
- Major component of fungal cell wall especially *Aspergillus* spp. and also found in
  - Family of Trichocomaceae ie. *Penicillium* spp., *Paecilomyces* spp.
  - *Fusarium* spp.
  - *Histoplasma capsulatum*
  - *Pneumocystis jirovecii*

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)



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- Clinical samples interpretation

	Recommended cut-off	Collected samples	Sens.	Spec.	Reference
Serum	Index 0.5 Index 1.0	• 2 aliquots: same positive sample + 1 sample collected at a different time point • 1 single sample	60-80%	80-95%	*Marisa 2015* *Lamoth 2016*
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

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

Infectious Disease Society of America (IDSA) guideline; 2016

- **Recommended** as marker to diagnose IA in adults & pediatric patients with
  - underlying hematologic malignancy
  - hematologic stem cell transplant (HSCT)
- **Not recommended** for routine screening in
  - patients who currently received antifungal therapy / prophylaxis
    - False pos: piperacillin-tazobactam** / amoxicillin-clavulanate / amoxicillin / ampicillin

    *** piperacillin-tazobactam: antibacterial therapy of febrile neutropenia* (Sulahian A et al N. Engl J Med 2003)
    *** even new formulations have been developed with a supposed lower risk of false positive, this point need to be concerned* (Mikulska M et al. J Antimicrob Chemother 2012, Vergidis P et al J. Clin Microbiol 2014)
  - patients with solid organ transplant (SOT)
    - Renal transplant with proven IA (56% sensitivity)
  - patients with recent
    - blood transfusion
    - mucositis due to chemotherapy
    - gastrointestinal tract graft-versus-host disease (GVHD)

Marisa et al. Sem in Res and Critical care Med 2015, Patterson TF et al. Clin Infect Dis 2016, Hoyo I et al. Transpl Infect Dis. 2014

The European Conference on Infections in Leukemia (ECIL) laboratory working group

- **Recommended** the cut-off of GM index that should prompt further diagnostic work-up *ie.* CT-scan for presumptive IFI.

  In serum

  - a single GM Index ≥ 0.7 or
  - two consecutive GM Index ≥ 0.5

  In BAL

  - a single GM Index ≥ 0.8

  In CSF

  - a single GM Index ≥ 1.0

Marchetti O et al. Bone Marrow Transplant, 2012

GM detection in other specimens

Urine *(Duettmann W et al. Med Mycol 2014)*

- Study in 75 proven IA cases underlying hematological malignancies
- GM index cut-off : 0.5
  - 47.6% Sensitivity
  - 86% Specificity
  - 24.4 Positive predictive value (PPV)
  - 94.5 Negative predictive value (NPV)

Moreover, GM positive also reported in

- Cyst fluids in patients with polycystic kidney disease *(Miller-Hjelle MA et al. Emerg Infect Dis 1997)*
- Subphrenic abscess in a pediatric patient with proven IA undering chronic granulomatous *(Verweij PE et al. J ClinMicrobiol 2000)*
- Purulent material, study in patients with fungal rhinosinusitis *(Klont RR et al. Clin Infect Dis 2004)*

However, the study of GM in other specimen is very limited, using for routine diagnosis is not recommended.

!! Caution !! GM Negative was found in some species of *Aspergillus* !!

Features of selected non-*A. fumigatus* infections *(modified from Lass-Florl et al. J Antimicrob Chemother 2017)*

Species	Diseases	Specific characteristics
Emericella nidulans	IA in CGD	• More virulent than A. fumigatus • Higher mortality • Propensity to spread from the lung to adjacent structures and to disseminate • Intrinsic resistance to amphotericin B
Emericella quadrilineata	IA in CGD and IA	• Resistant to caspofungin?
Aspergillus calidoustus	IA	• Propensity to disseminate • Intrinsic resistance to azoles • Intrinsic resistance to caspofungin?
Aspergillus terreus	IA	• Propensity to disseminate (63%) • Intrinsic resistance to amphotericin B
Aspergillus tubingensis	IA, airway colonization and ear infections	• Acquired resistance to azoles • Lower propensity to disseminate (10%-30%)
Aspergillus lentulus	IA	• Resistant to azoles and echinocandins • Resistant to amphotericin B
Aspergillus alliaceus	**IA**	**• GM negative** **• High MICs of amphotericin B and caspofungin**
Aspergillus carneus	**IA**	**• GM low positive**
Aspergillus novofumigatus	IA	• Resistant to azoles
Aspergillus alabamensis	Mainly airway colonization	• Resistant to amphotericin B
Aspergillus ustus	IA	• Resistant to amphotericin B, azoles and echinocandins
Aspergillus felis	IA	• High MICs against voriconazole and caspofungin

IA, invasive aspergillosis; CGD, chronic granulomatous disease; GM, galactomannan.

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GM as a marker of IA treatment response

- A meta-analysis reported the role of serum GM as a marker of response to therapy and predictor of IA outcomes
  - A decline of GM values within the first 1-2 week(s) after initiation of antifungal therapy was associated with better prognosis compared to persistently high values.
  - GM should be follow-up at day 7 and 14 after treatment.

(Bergeron A et al. J Clin Microbiol 2012, Koo S et al. J Clin Microbiol 2010, Mikel MH et al. Clin Infect Dis 2008, Woods G et al. Cancer 2007)

- **In CSF**
  - GM titers tend to decrease during effective therapy
  - Serial testing has been suggested useful for monitoring response to treatment.

(Antinori S et al. J Infect 2013, Viscoli C et al. J Clin Microbiol 2002)

BD: 1,3-β-D-glucan

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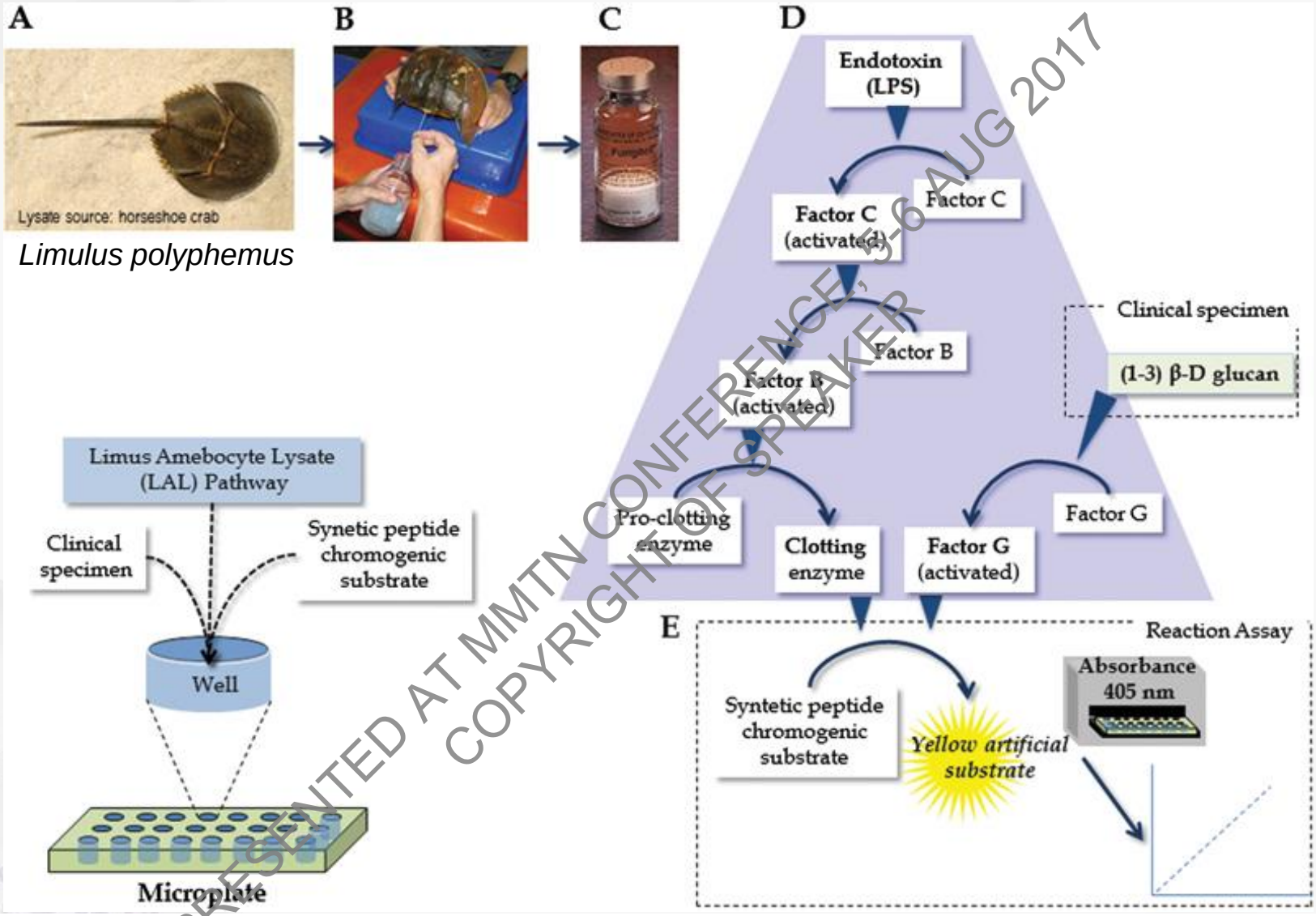
BD: 1,3-β-D-glucan

- 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)



Biological cascade-based assay

Measuring activation of Factor G through horseshoe crab substrates

- 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)

Infectious Disease Society of America (IDSA) guideline; 2016

- **Recommended** as marker to diagnose invasive fungal infection in high risk population including patients with
  - underlying hematologic malignancy
  - allogenic hematologic stem cell transplant (HSCT)
- **Note**
  - Data for serum BG use in patients with solid organ transplant (SOT) is very limited.
    - Lung transplant with proven IA (64% sensitivity, 9% specificity)
    - Liver transplant with proven IA (58% sensitivity, 83% specificity)

Patterson TF et al. Clin Infect Dis 20016

Role of BD to monitor the therapeutic response

Clinical samples	Proven disease	Study population	References
Serum	Invasive candidiasis	203 cases (90% PPV and 90% NPV)	*Jaijakul S et al. 2012*
Serum	*Pneumocystis jirovecii* Pneumonia	18 cases	*Held J et al. 2011*
Serum	Aspergillosis	11 cases	*Senn L et al. 2008* *Ellis M et al. 2008*
CSF	Hematogenous *Candida* Meningoencephalitis	7 cases	*Salvatore C M et al. 2016*
CSF	*Exserohilum rostratum* Meningitis	107 samples	*Litvintseva AP et al. 2014*

- However, the study of BD to monitor the therapeutic response is still limited.
- Using for routine is not recommended.

In summary

Biomarkers	GM	BD
Assay	Platelia Aspergillus EIA (Bio-Rad, France)	Fungitell (Cape Cod Inc. USA)
Principle	ELISA: Anti-GM monoclonal antibody (mAb EB-A2)	ELISA: Biological cascade-based assay
Clinical application	Invasive aspergillosis (IA)	Invasive fungal infection (IFI) *except mucormycosis, cryptococcosis*
FDA and CE approved specimens	Serum, BAL	Serum
Performance Result	Semi-quantitative	Quantitative
Recommended cut-off (sensitivity/specificity)	**Serum**; Index 0.5 (60-80% / 80-95%) **BAL**; Index 0.5-1 (85-90% / 90-95%) **CSF**; Index 0.5-2 (85-90% / 95-100%)	**Serum**; 60-80 pg/ml (60-80% / 80-95%)
Application for therapeutic monitoring	On research	On research

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

Another interesting clinical sample was applied for GM & BD detection

(1→3)-β-D-glucan and galactomannan testing for the diagnosis of fungal peritonitis in peritoneal dialysis patients, a pilot study

Navaporn Worasilchai[1,2,†], Asada Leelahavanichkul, MD, PhD[2,*,†], Talerngsak Kanjanabuch, MD[3,4], Nisa Thongbor[5], Pichet Lorvinitnun, MD[5], Kanya Sukhontasing[2], Malcolm Finkelman, PhD[6] and Ariya Chindamporn, PhD[2]

Peritoneal dialysis

- Study in PD patients with and without fungal peritonitis over 1 year in KCMH, Thailand
- 13 fungal peritonitis cases (proved by cultivation) showed

	GM index/Level	Cut-off	Sensitivity	Specificity
GM	3.41 ± 1.24	GM index = 0.5	77%	58%
BG	494 ± 19 pg/ml	240 pg/ml	100%	83%

- **Conclusion**: GM and BD in peritoneal fluid with provisional cut-off values were applicable as surrogate biomarkers for the diagnosis of fungal peritonitis in PD patients.

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