Diagnosis of Aspergillosis: Progress in Technologies and Approach

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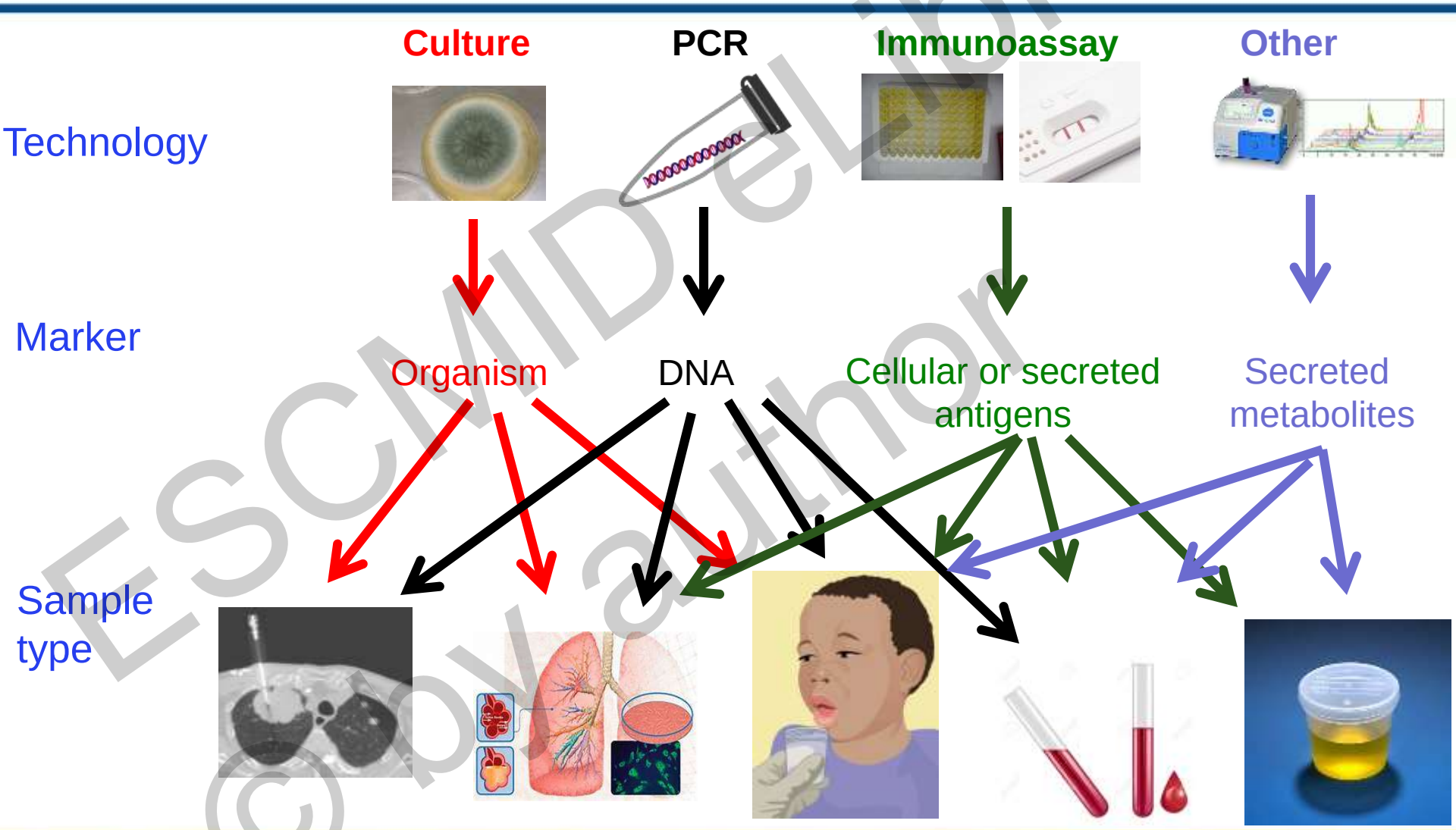
Disclosures

- Consultant / Advisory Board
  - Amplyx, Cidara, Merck
- Royalties / Equity
  - MycoMed Technologies

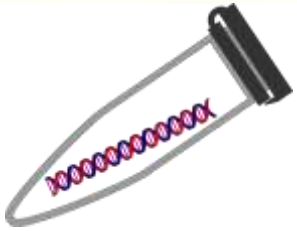
Background

- Focus on invasive aspergillosis
- This talk will address use of *non-culture based biomarkers* to:
  - Predict disease risk
  - Indicate presence of infection

Diagnostic Assays



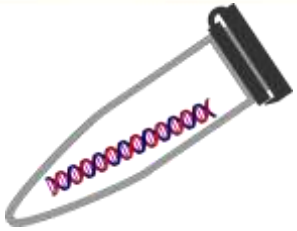
Nucleic acids PCR



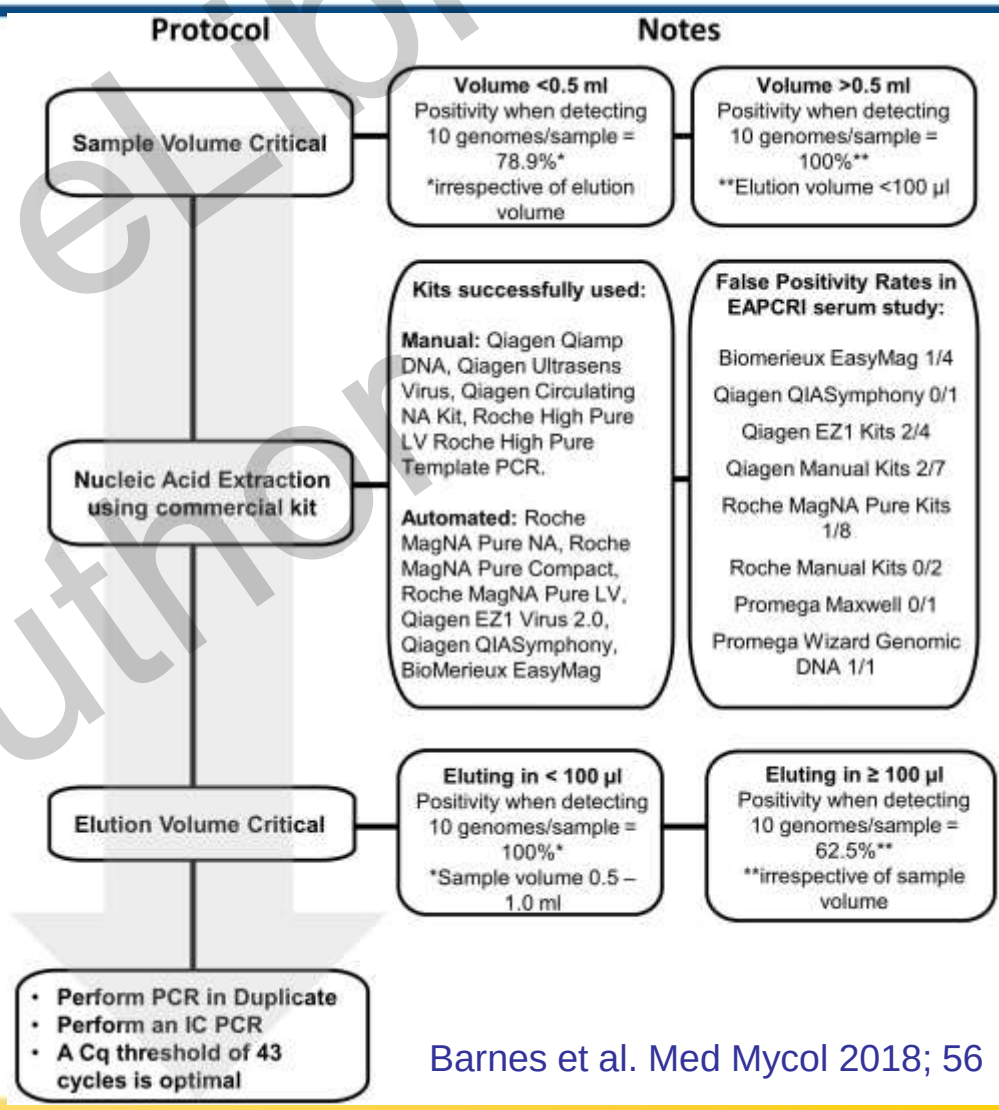
- Many years of research on PCR in tissue, BAL, blood
- Many in-house studies with non-standardized assays varied performance on BAL and blood, meta-analyses
- Several recent large studies on BAL:
  - 1555 samples / 3 yrs, tested using multiple in house and commercialized assays. Sensitivity 61 – 74% (GM 87%), up to 100% with GM combined[1]
  - 1248 samples / 12 yrs: lower sensitivity (40%), increased with combined (74%) [2]
- Results suggest utility with BAL, especially combined with GM, multiplexing enabling species ID & resistance genotype
- Recent efforts to optimize and standardize sample processing in EU cooperative

White et al. Clin Infect Dis 2015; 61
[1]Guegan et al. JI 2018; 76
[2]Hardak e al. IJID 2019; 3557
Cruciani et al. Cochrane DSR. 2015
Boch et al. CMI 22 (2016) 862
Chong et al. JAC 71 (2016) 3528

Nucleic acids PCR

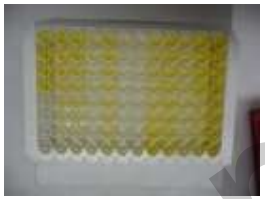


- Blood / plasma / serum complicated
  - Critical issues described for DNA extraction
    - Volume, buffers
    - Variation in commercial kits
    - Elution volumes
    - and more..



Barnes et al. Med Mycol 2018; 56

Immunodiagnostics

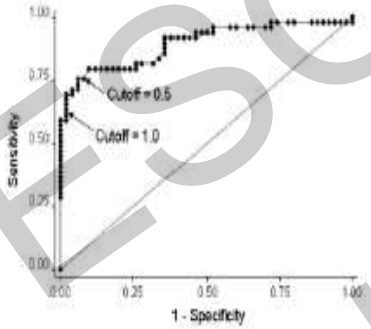


- Platelia galactomannan EIA widely studied in serum & BAL
  - Reported performance continues to be variable
    - Serum sensitivity ranging from 19% - 80%
    - BAL sensitivity ranging from 43% - 90%
      - Positive predictive value highest in BAL (highest prevalence)
  - Many variables impact performance
    - Hosts (neutropenic vs. not)
    - Other drugs (antifungals, contamination)
    - Biology (colonization vs. invasion)
    - Technical (cut-off variation)
  - Recent attention to cutoffs, “false positives”, and antigen

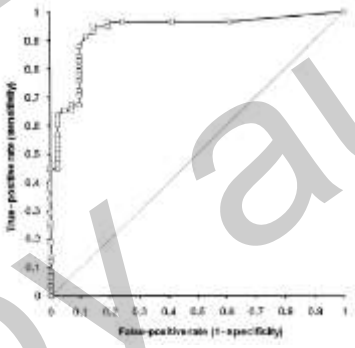
Leeflang MM, et al. *Cochrane Database Syst Rev* 2008.
Boch et al. Clin Microbiol Infect 2016; 22A

BAL Cut-offs

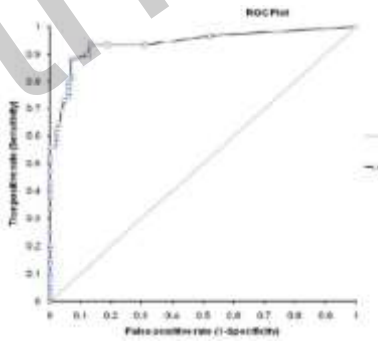
- Multiple cut-offs used: 0.5, 0.7 vs. 1.0?
  - Perhaps depends on host, and goal
  - Subject to a lot of bias: disease definitions, antifungal exposure (time) and *math*
    - *Lavage dilutions, variation in kit threshold control*



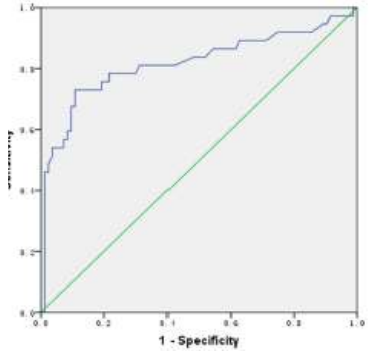
BMT – 0.5
Musher JCM2004



Heme – 1.0
Maertens CID2004



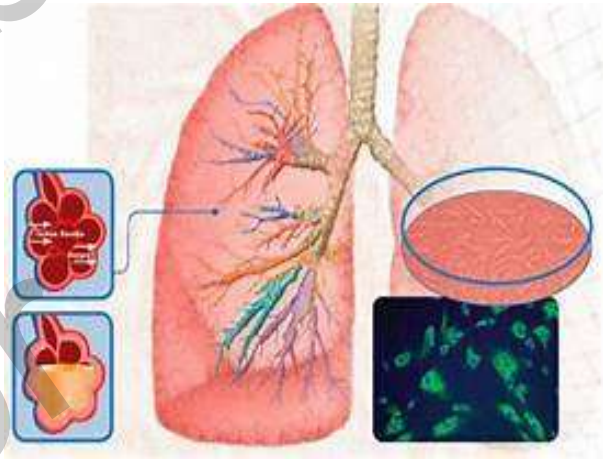
Mix – 0.8
D'Haese JCM 2012



Non-neut– 0.7
Zhou JCM 2017

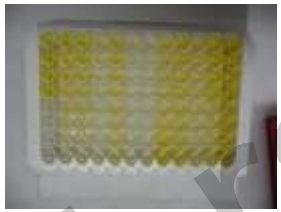
BAL - false positivity

- Consecutive patients (n=134) with BAL GM EIA to assess real-world predictive value
  - 42% of BAL positive at >0.5 were falsely positive, PPV = 58%
  - Is 0.5 too low?
- How do we really distinguish between FP test vs. FN gold standards, or definitions that are too conservative?
- Biology is vexing: airway antigen not necessarily disease
  - *Understanding nature of antigen critical*



Farmakiotis et al. Mycoses 2019. 62

Immunodiagnostics



- "galactomannan" is a complex polymer (mannan & gal*f* side chains)
  - Secreted in differing amounts depending on growth conditions
- Antigenic moiety = galactofuranose
  - In mammals, galactose is common but only found in the hexopyranosyl form (Gal*p*)
  - Galactofuranose (Gal*f*) is found in bacteria, fungi, protozoa, starfish, sponges and green algae (and lichens)- abundant
  - Equilibrium favors Gal*p*

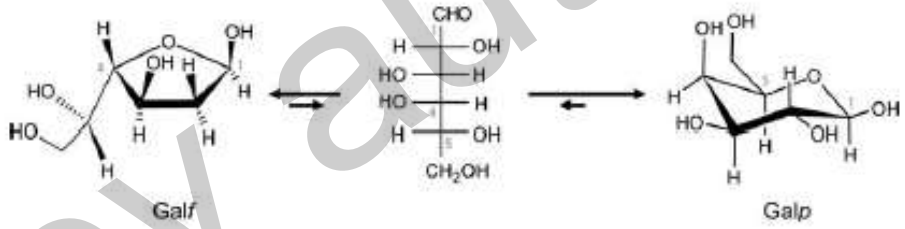
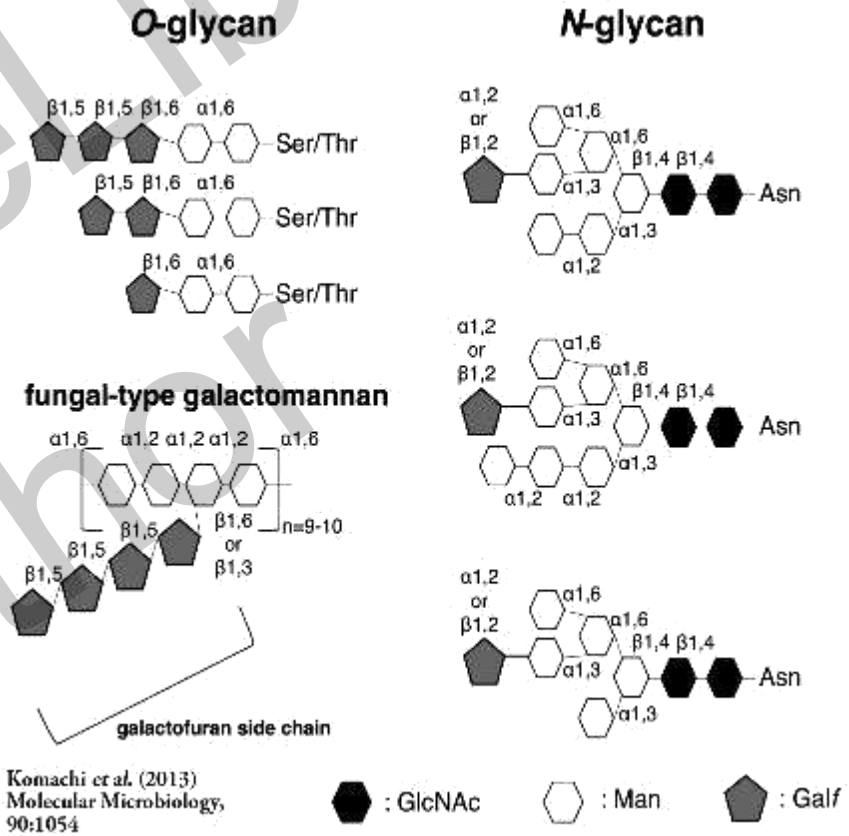


Fig. 1. The equilibrium in solution between Gal*f* and Gal*p*. Both cyclic forms of galactose are in equilibrium with the linear form. The equilibrium is heavily in favor of the six-membered Gal*p* form, as indicated by the arrows. The C-atoms involved in the formation of both cyclic forms are indicated with numbers.

Tefsen *et al.*, *Glycobiol*, 22:456, 2012; Komachi *et al*., Mol Microbiol, 90:1054, 2013; Kudoh *et al*., *Glycobiol*, 25:74, 2015

β-Galactofuranose (gal*f*)

- *A. fumigatus* cell wall contains many gal*f*-glycoconjugates, including
  - Fungal-type galactomannan (GM)
  - O-glycans
  - N-glycans



Tefsen *et al.*, *Glycobiol*, 22:456, 2012; Komachi *et al.*, Mol Microbiol, 90:1054, 2013; Kudoh *et al.*, *Glycobiol*, 25:74, 2015

galf - epitope recognition

- EB-A2 reactivity tested against galf-glycoarray
- Dimeric β5 (galf-galf) recognition a surprise
  - Terminal β5 (galf-galf)
- Potential specificity and sensitivity implications
  - Broader than previously thought but some limits on glycan recognition
- galf epitope specificity the most important driver – *mAb comparisons needed*

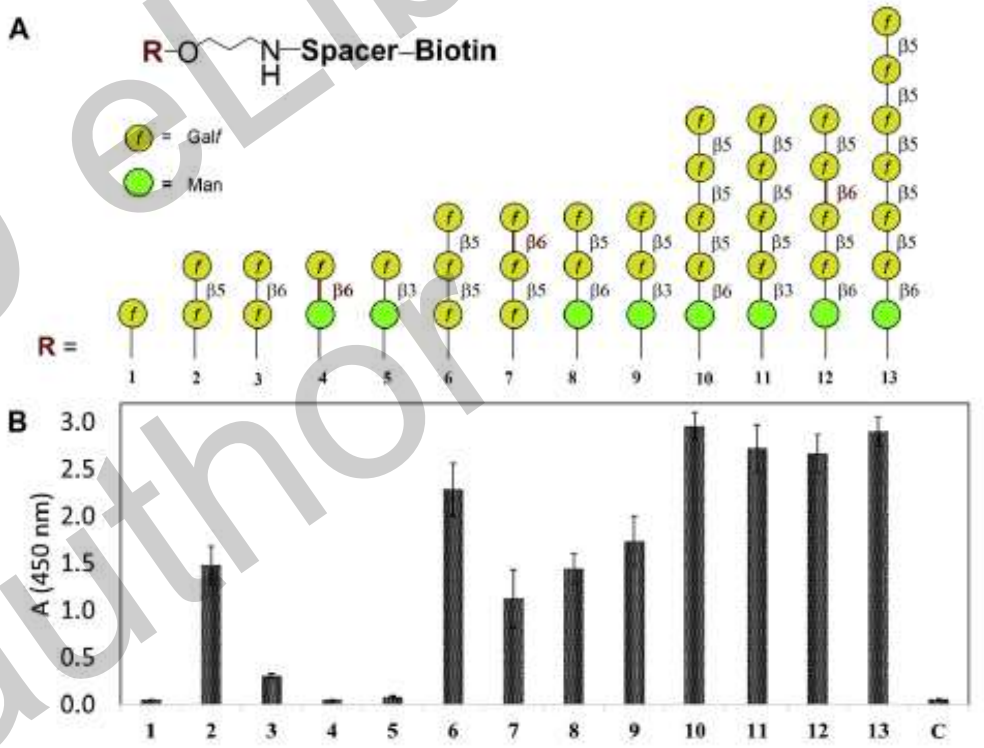
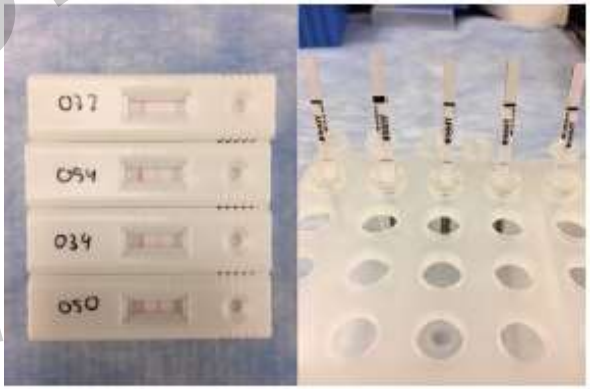


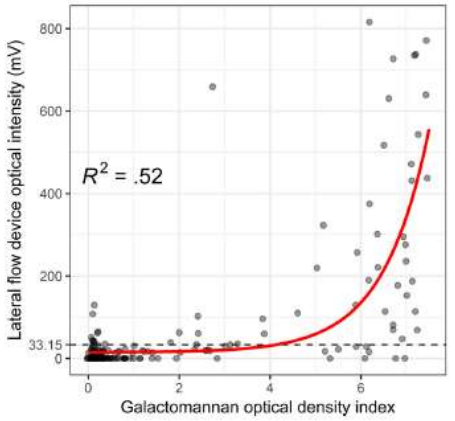
Fig. 1. Investigation of the oligosaccharide specificity of EB-A2 mAb. (A) The thematic glycoarray composed of oligosaccharide ligands representing key structural elements of the *A. fumigatus* galactomannan chain, and (B) the results of assaying the carbohydrate specificity of EB-A2 mAb on the glycoarray.

Krylov et al; Heliyon 2019: 5

Immunodiagnostics

- Multiple LFDs simplifying detection of Ag's in BAL
  - JF5 monoclonal reactive to "glycoprotein" released during active growth (OLM)
    - Different versions evaluated; new CE marked *BAL* LFD 71% sensitive; 100% specific[1]
    - Large BAL study with 247 heme / BMT patients[2]
      - BAL – sensitivity 82%; specificity 86% - 96% (visual vs. digital)
      - Correlation with serum GM EIA
      - Late reads – increased sensitivity, decreased specificity
  - "Galactomannan" LFD (IMMY)
    - Comparative studies
      - Non-neutropenic cohort sensitivity 58 – 69%; specificity 68 – 75%[3]

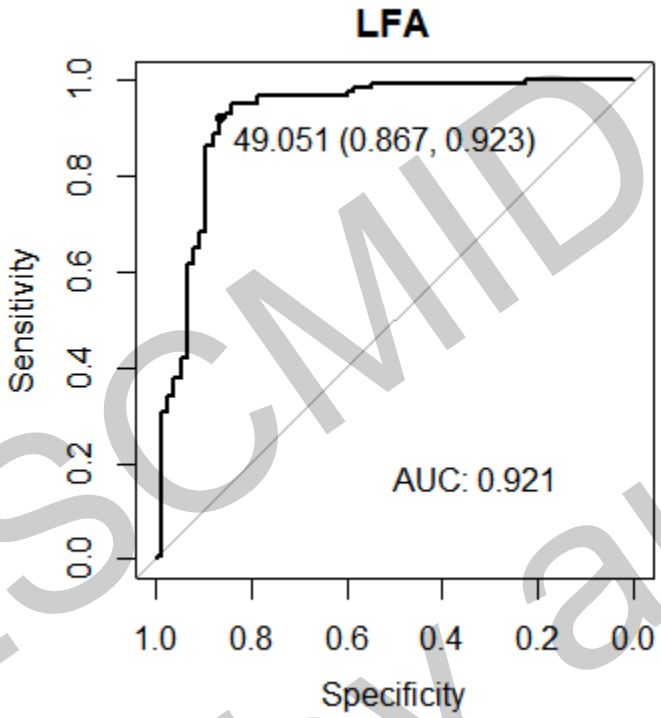




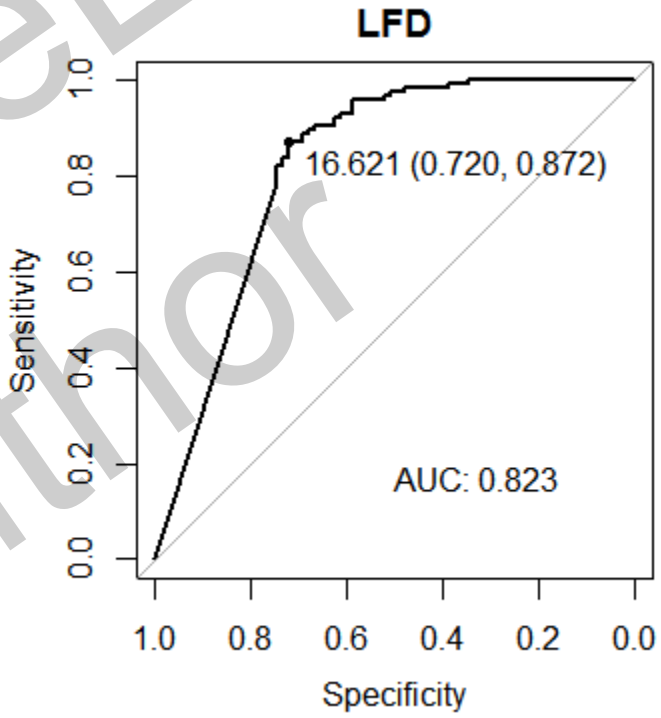
[1]Hoenigl et al. Mycoses 2018; 61
[2]Mercier et al. J Clin Microbiol; 2019: 57
[3]Jenks et al., Mycoses 2019; 62

Immunodiagnostics

Comparative study with digital readouts: both with good performance

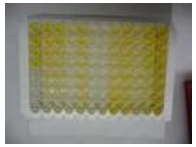


GM – higher sensitivity
more processing

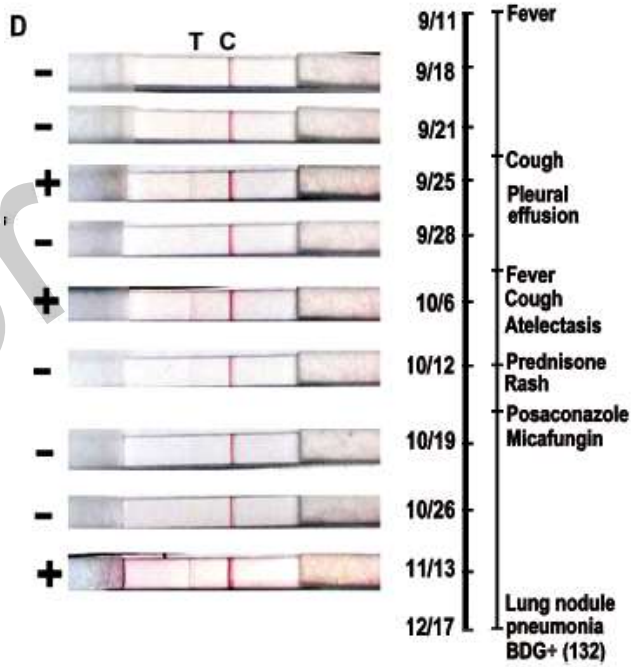


JF5 – easier, lower sensitivity

Immunodiagnostic

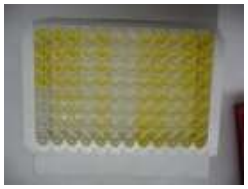


- mAb476 –recognizes *Aspergillus* glycans in infected animal serum, BAL, lung homogenate *but most antigen rapidly excreted in urine*
  - Proof of concept shown with human samples after urine processing step to optimize antigen recovery[1]
- LFD tested in 120 IA patients (MycoMed Tech.)[2]
  - Sensitive 89.5% (69-98); 92% specific (74-99)
  - Early in sequential samples from 'possible' cases
- Semi-quantitative ELISA developed (ASM, 2019)
- ECCMID 2019 (Tues): novel epitope specificity to include monomeric and terminal non β(1→5)-Gal*f* enables recognition of urinary Ag

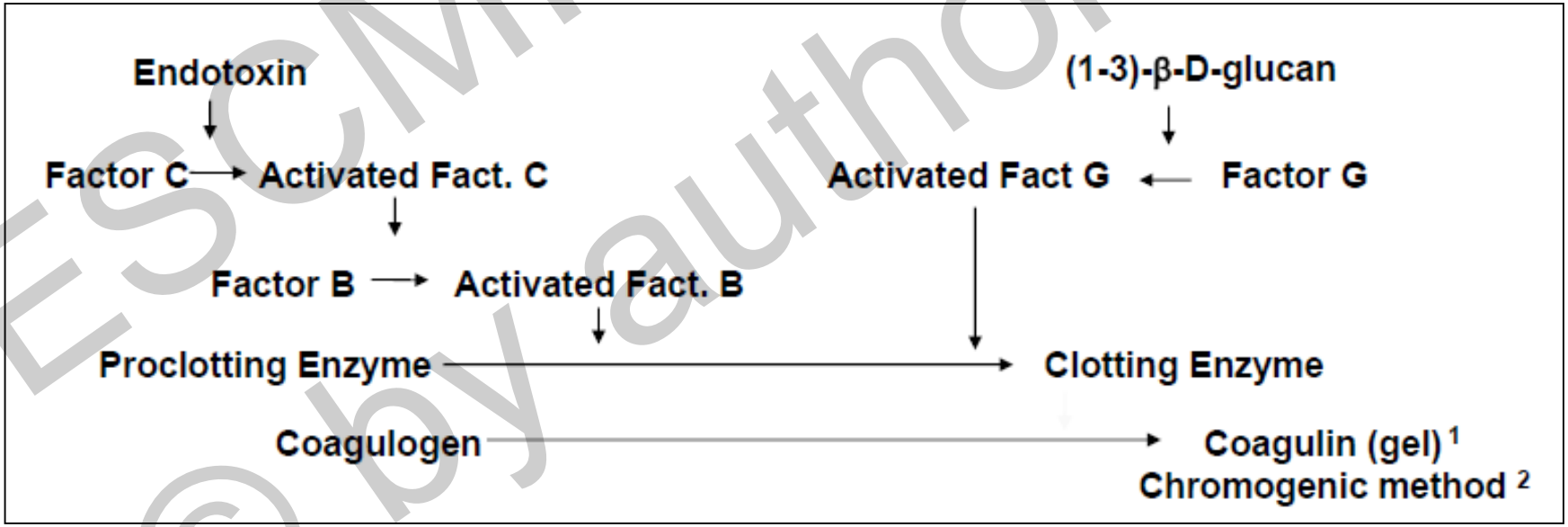


[1]Dufresne *et al*., *PLOS ONE*, 7: e42736, 2012
[2]Marr et al., Clin Infect Dis 2018

β-D-Glucan

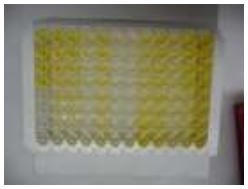


- Activates *Limulus* amebocyte lysate
- Factor G initiates cascade. Output measured by multiple substrates in multiple kits: Wako BDG (Fujifilm Wako); Fungitec G test (Seikagaku); Fungitell (Assoc. Cape Cod); Dynamiker Fungus (Dynamiker BT); GKT-25M (Tianjin Era BT); Goldstream Fungus (Gold Mountain River Test Development)

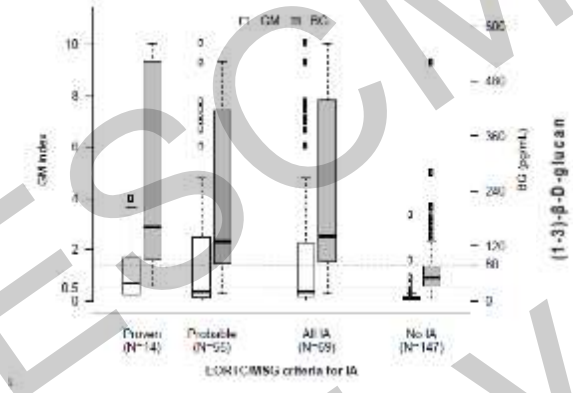


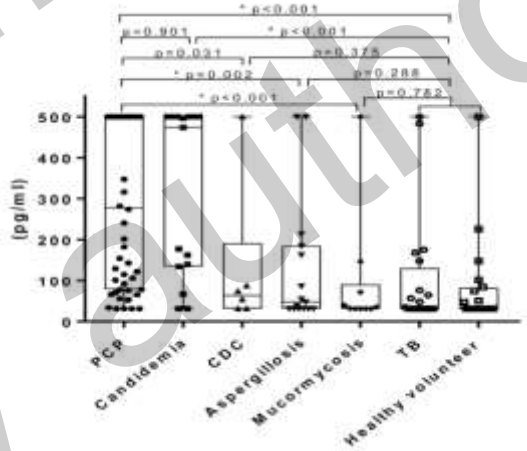
Nature June 10, 2008

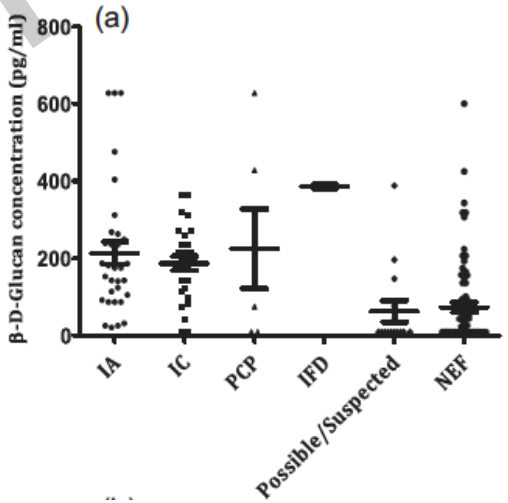
β-D-Glucan



- Highly sensitive, yet non-specific assays
- Likely different cut-offs needed for different IFIs – none yet optimized for IA
  - Fungitell and Goldstream assay – IA with lower values
  - Dynamiker – not as much variation
- More comparisons, ROCs needed

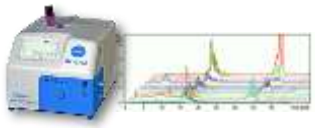






Sulahian A, et al. J Clin Microbiol. 2014 Jul;52(7):2328-33.
Son et al. Plos One 12 (2017)
White et al. Med Mycol 55 (2017)

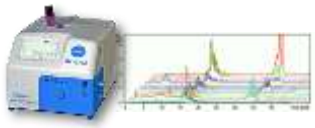
Other



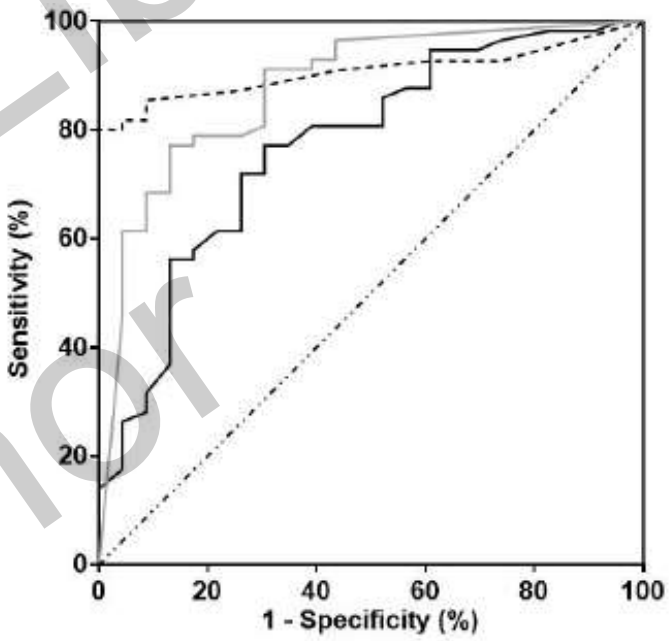
- Exhaled VOCs
  - Unique metabolic profiles predict IA (esp. sesquiterpenes)
    - Detected by MS-GC & Enose
  - Complicated studies and outputs
    - Growth conditions change VOCs
- Fungal siderophores including triacetylfusarine C (TIAC) in urine and serum

Koo et al., Clin ID 2014; de Heer J Breath Res 2016
Hoenigl A, et al. J Infect. 78 (2019)
Skriba et al. Frontiers Microbiol 2018
Rees et al. J Breath Res 11(3) 2018

Other



- MS – disaccharide (glycan) secreted during growth
  - Per-sample performance (blood) – less sensitive compared to BDG but more specific
  - Signals ? (trehalose)



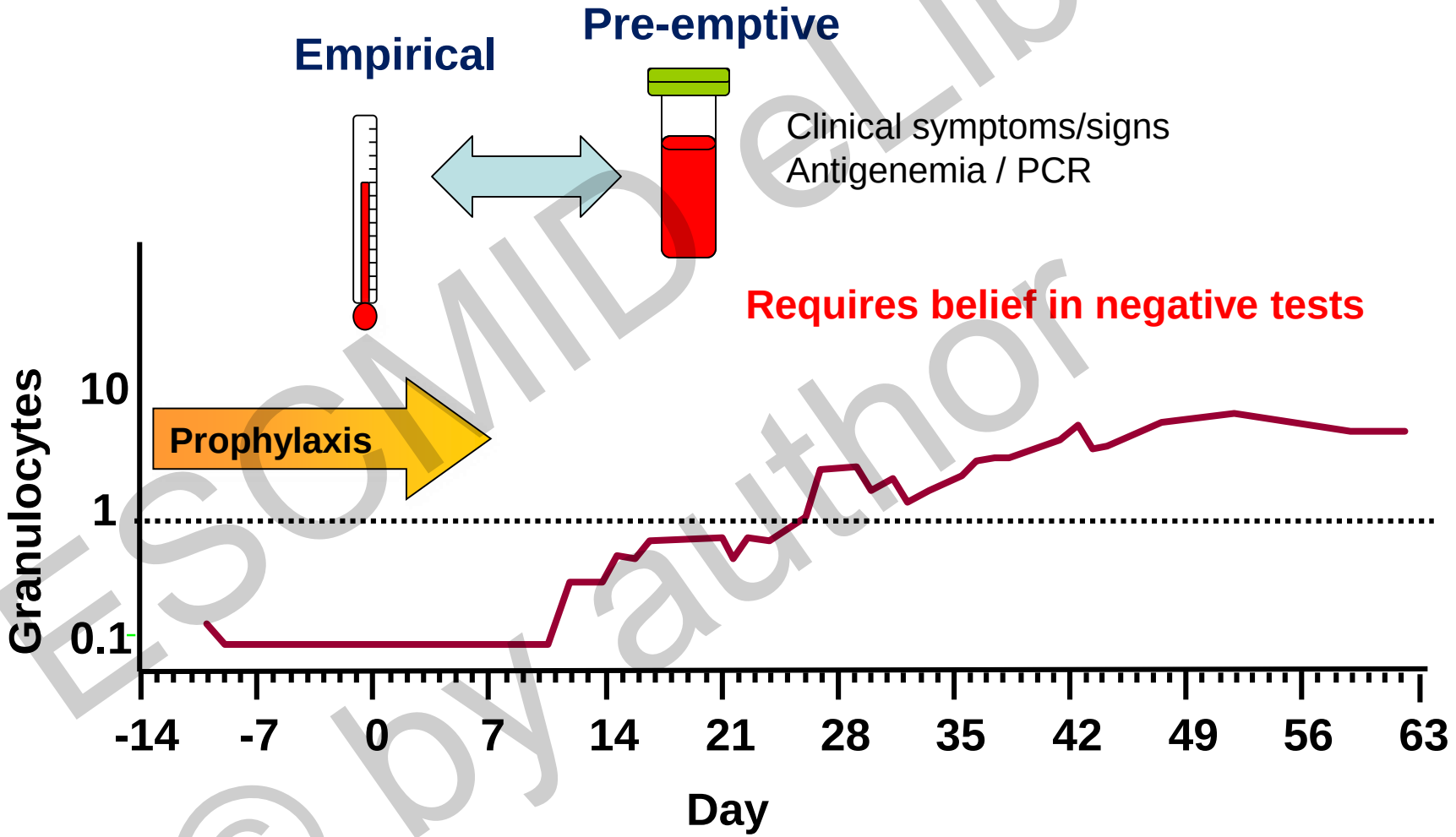
Test	Cut-off	Sensitivity	Specificity	AUC
BDG	80	79.0	73.9	0.872
Man	62.5	47.3	100	0.908
MS-DS	325	50.9	87.0	0.772

Cornu et al. *J Clin Microbiol*. 2019

And more….

- Next Generation Sequencing of cell-free DNA in plasma (Karius)
- Interpreting fungal markers in context of host inflammatory markers (Hoenigl)
- New technologies…

Historical Prevention Paradigm



The Math of (Negative) Tests

- Negative predictive value
  - NPV is the probability a negative test is correct – either stop a drug (or don't start it)
- A good test might have sensitivity & specificity = 85%
  - If likelihood is ~ 10% (low risk), NPV is ≥ 98% *(wrong 1 in 50)*
  - If likelihood is ~ 33% (medium risk), NPV is ≥ 92% *(wrong 1 in 11)*
- To get to an NPV of ≥ 98% at likelihood of 33%...
  - You need sensitivity and specificity of 96%
  - We don't have that with any test

We also need to think about risks differently with screening (10%) vs. suspected disease (33%) → NPV vs. PPV

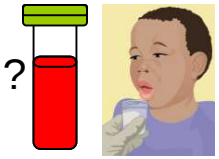
Slide: Adapted from J. Rex

Separate tests according to need
Tailoring diagnostics

Screening assays: High NPV
Easy, convenient: think fecal occult blood

Broad, sensitive, non-invasive predictor of *risk*

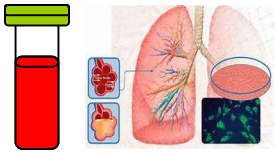
βDG ?
Urine glycan ?
Breath?

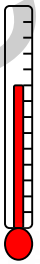


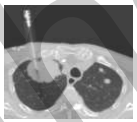
Diagnostic assays: High PPV
More invasive: think colonoscopy

Combined assays specific to pathogen

Serum GM
BAL PCR
BAL Ag







Conclusions

- Much learned about old tests
- New assays use easier technology
  - Need characterized Abs to understand biology
- New sample systems to enable screening (urine, breath)
- Need to think about NPV vs. PPV and combine tests when disease is highly likely

JOHNS HOPKINS
MEDICINE

Thank you