Early laboratory diagnosis of candida infection in non-neutropenic critically ill patients

~~Early identification of fungal sepsis in critically ill~~

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Early laboratory diagnosis of candida infection in non-neutropenic critically ill patients

- Overview of Candida infection in ICU
- Culture Methods
  - Limitations, Possible Improvements and Techniques of Early Species Identification
- Non-Culture Diagnostics
  - Overview, Current Status and Unresolved Issues
- Take Home Message

Magnitude of problem of *Candida* infection in ICU

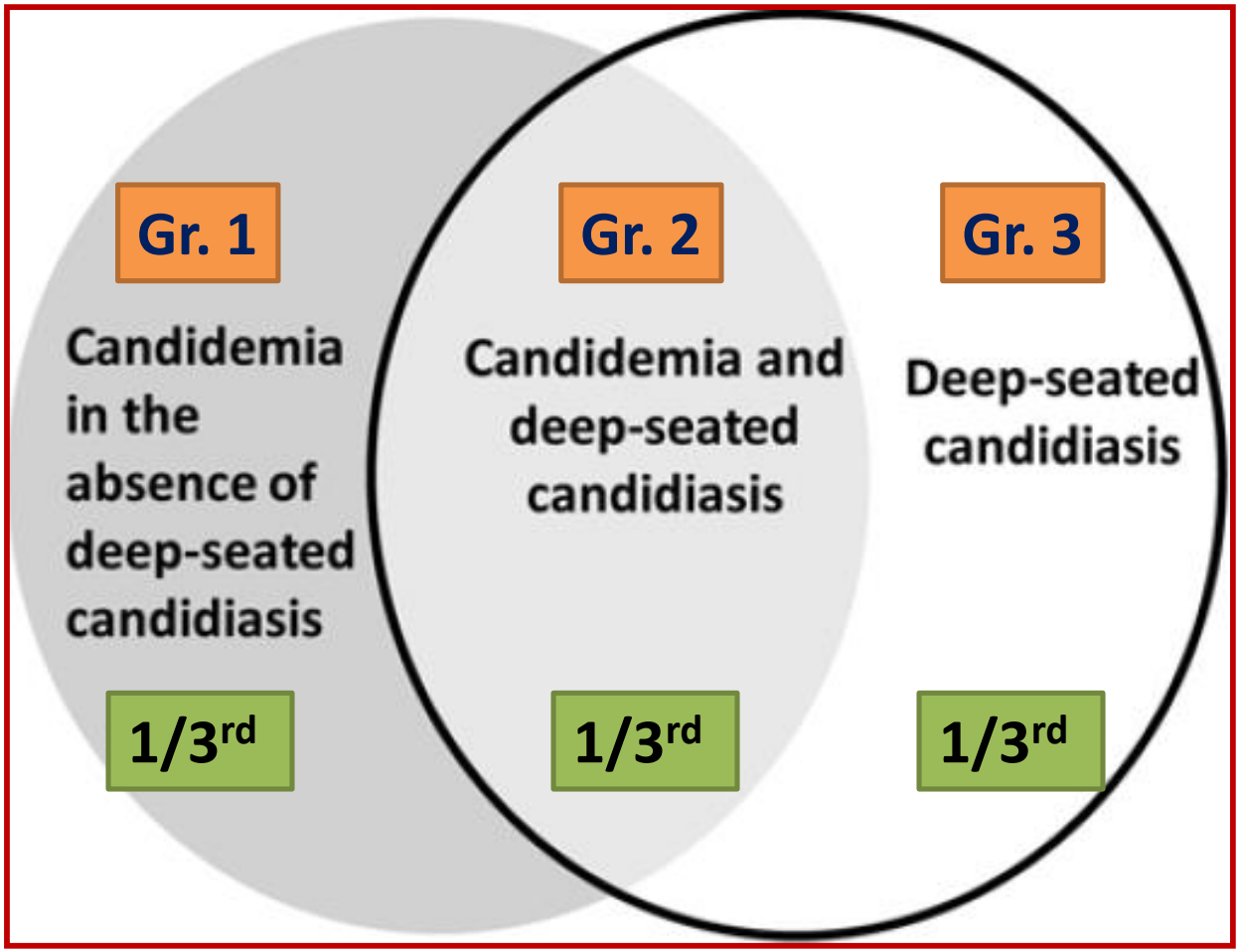
- 9-12% of all Blood Stream Infections (BSI)
- 4th most common cause of nosocomial BSI
- ICUs account for 33-55% of all candida-related hospital infections
- Overall mortality 31-70%, attributable mortality 12-62%
- Delay in effective therapy results in increased mortality, morbidity, LOS and cost of treatment

Performance of Blood Cultures in Autopsy Studies of Invasive Candidiasis

Reference	Year	No. of Patients	Underlying Disease	Sensitivity
Louria (from [13])	1962	19	Hematologic malignancies, solid tumors, medical and surgical conditions	42%
Bodey (from [13])	1966	61	Acute leukemia	25%
Taschdjian (from [13])	1969	17	Malignancies and other medical conditions	47%
Hart (from [13])	1969	16	Hematologic malignancies, solid tumors, transplant, medical and surgical conditions	44%
Bernhardt (from [13])	1972	14	Transplant and surgical conditions	36%
Gaines (from [13])	1973	26	Hematologic malignancies, solid tumors, medical and surgical conditions	54%
Myerowitz (from [13])	1977	39	Hematologic malignancies, solid tumors, medical and surgical conditions	44%
Ness [9]	1989	7	Hematologic malignancies and bone marrow transplant recipients	71%
Singer [37]	1977	16	Hematologic malignancies	31%
Berenguer [13]	1993	37	Mostly hematologic malignancies and solid tumors	43%
Van Burik [38]	1998	62	Bone marrow transplant recipients	52%
Kami [39]	2002	91	Hematologic malignancies	21%
Thorn [40]	2010	10	Hematologic malignancies, gastrointestinal disease, transplant, prematurity	50%

- **Sensitivity = 21-71%, Average 38%**
- **Long median time to positivity - 2-3 days, may take as long as 8 days**
- **No correlation with patient outcome**

Complete Spectrum of Candida Infection in ICU



Gr. 3 patients can not be diagnosed by blood culture

Overall sensitivity of blood culture for all groups remains < 50%

Performance of Deep Seated Cultures

- Gold standard for Gr. 3
- Optimal sampling is not known
- Difficulties and risks in obtaining culture samples from deep seated tissues
- Poor sensitivity has been documented in hepatic candida infection – sensitivity 42%

Improvements in Culture Methods

Improvements in blood culture

- Optimal sampling
  - Repeat sets of blood cultures on baseline day 1 of therapy, day 3, and day 5 or until clearance of the infection is detected.
  - The optimum detection of microorganisms is achieved with ≥3 sets of blood cultures.
  - In adults, 20–30 ml of blood should be collected per blood culture set
- Development of lysis-centrifugation system
  - System increases the yield of Candida from blood by using a detergent to release fungi trapped within host phagocytes
  - This method reduces time between inoculation and detection of growth.
  - This system is expensive, labor intensive and prone to contamination
- Commercially available automated blood culture systems
  - Colorimetric (BacT/ALERT 3D) or Fluorescent (BACTEC 9240)
  - Continuous growth monitoring - every 10 minute

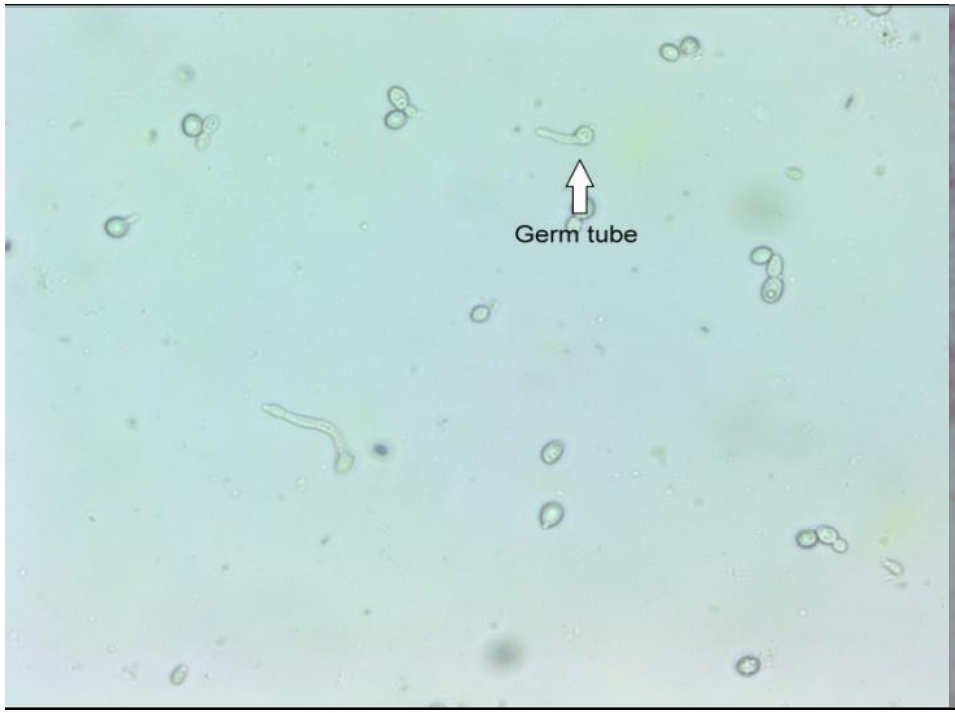
Early Species Identification

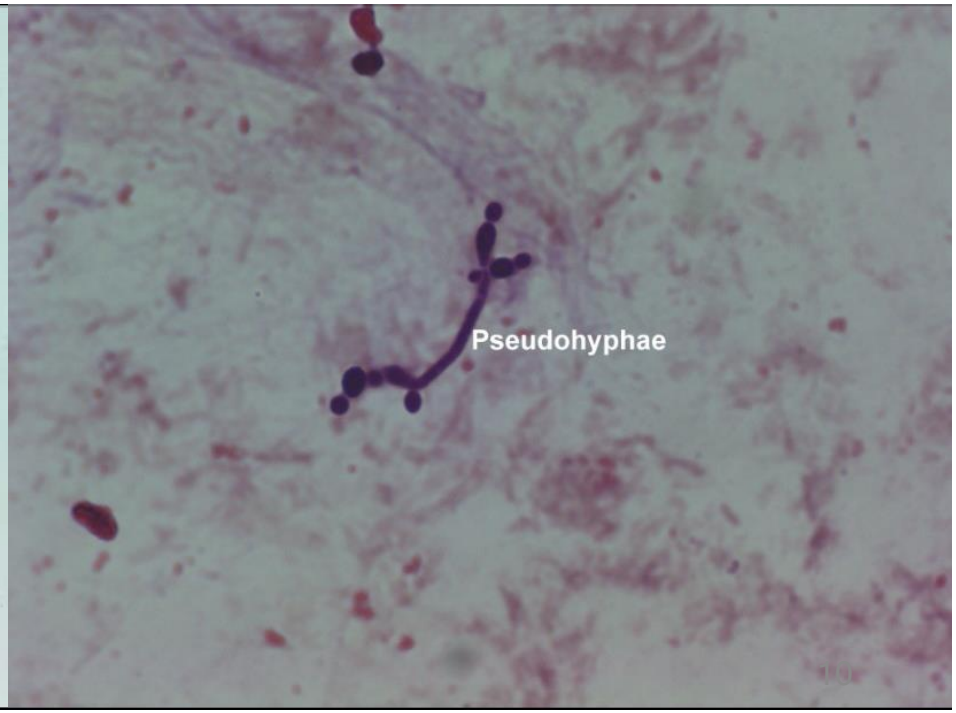
- Speciation of Candida is done on the basis of colony characteristics, germ tube test, physiological and biochemical characteristics or sero-diagnostic tests
- Tests
  - Germ tube test
  - Chromogenic media
  - Biochemical characterization
  - Others

Int.J.Curr.Microbiol.App.Sci (2014) 3(1): 206-218

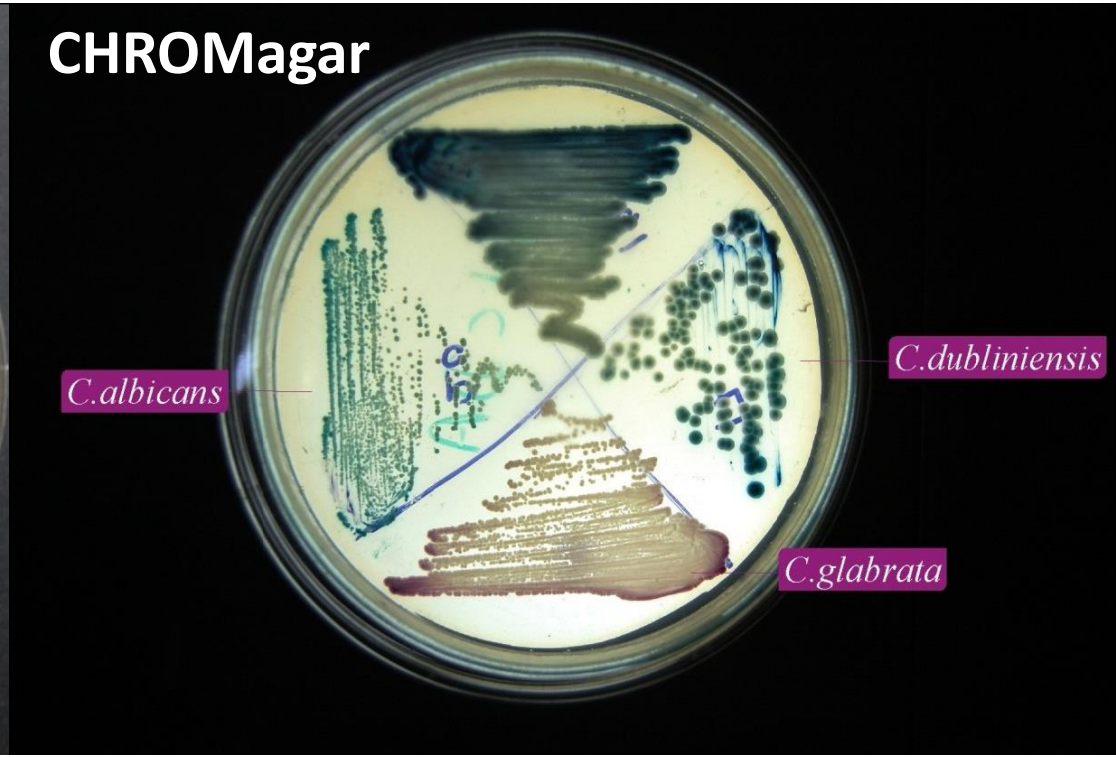
Germ tube test

- This is a rapid method for identifying *C. albicans and C. dubliniensis* by their ability to produce short, slender, tube like structures called germ tubes when it is incubated in serum at 37^0C for 2 hours.
- Non-albicans Candida spp. do not grow germ tubes.
- **Germ tubes** are elongated daughter cells arising from the mother cell without constriction at their origin whereas **pseudohyphae** have constriction at the origin of mother cells.





Chromogenic media



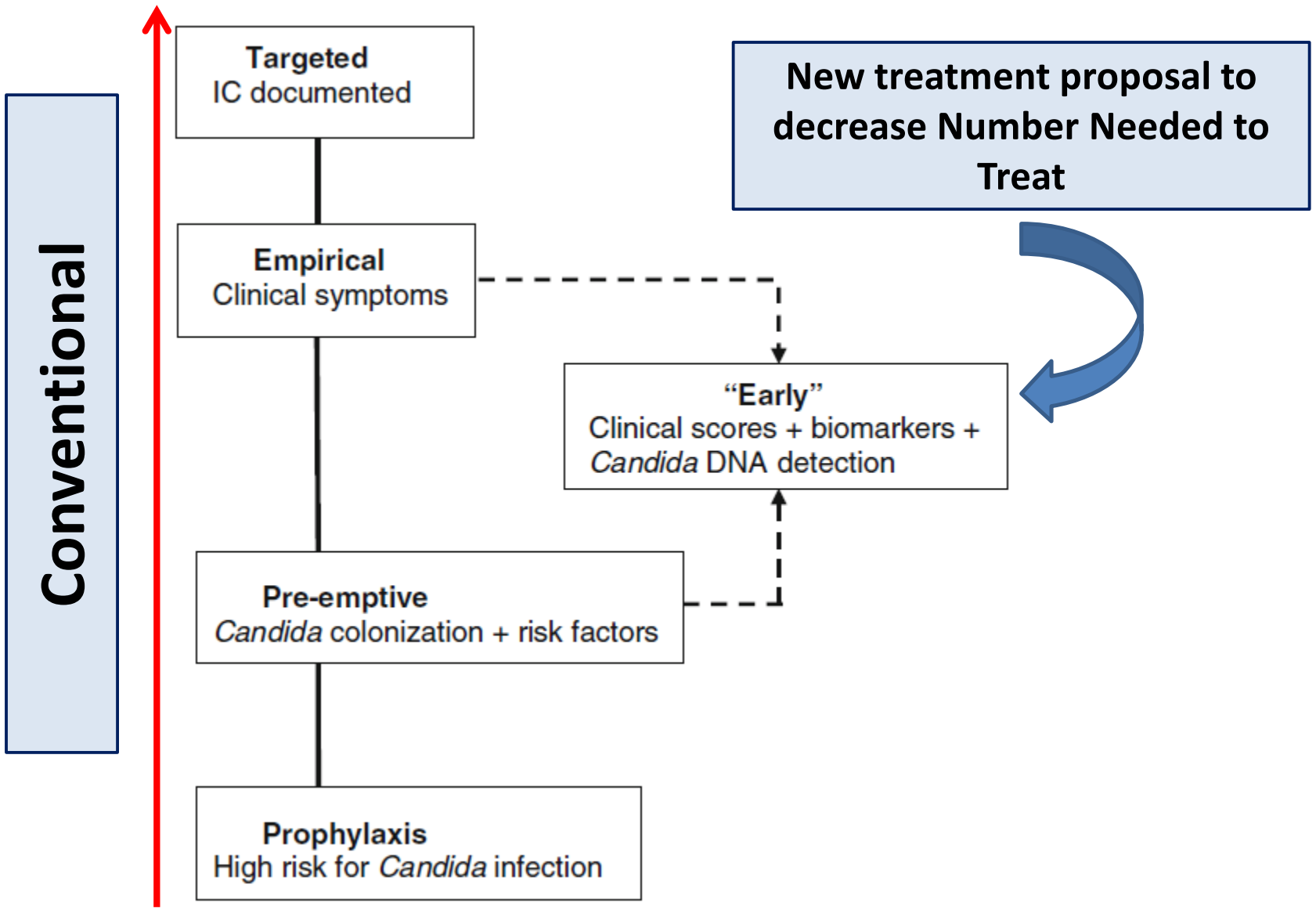
Other Methods

- Biochemical characterization
  - Biochemical identification of *Candida* spp. is based on assimilation and fermentation of carbohydrates.
  - Many manual and automated techniques like VITEK system
- Others
  - The formation of true hyphae, pseudohyphae, chlamydospores and arthroconidia aids in identification of Candida spp on variety of nutritionally deficient media which suppress the vegetative growth and promote sporulation.
  - Urease test can be used for identification of C. krusei
  - Methyl blue SDA and Staib agar (niger seed agar) can be used to distinguish C. albicans and C. dubliniensis which shares many phenotypic properties

Non-Culture Diagnostics

Why do we need Non-Culture Diagnostics?

Treatment of IC in critically ill patients



Serological and molecular methods

- Serological
  - Antigen
  - Antobody
  - Antigen and antibody both
  - Fungal cell wall component
- Molecular
  - Polymerase chain reaction (PCR) technology
  - Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)
  - Others
    - Restriction fragment length polymorphism (RFLP) analysis
    - Southern hybridization analysis
    - tRNA profile analysis

These identification methods are not standard routine procedures

Antigen detection

- Important diagnostic tool in immunocompromised patients where antibody production can be variable or nonexistent
- **Mannan** is a major cell wall component of *Candida* and it is released in blood circulation during infection
- **Mannan** can be detected in serum and other body fluids by a number of serological reactions
  - Enzyme linked immunosorbent assay (ELISA), Radioimmunoassay (RIA), Latex agglutination (LA) and Reverse passive agglutination test (RPLA)
- Other antigens that can be detected include **47 kDA protein**, enolase, specific mannosides and extracellular secreted proteinases

Antibody detection

- The clinical utility of antibody detection for diagnosis is limited because of 2 main reasons
  - False negative results in immunocompromised patients, where there is low or undetectable levels of antibodies.
  - False positive results in patients with superficial colonization
- Currently two tests are available
  - ELISA based test for detection of **antimannan antibodies** (**Platelia** *Candida* antibody test, Bio-Rad Laboratories, France)
  - Indirect immunofluorescence assay for detection of *C. albicans germ tube antibody (**CAGTA**,* C. albicans IFA IgG; Virvell Laboratories, Spain*)*

Fungal Cell Wall Component

- 1, 3,-β -D-glucan (BDG), a polysaccharide, is a structural component of Candida cell wall
- Its presence in the circulation signifies systemic infection
- BDG can be detected by its ability to activate factor G in the coagulation cascade of Japanese horseshoe crab (Tachypleus tridentalis)
- False positive results in several conditions like haemodialysis, abdominal surgery and treatment with β–lactam antibiotics

Molecular diagnostic techniques

- PCR and MALDI TOF-MS are well known molecular techniques
- Various polymerase chain reaction (PCR) techniques detect nucleic acids
- Rapid, sensitive and specific
- False positive results may be due contamination
  - To be used only for detection of *Candida spp. from* normally sterile sites such as blood, CSF and peritoneal fluid.
- Early identification of all clinically relevant *Candida spp.*
- **Technique can be used for detecting *Candida spp* directly from clinical specimens**

MALDI TOF-MS

- Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI TOF-MS)
- MALDI TOF-MS usually identifies microorganisms by analysing cellular proteins and peptides, the technology may also be used for the analysis of carbohydrates, polymers, oligonucleotides, single nucleotide polymorphisms and metabolites
- Identification requires organisms to be obtained from culture media
- Accurate, rapid, and reliable technique for 100% Candida isolates.

Diagn Microbiol Infect Dis. 2012 May;73(1):65-7
Clin Lab. 2012;58(9-10):927-31

Current Status and Unresolved Issues

Unresolved Issues for All Non-Culture Diagnostics

- How do tests perform in blood culture negative cases?
- How do tests perform in deep seated candidiasis?
- How do tests perform in specific patient populations?
- What is the impact of antifungal therapy on performance?
- What is the impact of colonization, mucosal candidiasis, or prior invasive candidiasis on performance?
- What are the kinetics of the tests, and do baseline values change over time
- Do they have prognostic value?
- How should tests be incorporated into patient management strategies?
- How do the tests perform in samples other than blood/ serum?

Unresolved Issues

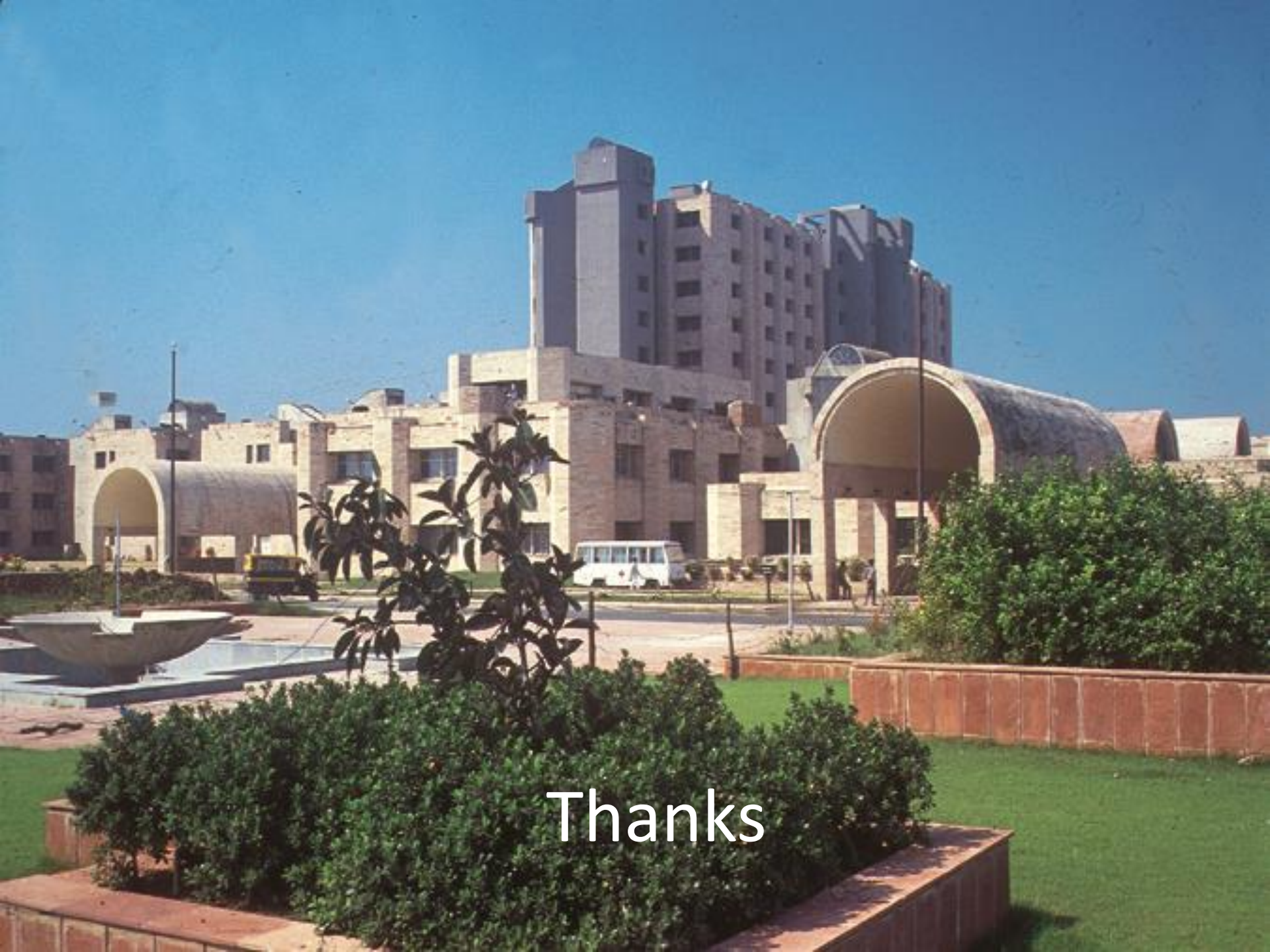
- Mannan/Antimannan Diagnostics
  - How does the assay perform for infections caused by various Candida spp?
  - What is the impact of immunosuppression on performance?
  - What is the timeline of immunoglobulin G responses during the pathogenesis of invasive candidiasis?
  - How does the assay perform in patients who have ongoing, subclinical invasive disease?
- β-D-Glucan Diagnostics
  - What is the specificity, and what are the positive predictive values (especially in high-risk populations)?
  - What is the impact of β-D-glucan synthesis inhibition by echinocandins on performance?
- Polymerase Chain Reaction Diagnostics
  - Will a standardized assay be developed?
  - Will an assay be validated in multicenter studies?

Take Home Message

- Candida infection in critically ill patients is difficult to predict
- Diagnosis is a major challenge
- Microbiological documentation has low sensitivity and occurs late
- Empiric antifungal treatment without documented invasive candidiasis is a common practice leading to antifungal overuse and upto 70% of antifungal treatment in ICU remains pre-emptive/empirical
- Non-culture diagnostics are very much needed to compliment cultures particularly for identifying the missing 50% of patients of IC who remain blood culture negative

Take Home Message

- Mannan antigen (Mn) and anti-mannan antibodies (A-Mn), β-D-glucan (BDG), Polymerase chain reaction (PCR), Candida albicans germ tube antibody (CAGTA) are promising non-culture diagnostics for early diagnosis of IC
- Galactomannan is promising in the diagnosis of IA
- Turn around time is usually in hours only
- They have promising sensitivity and specificity
- In some cases PPV and NPV is limited because of the low prevalence of IC and IA in non-neutropenic ICU patients
- When used in conjunction with other risk prediction tools, they can improve pre-emptive treatment strategy
- Their good NPV is very useful in ruling out fungal infections and avoinding unnecessary anti-fungal therapy


Thanks

Current Status of Non-Culture Methods

Mn and A-Mn – Meta-Analysys

- 14 studies 453 patients and 767 controls (07 haematological and cancer cases and 07 mainly ICU and surgery cases)
- All studies but one were retrospective in design.

Test	Sensitivity	Specificity	Diagnostics Odds Ratio
Mn	58% (95% CI, 53-62)	93% (95% CI, 91-94)	18 (95% CI 12-28).
A-Mn	59% (95% CI, 54-65)	83% (95% CI, 79-97)	12 (95% CI 7-21)
Mn + A-Mn	83% (95% CI, 79-87)	86% (95% CI, 82-90)	58 (95% CI 27-122)

- **Conclusions: Mn and A-Mn are useful for diagnosis of IC. The performance of combined Mn/A-Mn testing is superior to either Mn or A-Mn testing**

Critical Care 2010, 14:R222

β-D-glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis

16 studies, 2979 patients (594 with proven or probable IFIs)
Cutoff – 80 pg/mL in most of the studies

Test Methods	Sensitivity	Specificity	DOR	PLR	NLR	AUC
All types of assays	**76.8%** (95% CI, 67.1% - 84.3%)	**85.3%** (95% CI, 79.6% - 89.7%)	**19.2** (95% CI, 10.5 -35.4)	**5.2** (95% CI, 3.7 - 7.5)	**0.27** (95% CI, 0.19 -0.40)	**0.89** (95% CI, 0.86 -0.91)
Fungitell or Glucatell assay	**71.3%** (95% CI, 59.9%-80.6%)	**82.0%** (95% CI, 69.6% - 90.1%).	**11.3** (95% CI, 4.7 - 27.5)	**4.0** (95% CI, 2.2 - 7.2)	**0.35** (95% CI, 0.24 - 0.52)	**0.81** (95% CI, 0.78 - 0.85)

Clinical Infectious Diseases 2011;52(6):750–770

Systematic Review and Meta-Analysis PCR Diagnosis of Invasive Candidiasis

- Proven candidemia cases and healthy controls – sensitivity 100% and specificity 100%
- Suspected invasive candidiasis cases and healthy controls – Sensitivity 0.95 (CI, 0.88 to 0.98) and specificity 0.92 (CI, 0.88 to 0.95)
- A specificity of >90% in different control groups.
- Use of whole-blood samples, rRNA, or P450 gene targets and a PCR detection limit of <10 CFU/ml improves test performance.
- PCR positivity rates among patients with proven or probable IC were 85% (78 to 91%), while blood cultures were positive for 38% (29 to 46%).
- **Conclusion -** direct PCR using blood samples had good sensitivity and specificity for the diagnosis of IC and offers an attractive method for early diagnosis of specific *Candida spp.* Its effects on clinical outcomes should be investigated.

J. Clin. Microbiol. 2011, 49(2):665.

Summary of Commercially Available Molecular Assays

Assay	Method	Targets	Results	Specimen	TAT	FDA A/C
* Yeast Traffic Light	PNA FISH	26S rRNA for Candida spp	Qualitative, Speciation of most Candida spp	Blood culture bottles +ve for growth	2-3 h	Yes
** Multiplex xTAG Fungal ASR Assay	Multiplex PCR & bead-based flow Cytometry	23 clinically significant fungi (yeasts and molds)	Qualitative, with Speciation when possible	Respiratory specimens; blood culture bottles +ve for growth	5–6 h post extraction	No
*** Aspergillus Real-Time PCR Panel	Real-time PCR	18S rRNA and ITS1 For Aspergillus spp	Qualitative, Detection of Aspergillus spp, A. fumigatus, or A. terreus	BAL; bronchial washing	8-12 h	No

* AdvanDx, USA, ** Luminex USA *** Viracor-IBT USA

Clin Lab Med 33 (2013) 505–525

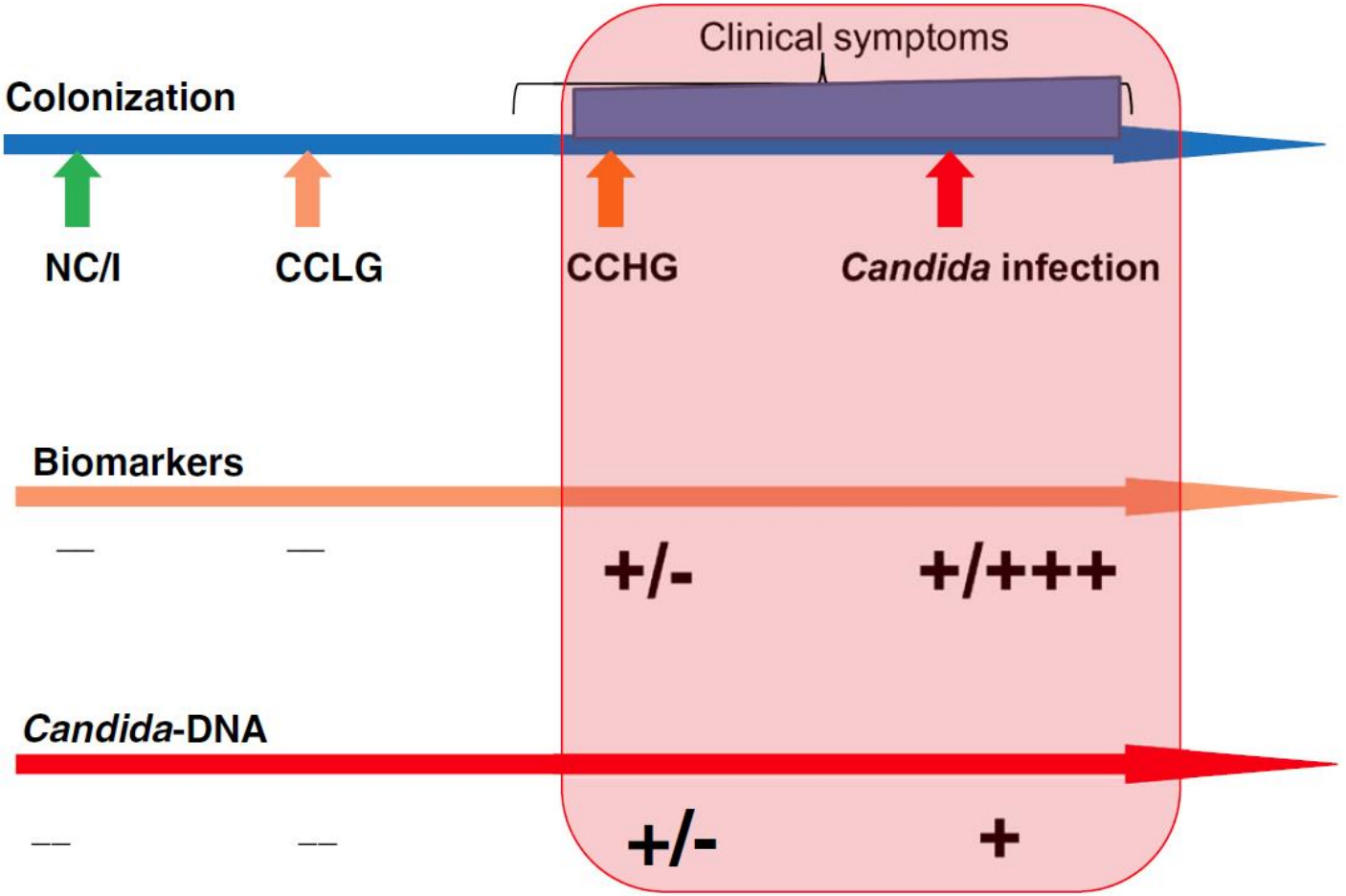
Summary of Commercially Available Molecular Assays

Assay	Method	Targets	Results	Specimen	TAT	FDA A/C
* Candida Real-time PCR Panel	Real-time PCR	ITS1 for Candida spp	Qualitative, Detection of C. alb and/or C. trop; C. glab and/or C. krus; and C. parapsilosis complex	Plasma; serum	Same day	No
** PLEX-ID Broad Fungal Assay	Multiplex PCR and mass spectrometer	Up to 75 fungi	Qualitative, unique Organism identification	BAL; blood	6-12 h	No
# MycAssay Aspergillus	Real-time PCR	18S rRNA for Aspergillus spp	Qualitative	Serum; BAL	3 h	No
## SeptiFast	Real-time PCR	5 species of Candida & A. fumigatus	Qualitative	Blood	6 h	No

* Viracor-IBT, USA, **Abbott, USA, # Myconostica UK, ## Roche, USA

Clin Lab Med 33 (2013) 505–525

Interrelation between microbiology, clinical, biomarkers, and Candida DNA



NC/I - no colonized/infected
CCLG - Candida colonization low grade
CCHG - Candida colonization high grade

Non-Culture Diagnostics for IC

Goals

- Finding the "Missing 50%" of IC which are culture -ve
- Use of their NPV to rule out IC and to discontinue unnecessary antifungal therapy
- Use of their PPV in conjunction with other risk prediction tools to improve empirical treatment strategy
- Improved diagnostics compared to culture alone

Tests

- Mannan antigen (Mn) and anti-mannan antibodies (A-Mn)
- β-D-glucan (BDG)
- Polymerase chain reaction (PCR)
- Candida albicans germ tube antibody (CAGTA)
- Enolase and arabinitol

β-D-glucan and CAGTA in Severe Abdominal Conditions

Diagnostic accuracy of CART-derived prediction rule, BDG (cutoff - 259 pg/mL), CAGTA (cutoff, any positive value), and CS for the diagnosis of invasive candidiasis

	Area under ROC curve (95 % CI)[a]	Sensitivity % (95 % CI)	Specificity % (95 % CI)	Predictive value	
				Positive % (95 % CI)	Negative % (95 % CI)
CART analysis	0.78 (0.76–0.81)	90.3 (75.1–96.6)	54.8 (44.1–65.0)	42.4 (31.2–54.4)	93.9 (83.5–97.9)
BDG	0.66 (0.59–0.74)	51.6 (34.8–68.0)	86.9 (78.0–92.5)	59.3 (40.7–75.5)	83.0 (73.8–89.4)
CAGTA	0.67 (0.64–0.71)	71.0 (53.4–83.9)	57.3 (46.5–67.5)	38.6 (27.1–51.6)	83.9 (72.2–91.3)
CS	0.62 (0.58–0.66)	93.5 (79.2–98.2)	18.1 (11.3–27.7)	29.9 (21.7–39.6)	88.2 (65.7–96.7)

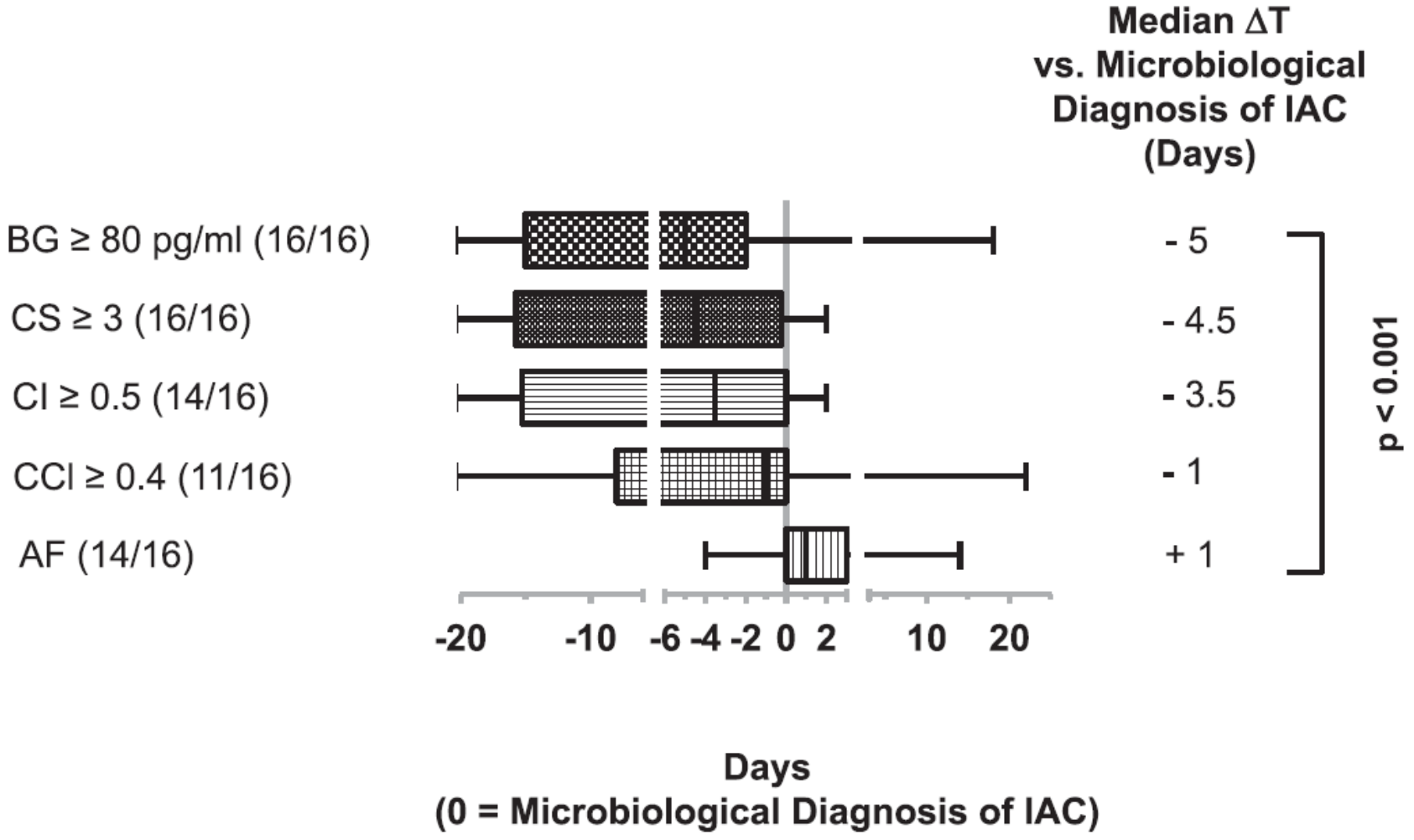
CART-derived prediction rule applied for all the study population

	Node BDG <259 and CAGTA negative	Node BDG <259 and CAGTA positive	BDG >259	Total
Neither colonized nor infected, *n* (%)	31 (50.8)	18 (29.5)	12 (19.7)	61
Candida spp. colonization, *n* (%)	46 (54.8)	27 (32.1)	11 (13.1)	84
Invasive candidiasis, *n* (%)	3 (9.7)	12 (38.7)	16 (51.6)	31
Total, *n* (%)	80 (45.5)	57 (32.4)	39 (22.2)	176

CART - classification and regression tree analysis

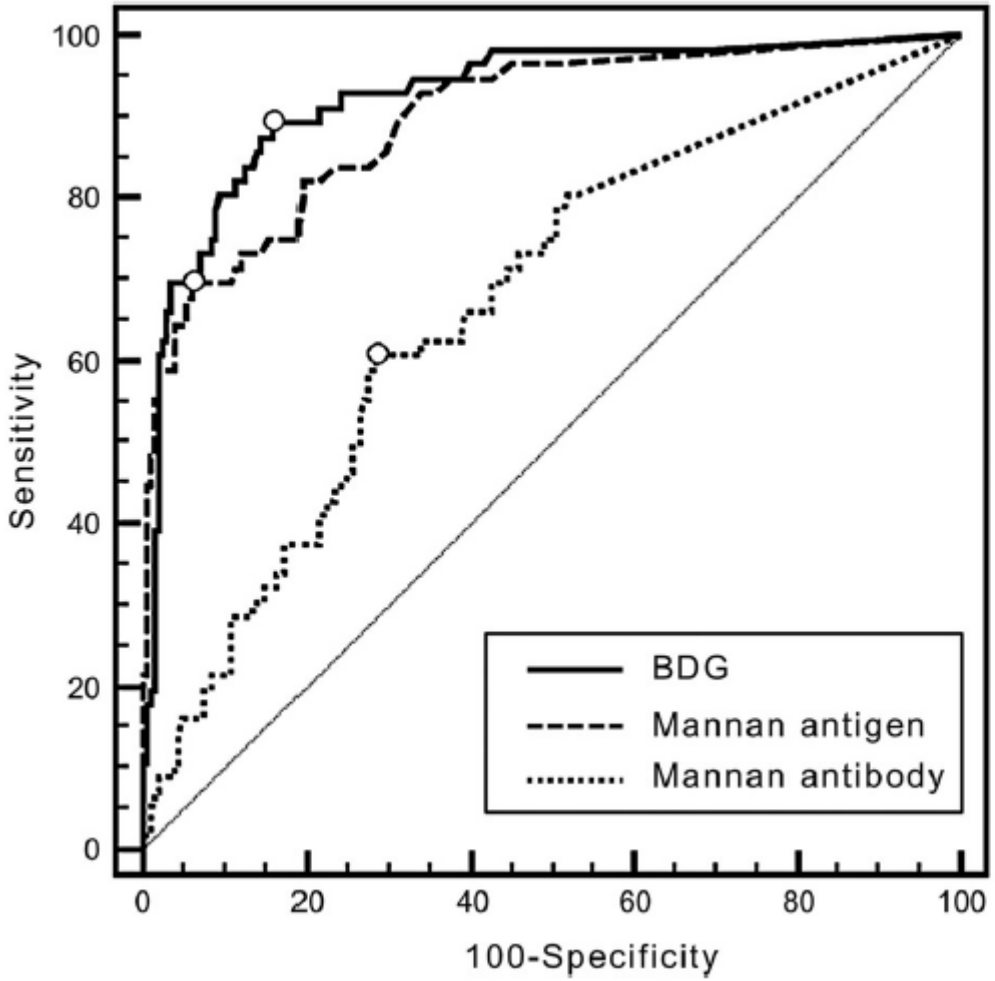
Intensive Care Med (2012) 38:1315–1325

β-D-glucan anticipates Diagnosis of Blood Culture–Negative Intra-abdominal Candidiasis



Am J Respir Crit Care Med Vol 188, Iss. 9, pp 1100–1109, Nov 1, 2013

Comparison of β-D-glucan , Mn/A-Mn, and Cand-Tec *Candida* Antigen as Serum Biomarkers for Candidemia



	AUC (95%-CI)
BDG	0.925 (0.885-0.954)
Mannan-Ag	0.898 (0.854-0.932)
Mannan-Ab	0.673 (0.612-0.730)
	Optimized cut-off
BDG	≥ 70 pg/ml
Mannan-Ag	> 50 pg/ml
Mannan-Ab	≥ 15 AU/ml

ROC curves for BDG, mannan Ag, and mannan Ab.

J. Clin. Microbiol. 2013, 51(4):1158

Causes of False +ve β-D-Glucan Results for IC

False-positive Results

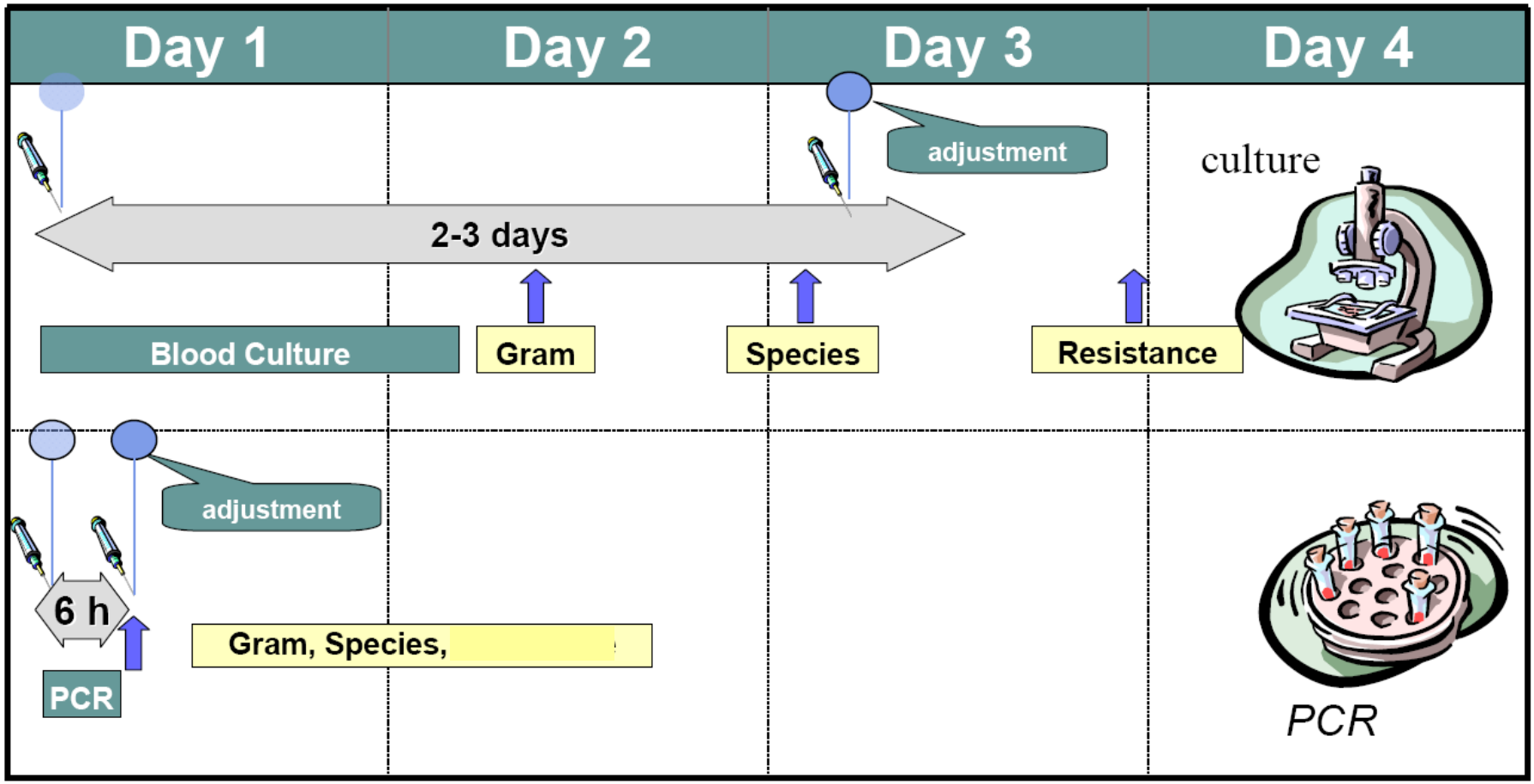
- Human blood products (albumin, immunoglobulin, coagulation factors, plasma protein fractions)
- Hemodialysis*
- Surgical gauze or other materials containing glucan
- Antibiotics such as piperacillin-tazobactam and ampicillin-clavulanate
- Systemic bacterial infections
- Excess manipulation of sample
- Severe mucositis

Fungi That Yield Positive β-D-Glucan Results

- Yeasts: Candida spp, Trichosporon spp, Saccharomyces cerevisiae
- Molds: Acremonium, Aspergillus spp, Fusarium spp
- Dimorphic fungi: Coccidioides immitis, Histoplasma capsulatum, Sporothrix schenckii
- Others: Pneumocystis jiroveci

***Initial reports ascribed false-positive results to cellulose membranes, but more recent studies have described associations with hemodialysis in the absence of such membranes also.**

PCR provides early diagnosis



Species Identification

- Direct Examination of Clinical Samples
  - Classical stains used in histopathology include Gomori methenamine silver, periodic acid-Schiff, Gridley fungus, and hematoxylin and eosin stains.
  - Alternatively, calcofluor white (CW) can be used with a fl uorescent microscope to observe fungal elements in clinical samples.
- Culture-Based Methods for Fungal Detection
  - Non-specific
    - Chromogenic media
    - Automated blood culture systems
  - Specific for *C. albicans*
    - Germ tube test
    - *N -acetyl- b - D -galactosaminidase and L -proline arylamidase*
    - CHROMagar™ for *C. glabrata*
- Post-culture Identification Methods
  - Manual identification methods
  - Automated Identification Systems
  - Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)

Mannan antigen (Mn) and anti-mannan antibodies (A-Mn)

Mannan antigen (Mn) and anti-mannan antibodies (A-Mn)

- Mn is a major component of the C. albicans cell wall, composing up to 7% of the cell dry weight, and is one of the main Candida antigens that circulate during infection
- There is a balance between Mn epitope circulation and A-Mn antibody response
- Combined detection of mannanemia and A-Mn antibodies by enzyme-linked immunosorbent assays (ELISAs) is the recommended diagnostic procedure
- Platelia™ Candida Antigen (Bio-Rad Laboratories, France) and Platelia™ Candida Antibody

Critical Care 2010, 14:R222

β-D-glucan

β-D-glucan Test

- BDG is a component of the cell wall of most fungi.
- The main exceptions are Zygomycetes and cryptococci
- The measurement of BDG is based on the Limulus test
- BDG activates factor G, a serine protease zymogen of the Limulus amebocyte lysate, which is extracted from amebocytes of horseshoe crab species. This in turn activates a coagulation cascade. The activity of this reaction can be measured with use of colorimetric or turbidimetric methods.

Comparison of BDG test findings in non-neutropenic critically ill adult patients (ICU)

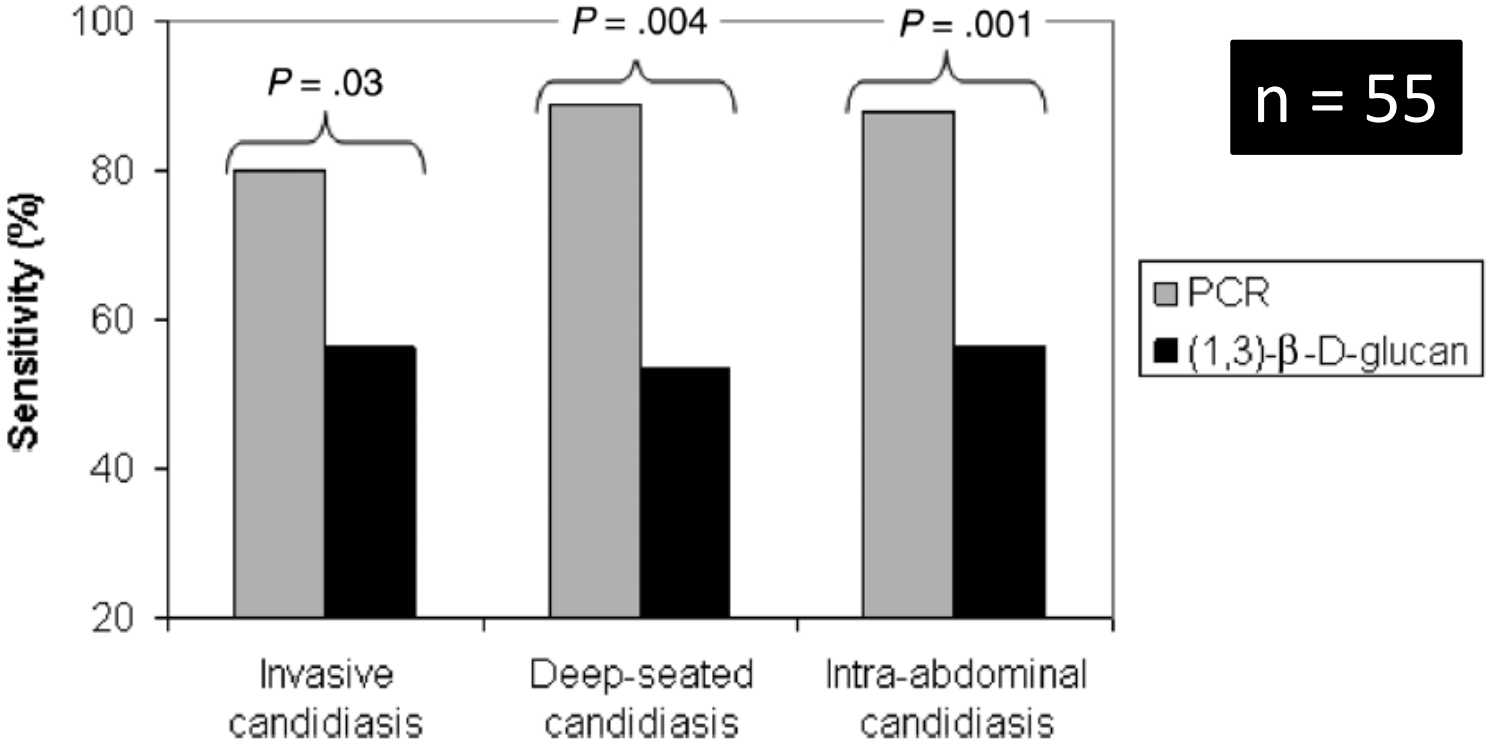
At the cutoff value of > 80 pg/mL

Sensitivity (%) (95 % CI)	Specificity (%) (95 % CI)	PPV (%) (95 % CI)	NPV (%) (95 % CI)	Proven IC BG[b] (median)
65 (46–82)[a]	78 (63–90)[a]	68 (48–84)[a]	77 (61–88)[a]	223
51.6 (34–69) 62	86.9 (78–92) 98	59.3 (40–75) 98.4	83.0 (73–89) 57.3	259 324
92.9 (66–99)	93.7 (85–90)	72.2 (46–90)	98.7 (92–99)	500
100[a]	59[a]	NDA	NDA	171

[a] Two consecutive BG determinations: maximal BG to time of the IC diagnosis

Mixed patient population - invasive candidiasis, candidemia, intra-abdominal candidiasis, severe abdominal conditions, hepatic candidiasis, mediastinitis

Sensitivity of serum polymerase chain reaction (PCR) and β-D-glucan (BDG) in diagnosing IC



PCR was superior to BDG, particularly among patients with deep-seated candidiasis.
60% had deep-seated candidiasis in the absence of positive blood cultures (Gr III)
31% had candidemia without evidence of deep-seated candidiasis (Gr I)
9% had both candidemia and deep-seated candidiasis (Gr II).
89% of deep-seated candidiasis was intra-abdominal infections.
Deep-seated and IA candidiasis include patients with and without +ve blood cultures.
Results for deep-seated candidiasis without +ve blood cultures did not differ from the deep-seated candidiasis with +ve blood cultures.

DNA detection by polymerase chain reaction (PCR)

The versatility of PCR has led to a large number of variants

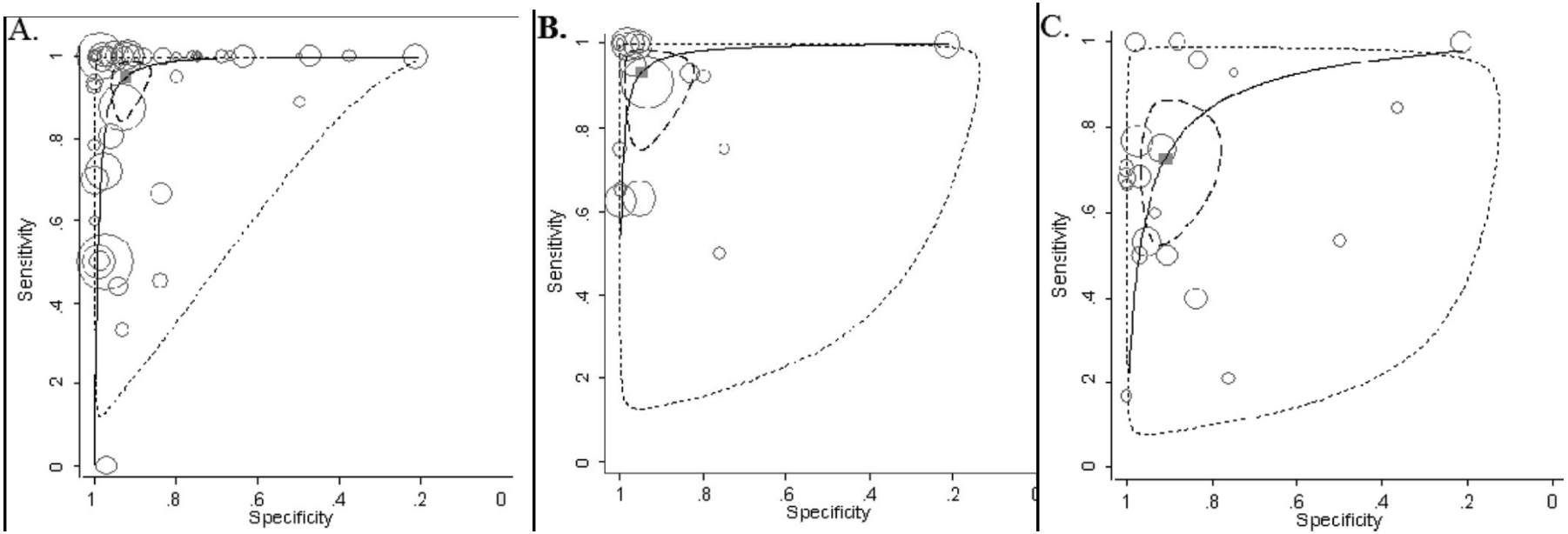
- Multiplex-PCR uses several pairs of primers annealing to different target sequences.
- Nested PCR is used to increase the specificity of DNA amplification
- RT-PCR (or Reverse Transcription PCR) is used to reverse-transcribe and amplify RNA to complementary DNA
- Teal-time PCR is used to amplify and simultaneously detect or quantify a targeted DNA molecule

Systematic Review and Meta-Analysis PCR Diagnosis of Invasive Candidiasis

- 54 studies with 4,694 patients, 963 of whom had proven/probable or possible IC.
- Samples - serum, whole-blood samples, fresh blood samples, frozen stored blood samples.
- Target genes - rRNA, cytochrome P450 L1A1, SAP, EO3, HSP, ERG11, CHS1, or ACT1
- PCR sample-processing time - 4 to 12 h
- Reporting of results within 1 working day

J. Clin. Microbiol. 2011, 49(2):665.

Hierarchical Ordinal Regression



- **(A) TP level I individuals (with candidemia) versus TN at-risk patients**
- **(B) TP level II individuals (with proven/probable IC) versus TN at-risk patients**
- **(C) TP level III individuals (with proven/probable/possible IC) versus TN at-risk patients**
- **Shaded square marks the summary point**
- **Open circles mark study estimates**
- **Solid lines mark HSROC curves**
- **Dashed lines mark 95% confidence regions**
- **Dotted lines mark 95% prediction regions**

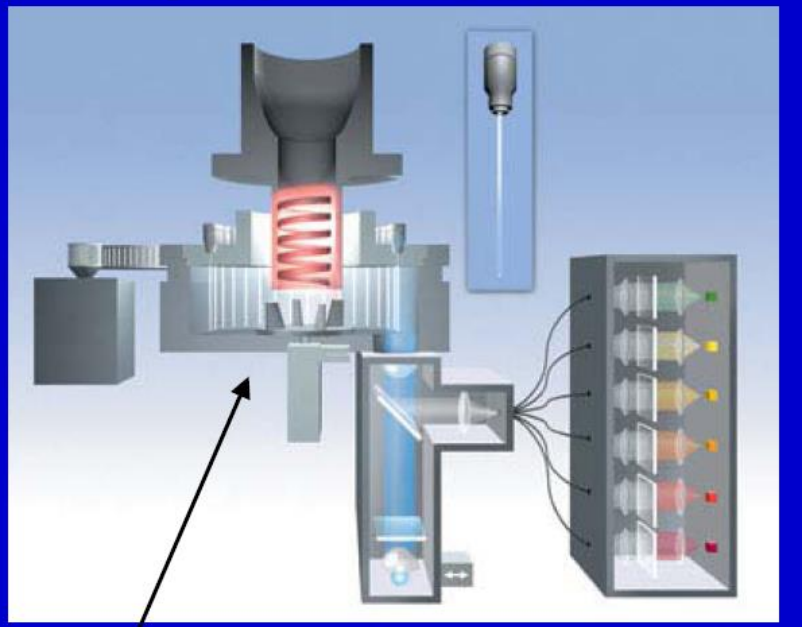
J. Clin. Microbiol. 2011, 49(2):665.

Phenotypic identification of Candida species

LightCycler Septi*Fast* assay *(Roche)*

LightCycler 2.0

LightCycler 2.0 Schematic



Organism Detected by Septi*Fast*

Gram (-)

- *Escherichia coli*
- *Klebsiella (pneumoniae/oxytoca)*
- *Serratia marcescens*
- *Enterobacter (cloacae / aerog.)*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Acinetobacter baumannii*
- *Stenotrophomonas maltophilia*

Gram (+)

- *Staphylococcus aureus*
- *CoNS* [1]
- *Strep. pneumoniae*
- *Streptococcus* spp. [2]
- *Enterococcus faecium*
- *Enterococcus faecalis*

Fungi

- *Candida albicans*
- *Candida tropicalis*
- *Candida parapsilosis*
- *Candida glabrata*
- *Candida krusei*
- *Aspergillus fumigatus*

LightCycler Septi*Fast* Fungemia Assay: Meta-Analysis

- Poor sensitivity (0.61; 95% CI: 0.48–0.72)
- Nearly perfect specificity (0.99; 95%: 0.99–0.99)
- High pooled LR+ (66.8, 95% CI: 39.8–112)
- Very poor pooled LR- (0.40, 95% CI: 0.29–0.54)
- The results suggested the LC-SF test was only good for ruling in fungemia.

PLoS ONE 2013, 8(5): e62323. doi:10.1371/journal.pone.0062323

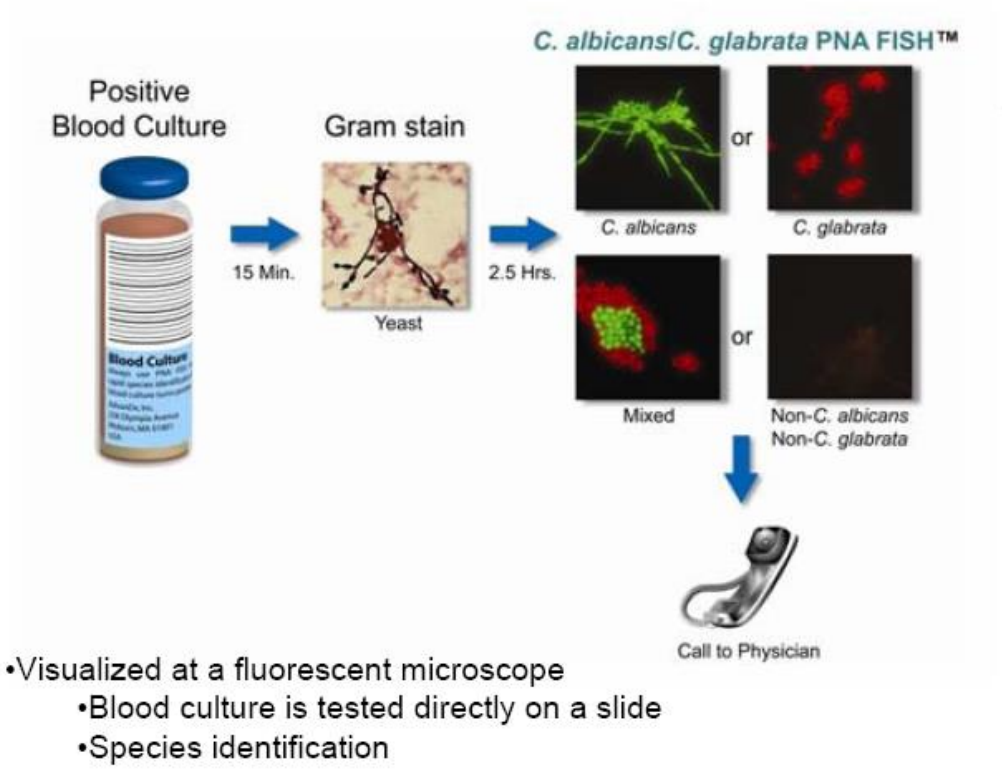
LightCycler Septi*Fast* assay

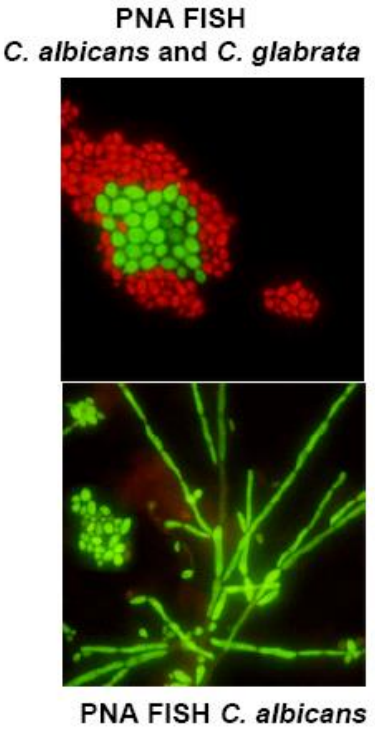
- State-of-the-art commercial PCR method that target bacterial and fungal DNA
- LightCycler Septi*Fast* assay enables detection of DNA from 25 human pathogens (Gram-positive and Gram-negative bacteria as well as fungi) in the blood of patients with suspected sepsis even after empirical antimicrobial therapy has been started.
- DNA Target Sequence – Internal transcribed spacer (ITS) region between:
  - 16s and 23s ribosomal DNA in bacteria
  - 18s and 5.8s ribosomal DNA in fung
- Septi*Fast* results were positive for six of the 10 patients (60 %), whereas blood cultures were positive in only two out of 10 patients (20 %).
- The declared analytical sensitivity of the Septi*Fast* assay ranges between 30 and 100 c.f.u. (depending on the micro-organism).
- The Septi*Fast* assay provides a rapid identification of the causative micro-organism within 6 h (3 h of technician hands-on time)

J Med Microbiol October 2008 vol. 57 no. 10 1306-1307

Peptide Nucleic Acid - Fluorescence In Situ Hybridization PNA-FISH (AdvanDx, USA)

PNA-FISH method employs fl uorescein-labeled probes that hybridize with 26S ribosomal RNA of target species that can be identi fi ed directly using smears of positive blood cultures.





Other Similar Commercial Techniques - Prove-it™ Fungi and BlackLight ® Fungal ID kit

Rapid identification of bacteria and *candida* using pna-fish from blood and peritoneal fluid cultures: a retrospective clinical study

- Time to species identification
  - Blood cultures - 83.6 hours (95% CI 56.7 to 110.5)
  - Blood PNA-FISH - 11.2 hours (95% CI 4.8 to 17.6)
  - Peritoneal fluid culture - 87.4 hours (95% CI −92.4 to 267.1).
  - Peritoneal fluid PNA-FISH - 16.4 hours (95% CI −57.3 to 90.0).
- Accuracy
  - Blood - 98.8% (83/84, 95% CI 93.5% to 99.9%) as compared to culture
  - Peritoneal fluid - 100% (13/13, 95% CI 75.3% to 100%)
- Yeast Traffic Light PNA FISH provides rapid, reliable identification of the five common Candida species found in blood cultures
- For *Candida* sp., pharmaceutical cost savings based on PNA-FISH identification could be $377.74/day.
- For coagulase-negative staphylococcus (CoNS), discontinuation of vancomycin could result in savings of $20.00/day.

Annals of Clinical Microbiology and Antimicrobials 2013, **12**:2 doi:10.1186/1476-0711-12-2
J. Clin. Microbiol. April 2012 vol. 50 no. 4 1446-1448

Invasive Aspergillosis

Antigen- and Antibody-Based Tests

Circulating galactomannan (GM)

- GM is a heteropolysaccharide component of the cell walls of *Aspergillus* and *Penicillium species*
- Interest of GM measurement in other specimens, e.g., urine, BAL, or CSF, because of higher sensitivity and potential early detection over the course of infection I immunocompromised patients
- BAL samples from immunocompetent patients seems to have no added value

Value of a single galactomannan determination for the diagnosis of IA in non-hematological patients

- 75 non-hematological from whom Aspergillus spp. were recovered 2003-2006.
- 10 of these patients (13.3%) had proven or probable invasive aspergillosis
  - 05 chronic obstructive pulmonary disease
  - 01 each HIV infection, lymphoma , liver transplant , solid malignancies and corticosteroid treatment
- Sensitivity 60% at cut-off $\geq$ 0.5 ng/ml and 50% at $\geq$ 1 ng/ml
- Specificity 89.23% at cut-off $\geq$ 0.5 ng/ml and 100% at $\geq$ 1 ng/ml
- PPV 46.15% at cut-off $\geq$ 0.5 ng/ml and 100% at $\geq$ 1 ng/ml
- NPV 93.55% at cut-off $\geq$ 0.5 ng/ml and 92.68% at $\geq$ 1 ng/ml
- p at cut-off $\geq$ 0.5 ng/ml = 0.001 and 50% at $\geq$ 1 ng/ml $\leq$0.001)
- **Conclusion -** The determination of galactomannan in the sera of non-neutropenic patients could prove to be a useful microbiological finding when diagnosing invasive aspergillosis

Medical Mycology 2008, 15, iFirst article

Galactomannan - Subgroup analysis of patients with COPD.

	All patients		COPD patients	
	≥0.5 ng/ml (95% CI)	≥1 ng/ml (95% CI)	≥0.5 ng/ml (95% CI)	≥1 ng/ml (95% CI)
Sensitivity (%)	60 (24.64–95.36)	50 (14.01–85.99)	60 (7.06–100)	40 (0–92.94)
Specificity (%)	89.23 (80.93–97.54)	100 (99.23–100)	92.31 (80.14–100)	100 (98.08–100)
PPV (%)	46.15 (15.21–77.10)	100 (90–100)	60 (7.06–100)	100 (75–100)
NPV (%)	93.55 (86.63–100)	92.86 (86.11–99.60)	92.31 (80.14–100)	89.66 (76.85–100)
VI (%)	85.33 (76.66–94.01)	93.33 (87.02–99.65)	87.10 (73.68–100)	90.32 (78.30–100)
p value	0.001	<0.001	0.020	0.022

NPV, negative predictive value; PPV, positive predictive value; VI, validity index.

Medical Mycology 2008, 15, iFirst article

Causes of false positivity or cross-reactivity in the galactomannan (GM) test

False-Positive Results Due to GM Contamination	Cross-Reactivity Caused by Similar Cell-Wall Antigens
Piperacillin-tazobactam	Histoplasma
Amoxicillin-clavulanate	Blastomyces
Other b-lactam antibiotics	Prototheca
Neonates colonized with Bifidobacterium	Fusarium
Enteral nutrition	Penicillium
Gluconate-containing Plasma-Lyte	Geotrichum
Other intravenous fluids containing gluconate	
Possibly cardboard or soybean protein	

Clin Lab Med 33 (2013) 505–525

Typical cutoff index values used for the galactomannan assay

	Negative	Indeterminate	Positive
Europe	<0.5	1.0	>1.5
United States	<0.5		>0.5

Relative NPV and PPV of galactomannan and 1,3-b-D-glucan in diagnosis of invasive aspergillosis and invasive candidiasis

		PPV	NPV
Galactomannan	Invasive aspergillosis	Excellent	Excellent
1,3-b-D-glucan	Invasive candidiasis Invasive aspergillosis	Low Low to moderate	Low Excellent

Clin Lab Med 33 (2013) 505–525

Diagnosis of Invasive Aspergillosis Using a Galactomannan Assay: A Meta-Analysis

- In immuno-compromised patients
- 27 studies from 1966 to 2005
- Sensitivity of 0.71 (95% CI, 0.68–0.74)
- Specificity of 0.89 (95% CI, 0.88–0.90) for proven cases of invasive aspergillosis.
- Subgroup analyses showed that the performance of the test differed by patient population and type of reference standard used.
- Significant heterogeneity was present.

Clinical Infectious Diseases 2006; 42:1417–27

Pooled sensitivity and specificity of the galactomannan assay for diagnosis of invasive aspergillosis (IA)

	Cases of proven IA				Cases of proven or probable IA			
Studies	TP/(TP + FP)	Pooled sensitivity (95% CI)	TN/(TN + FP)	Pooled specificity (95% CI)	TP/(TP + FN)	Pooled sensitivity (95% CI)	TN/(TN + FP)	Pooled specificity (95% CI)
All	163/229	0.71 (0.68–0.74)	3601/4055	0.89 (0.88–0.90)	250/407	0.61 (0.59–0.63)	2839/3060	0.93 (0.92–0.94)
Studies limited to patients with hematological malignancy	106/152	0.70 (0.62–0.77)	2570/2808	0.92 (0.90–0.93)	177/304	0.58 (0.52–0.64)	2324/2457	0.95 (0.94–0.96)
Studies limited to patients undergoing BMT	49/60	0.82 (0.70–0.90)	722/843	0.86 (0.83–0.88)	32/49	0.65 (0.60–0.78)	17/26	0.65 (0.44–0.83)
Studies limited to solid-organ transplant recipients	2/9	0.22 (0.03–0.60)	180/215	0.84 (0.78–0.88)	9/22	0.41 (0.21–0.64)	210/247	0.85 (0.80–0.89)
Studies using EORTC/MSG criteria	74/116	0.64 (0.54–0.73)	2549/2869	0.89 (0.88–0.90)	211/354	0.60 (0.54–0.65)	2628/2823	0.93 (0.92–0.94)
Studies not using EORTC/MSG criteria	89/113	0.79 (0.70–0.86)	1052/1186	0.89 (0.87–0.90)	39/53	0.74 (0.60–0.85)	211/237	0.89 (0.84–0.93)
Studies involving pediatric population only	8/9	0.89 (0.51–1.00)	316/370	0.85 (0.85–0.89)	11/12	0.92 (0.82–1.00)	12/20	0.60 (0.36–0.81)
Studies involving adult population only	58/93	0.62 (0.52–0.72)	1211/1398	0.87 (0.85–0.88)	102/140	0.73 (.46-.61)	802/889	0.90 (.88–0.92)
Studies of both pediatric and adult populations	70/93	0.75 (0.65–0.84)	1726/1875	0.92 (0.91–0.93)	92/196	0.47 (0.40–0.54)	1601/1701	0.94 (0.93–0.95)
Studies using a cutoff value of 0.5 for defining positivity	3/11	0.27 (0.06–0.61)	27/341	0.79 (0.74–0.83)	69/87	0.79 (0.69–0.87)	493/571	0.86 (0.83–0.89)
Studies using a cutoff value of 1.0 for defining positivity	85/107	0.79 (0.71–0.87)	1385/1598	0.87 (0.85–0.88)	103/159	0.65 (0.57–0.72)	1163/1242	0.94 (0.92–0.95)
Studies using a cutoff value of 1.5 for defining positivity	75/111	0.68 (0.58–0.76)	1946/2116	0.92 (0.91–0.93)	78/161	0.48 (0.41–0.56)	1183/1247	0.95 (0.93–0.96)

Most common variables associated with false-positive and false-negative results for the Platelia™ Aspergillus EIA test (Bio-Rad)

Host related	Renal failure Mucositis Food intake of galactofuranose[a] Gut colonization and potential translocation of *Bifidobacterium* Gastrointestinal microflora of neonates
Iatrogenic	Blood derivatives Intravenous solutions containing gluconate Treatment with antibiotics derived from the fermentation of *Penicillium* species (e.g., piperacilin-tazobactam, amoxicilin-clavulanic acid) Use of cyclophosphamide in cancer patients
Sample collection and/or processing	Use of materials such as cotton swabs and cardboard Inappropriate cut-off value (too low)
Environmental	Presence of other non-*Aspergillus* fungi such as *Penicillium*, *Alternaria*, *Paecilomyces*, *Geotrichum*, *Histoplasma*, and even *C. neoformans*[b]
Factor and/or situation that can lead to false-negative results	
Host conditions	Chronic granulomatose disease
Iatrogenic	Treatment with antifungals
Sample collection and/or processing	Long-term storage of samples Inappropriate cut-off value (too high)

Test Performance Characteristics of an Ideal Diagnostic Test for IC

- Minimally invasive (eg, blood test rather than test of a deep tissue sample)
- Requires low volume samples
- Rapid turn-around time
- Requires minimal labour and fits within the normal flow of activities in clinical microbiology laboratories
- Sensitive and specific
- Provides speciation and antifungal susceptibility data
- Multiplex capabilities

Testing goals of an Ideal Diagnostic Test for IC

- Identify patients early in the course of IC
- Identify patients with candidemia who have deep-seated candidiasis
- Identify patients with candidemia who are likely to develop deep-seated candidiasis
- Identify patients with deep-seated candidiasis but negative blood cultures
- Provide prognostic information (eg, identify patients who are likely to have poor outcomes or fail antifungal therapy)

Potential Advantages of Nonculture Diagnostic Tests

- Rapid turn-around time
- Not dependent on viable organisms*
- May be positive prior to cultures, and stay positive during antifungal therapy*
- May offer quantitative data with prognostic significance
- Multicopy targets and amplification may improve sensitivity
- May be coupled with detection of markers for drug resistance or other relevant phenotypes

* These may also be liabilities - may detect dead organisms or remnants of old infections with no active disease.

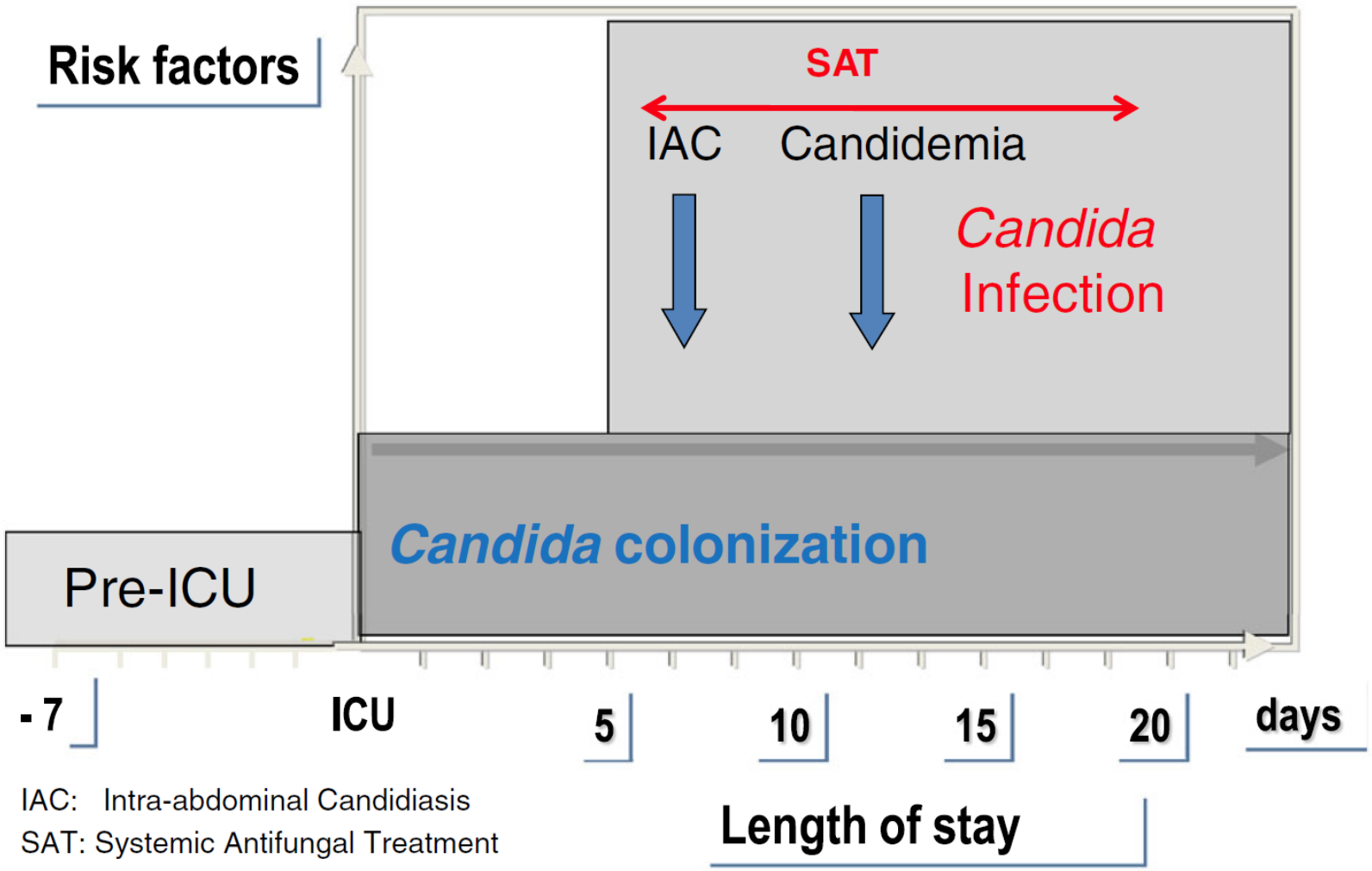
* Persistence of positivity may confound interpretations if kinetics are not linked to outcomes and may limit the subsequent ability to diagnose recurrent or relapsing infections.

Potential Disadvantages of Nonculture Diagnostic Tests

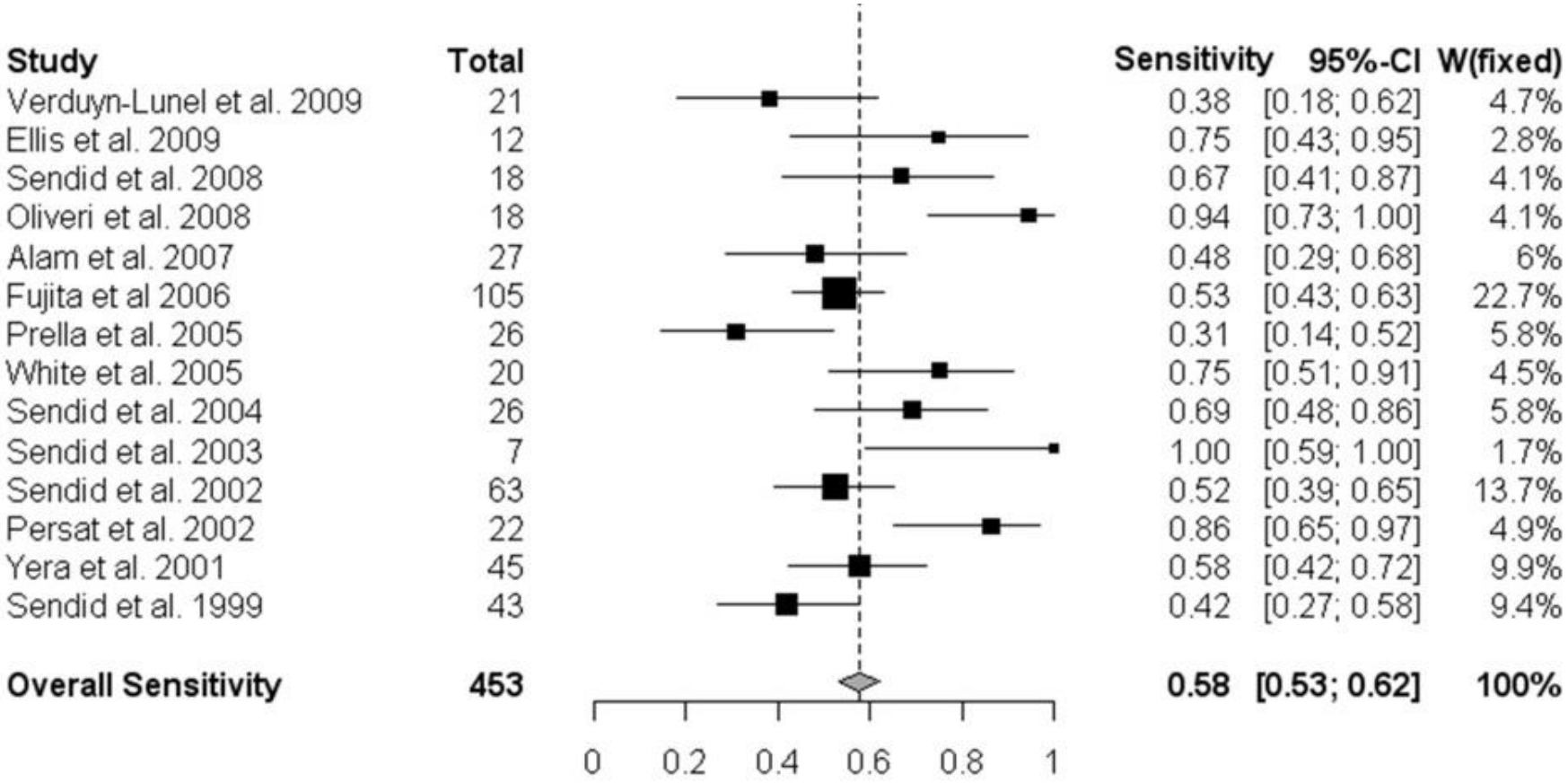
- Do not recover organisms
- May not speciate Candida or distinguish between fungi
- Narrow-spectrum (may detect only Candida among multiple pathogens)
- May need to be run in batch due to limited number of samples
- May have low threshold for contamination
- Financial costs to patients and clinical microbiology laboratory

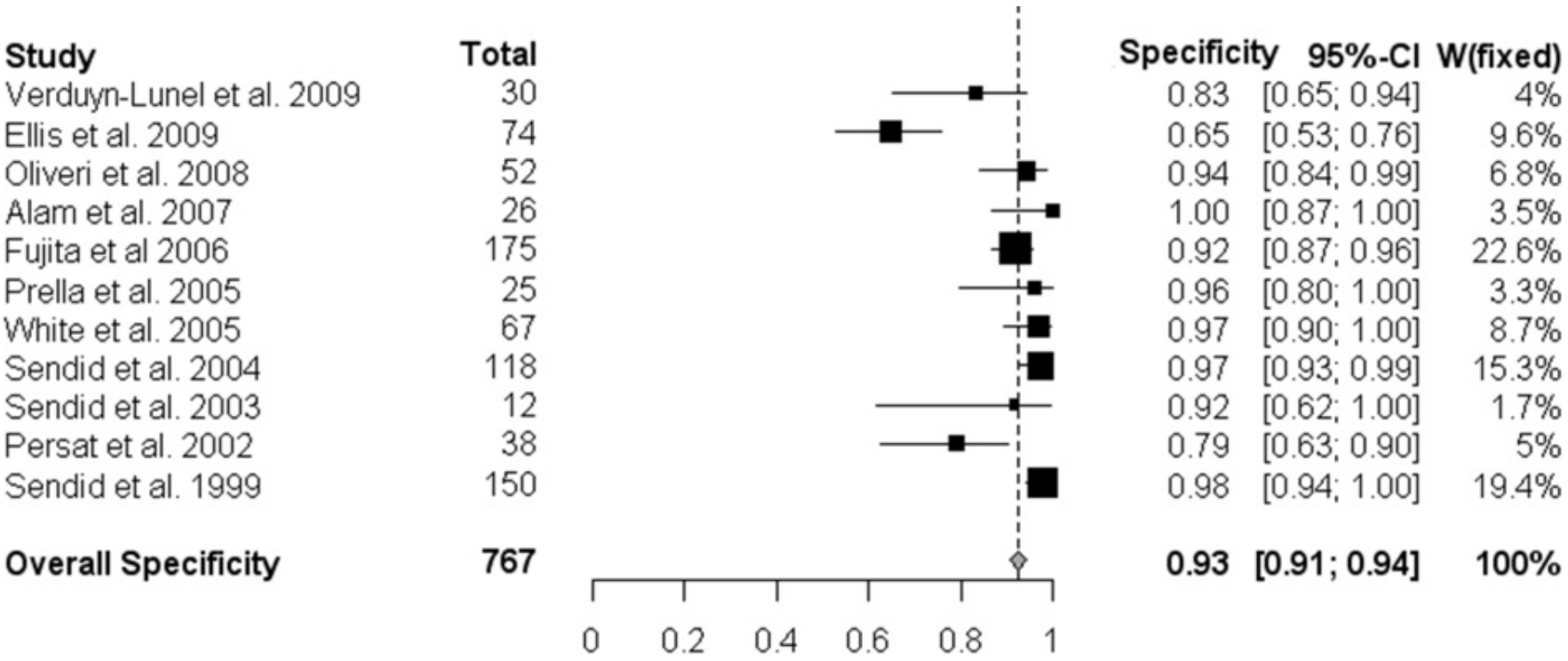
- Flow cytometric technology
- PCR Electrospray Ionization Mass Spectrometry

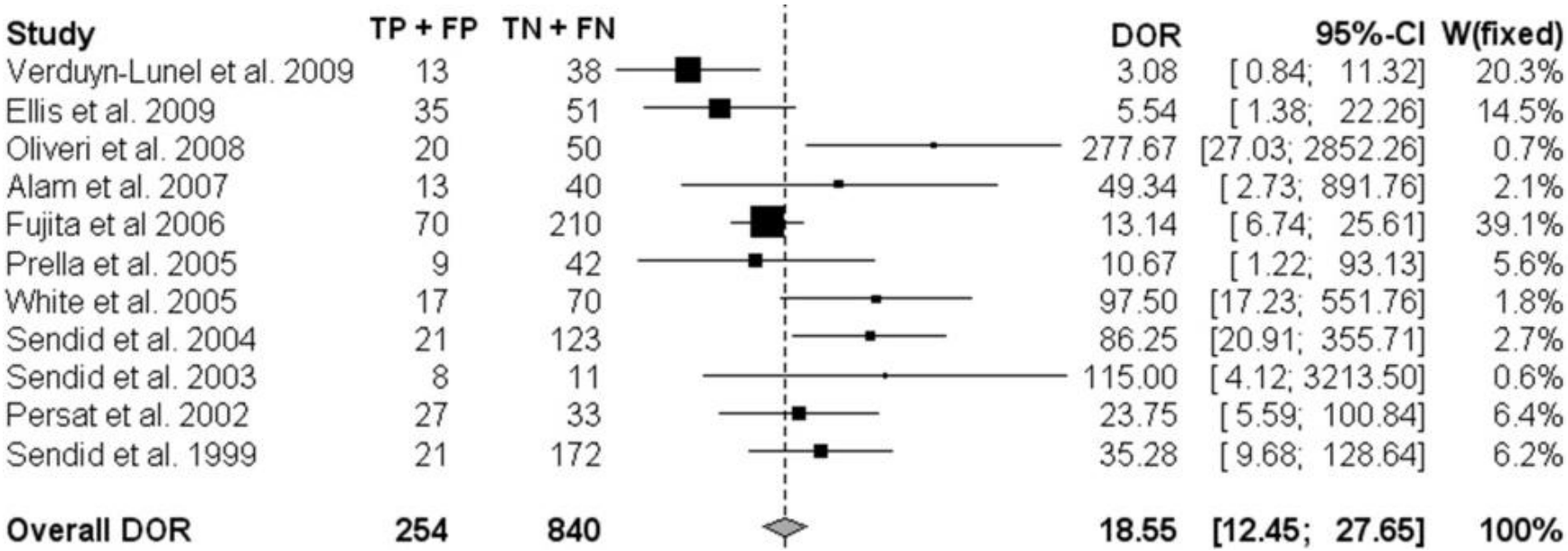
Natural history of invasive candidiasis in ICU.



Test	Sensitivity	Specificity	Diagnostics Odds Ratio
Mn	58% (95% CI, 53-62)	93% (95% CI, 91-94)	18 (95% CI 12-28).
A-Mn	59% (95% CI, 54-65)	83% (95% CI, 79-97)	12 (95% CI 7-21)
Mn + A-Mn	83% (95% CI, 79-87)	86% (95% CI, 82-90)	58 (95% CI 27-122)


Study
Total
Sensitivity
95%-CI
W(fixed)
Verduyn-Lunel et al. 2009 21 0.38 [0.18; 0.62] 4.7%
Ellis et al. 2009 12 0.75 [0.43; 0.95] 2.8%
Sendid et al. 2008 18 0.67 [0.41; 0.87] 4.1%
Oliveri et al. 2008 18 0.94 [0.73; 1.00] 4.1%
Alam et al. 2007 27 0.48 [0.29; 0.68] 6%
Fujita et al 2006 105 0.53 [0.43; 0.63] 22.7%
Prella et al. 2005 26 0.31 [0.14; 0.52] 5.8%
White et al. 2005 20 0.75 [0.51; 0.91] 4.5%
Sendid et al. 2004 26 0.69 [0.48; 0.86] 5.8%
Sendid et al. 2003 7 1.00 [0.59; 1.00] 1.7%
Sendid et al. 2002 63 0.52 [0.39; 0.65] 13.7%
Persat et al. 2002 22 0.86 [0.65; 0.97] 4.9%
Yera et al. 2001 45 0.58 [0.42; 0.72] 9.9%
Sendid et al. 1999 43 0.42 [0.27; 0.58] 9.4%
Overall Sensitivity 453 0.58 [0.53; 0.62] 100%
0
0.2
0.4
0.6
0.8
1


Study
Total
Specificity
95%-CI
W(fixed)
Verduyn-Lunel et al. 2009 30 0.83 [0.65; 0.94] 4%
Ellis et al. 2009 74 0.65 [0.53; 0.76] 9.6%
Oliveri et al. 2008 52 0.94 [0.84; 0.99] 6.8%
Alam et al. 2007 26 1.00 [0.87; 1.00] 3.5%
Fujita et al 2006 175 0.92 [0.87; 0.96] 22.6%
Prella et al. 2005 25 0.96 [0.80; 1.00] 3.3%
White et al. 2005 67 0.97 [0.90; 1.00] 8.7%
Sendid et al. 2004 118 0.97 [0.93; 0.99] 15.3%
Sendid et al. 2003 12 0.92 [0.62; 1.00] 1.7%
Persat et al. 2002 38 0.79 [0.63; 0.90] 5%
Sendid et al. 1999 150 0.98 [0.94; 1.00] 19.4%
Overall Specificity 767 0.93 [0.91; 0.94] 100%
0
0.2
0.4
0.6
0.8
1


Study
TP + FP
TN + FN
DOR
95%-CI
W(fixed)
Verduyn-Lunel et al. 2009 13 38 3.08 [0.84; 11.32] 20.3%
Ellis et al. 2009 35 51 5.54 [1.38; 22.26] 14.5%
Oliveri et al. 2008 20 50 277.67 [27.03; 2852.26] 0.7%
Alam et al. 2007 13 40 49.34 [2.73; 891.76] 2.1%
Fujita et al 2006 70 210 13.14 [6.74; 25.61] 39.1%
Prella et al. 2005 9 42 10.67 [1.22; 93.13] 5.6%
White et al. 2005 17 70 97.50 [17.23; 551.76] 1.8%
Sendid et al. 2004 21 123 86.25 [20.91; 355.71] 2.7%
Sendid et al. 2003 8 11 115.00 [4.12; 3213.50] 0.6%
Persat et al. 2002 27 33 23.75 [5.59; 100.84] 6.4%
Sendid et al. 1999 21 172 35.28 [9.68; 128.64] 6.2%
Overall DOR 254 840 18.55 [12.45; 27.65] 100%

	Sensitivity	Specificity	PPV	NPV	Efficiency, %
BG ⩾ 80 pg/ml 1×					
At inclusion	0.76 (0.56–0.90)	0.59 (0.43–0.74)	0.56 (0.40–0.72)	0.78 (0.60–0.90)	66
At infection*	0.83 (0.64–0.94)	0.40 (0.26–0.57)	0.49 (0.34–0.64)	0.77 (0.55–0.92)	58
BG ⩾ 80 pg/ml 2×†					
At inclusion	0.66 (0.45–0.82)	0.83 (0.69–0.93)	0.73 (0.52–0.88)	0.78 (0.63–0.89)	76
At infection*	0.65 (0.46–0.82)	0.78 (0.63–0.90)	0.68 (0.48–0.84)	0.77 (0.61–0.88)	73
CS ⩾ 3					
At inclusion	0.86 (0.68–0.96)	0.50 (0.34–0.66)	0.54 (0.39–0.69)	0.84 (0.64–0.95)	65
At infection*	0.86 (0.68–0.96)	0.38 (0.23–0.54)	0.49 (0.35–0.63)	0.80 (0.56–0.94)	58
CI ⩾ 0.5					
At inclusion	0.26 (0.10–0.48)	0.76 (0.61–0.87)	0.35 (0.14–0.62)	0.67 (0.53–0.80)	59
At infection*	0.88 (0.69–0.97)	0.34 (0.19–0.52)	0.49 (0.34–0.64)	0.80 (0.52–0.96)	57
CCI ⩾ 0.4					
At inclusion	0.14 (0.03–0.36)	0.77 (0.61–0.88)	0.23 (0.05–0.54)	0.65 (0.50–0.77)	56
At infection*	0.50 (0.29–0.71)	0.43 (0.28–0.60)	0.35 (0.20–0.53)	0.59 (0.39–0.76)	46

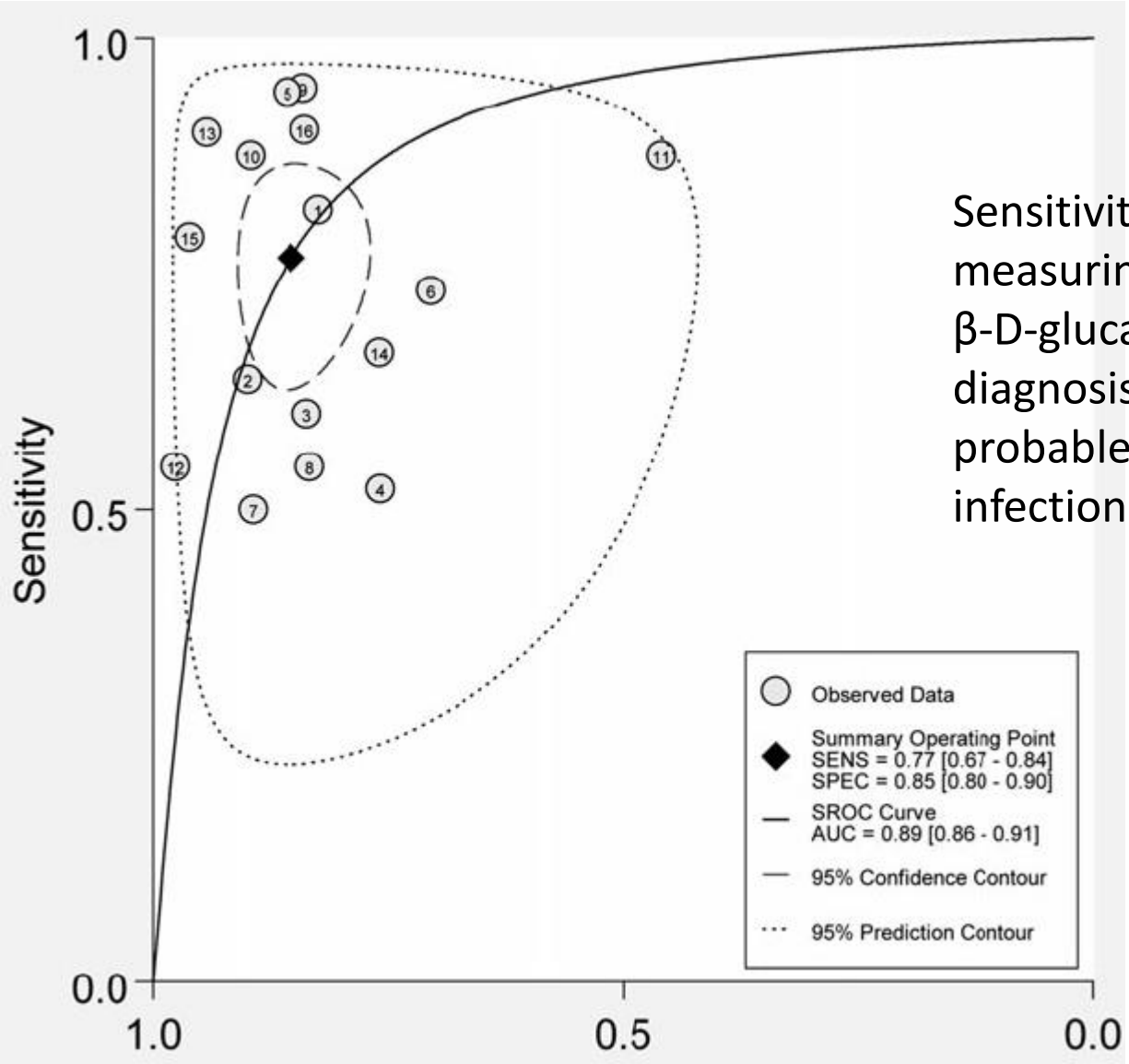
Am J Respir Crit Care Med Vol 188, Iss. 9, pp 1100–1109, Nov 1, 2013

β-D-glucan and CAGTA in Severe Abdominal Conditions

- The probabilities of IC
- were 59.3 % for the terminal node of
- BDG greater than 259 pg/mL and
- 30.8 % for BDG less than 259 pg/mL
- and CAGTA positivity, whereas there
- was a 93.9 % probability in predicting
- the absence of IC for BDG less
- than 259 pg/mL and negative CAGTA.
- Using a cutoff of 30 % for IC
- probability, the prediction rule
- showed 90.3 % sensitivity, 54.8 %
- specificity, 42.4 % positive predictive
- value, and 93.9 % negative predictive
- value with an AUC of 0.78 (95 %
- confidence interval 0.76–0.81).
- Significant differences in CRP
- (p = 0.411) and PCT (p = 0.179)
- among the studied groups were
- not found. Conclusions: BDG with
- a positive test for CAGTA accurately
- differentiated Candida colonization
- from IC in patients with SAC,
- whereas CRP and PCT did not.

Intensive Care Med (2012) 38:1315–1325

β-D-glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis



Sensitivity and specificity of measuring serum or plasma β-D-glucan levels for the diagnosis of proven or probable invasive fungal infections.

Organisms detected by PCR method

Gram Positive

CoNS[1]
Enterococcus faecium
Enterococcus faecalis
Staph. aureus
Strep. pneumoniae
Strep. sp.[2]
MRSA (mec A gene)[3]

Gram Negative

Acinetobacter baumannii
Enterobacter aerogenes/ cloacae[4]
E. coli
Klebsiella pneumoniae/ oxytoca[4]
Proteus mirabilis
Pseudomonas aeruginosa
Serratia marcescens
Stenotrophomonas maltophilia

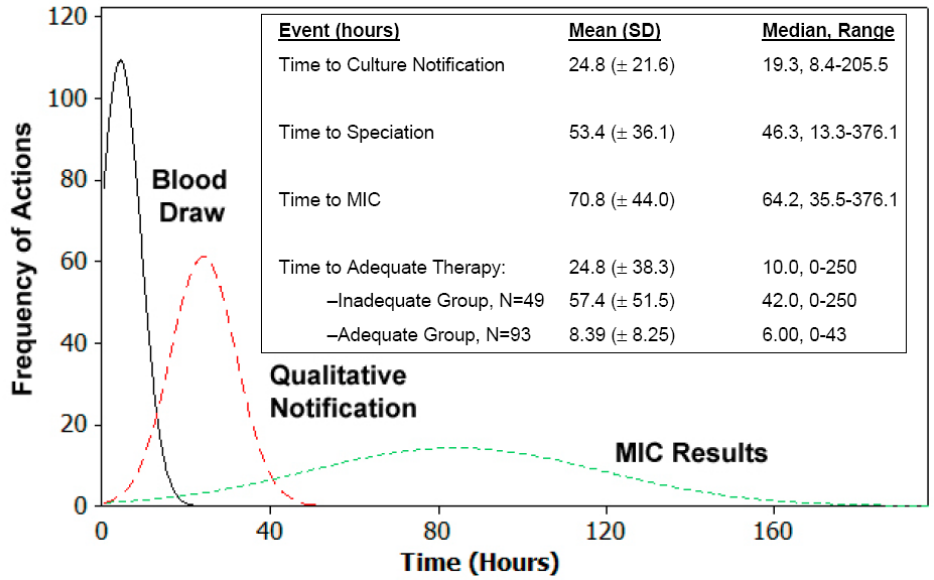
Fungi

Aspergillus fumigatus
Candida albicans
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis

1-Staphylococcus hemolyticus, epidermidis = CoNS
2-Streptococcus agalaciae, pyogenes, viridans = Strep. Sp.
3-Separate test kit
4-No differentiation between these two subspecies

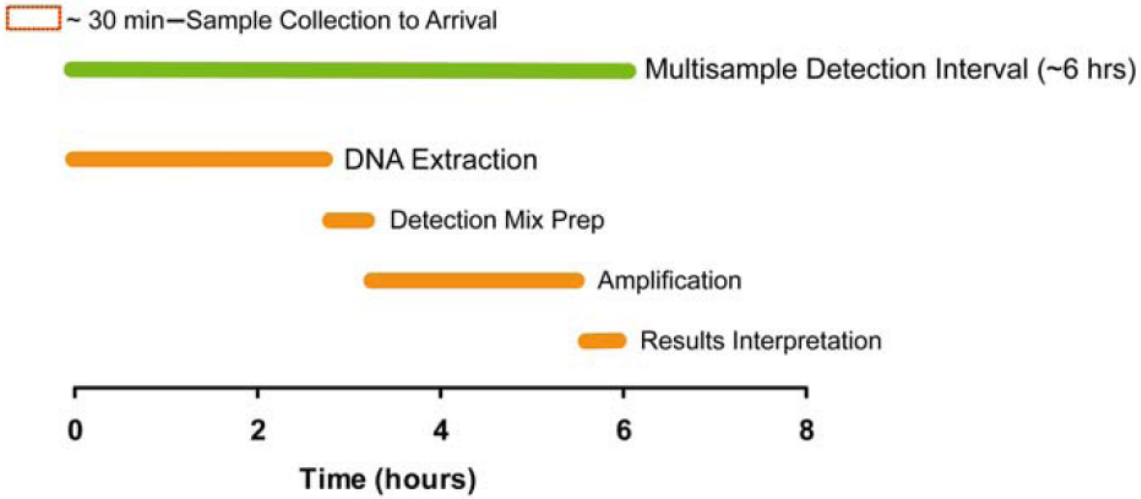
Timelines of Blood Culture and PCR

Timeline of Blood Culture Processing (N=142)



Event (hours)	Mean (SD)	Median, Range
Time to Culture Notification	24.8 (± 21.6)	19.3, 8.4-205.5
Time to Speciation	53.4 (± 36.1)	46.3, 13.3-376.1
Time to MIC	70.8 (± 44.0)	64.2, 35.5-376.1
Time to Adequate Therapy:	24.8 (± 38.3)	10.0, 0-250
–Inadequate Group, N=49	57.4 (± 51.5)	42.0, 0-250
–Adequate Group, N=93	8.39 (± 8.25)	6.00, 0-43

Timelines of Blood Culture and PCR



Problems with the Cultures - Blood Cultures

- Need of viable candida cells
- Viable candida cells are rapidly eliminated from the circulation
- Need of median candida concentration – 1 CFU/ml of blood
  - Translocation across the gut – lower organism burden – *Candida Glabrata* – lower sensitivity
  - Director inoculation via intravascular catheter – higher organism burden – *Candida Parapsilosis* – higher sensitivity
- Zero sensitivity for Gr. 3
- Long median time to positivity
  - 2-3 days (higher for Glabrata and lower for Parapsilosis)
  - May take as long as 8 days
- No correlation with patient outcome

Blood Culture: Recommendations

- Repeat sets of blood cultures - baseline day 1 of therapy, day 3, and day 5 or until clearance of the infection is detected.
- The optimum detection of microorganisms is achieved with ≥3 sets of blood cultures.
- In adults, 20–30 ml of blood should be collected per blood culture set
- Daily blood culture till febrile or in shock – at least 2-3 consecutive days
- Additional blood culture during febrile episodes
- The number of BC recommended in a single session is 2 to 4 (average 3)
- Total volume varying according to the age of the patient
  - 40–60 mL for adults
  - 2–4 mL for children under 2 kg
  - 6 mL between 2 and 12 kg
  - 20 mL between 12 and 36 kg
- The timing for obtaining the BC is one right after the other from different sites, and venipuncture remains the technique of choice.
- One Blood Culture session
  - 03 sets comprises of 20 mL each (total 60 mL) blood for adults obtained in a single session within a 30-min period

Negative Blood Culture

- Absence of viable Candida within the circulation
- Insufficient concentration of viable Candida within the circulation
- Intermittent or transient secondary candidemia from deep-seated candidiasis
- Deep-seated candidiasis with no candidemia
- Even with lysis centrifugation system, blood culture was found to be positive for only 43% of autopsy confirm cases of Candida
- Blood cultures are negative for candida species in approximately 50% of autopsy proven cases of disseminated candidiasis
- No candida infection in $\leq$ 50% candida infections

Summary of Commercially Available Molecular Assays for the Diagnosis of Fungal Infections

Assay	Method	Targets	Results	Specimen	TAT	FDA A/C
MycAssay Aspergillus	Real-time PCR	18S rRNA for Aspergillus spp	Qualitative	Serum; BAL	3 h	No
SeptiFast	Real-time PCR	5 species of Candida and A. fumigatus	Qualitative	Blood	6 h	No

* Myconostica UK, ** Roche, USA

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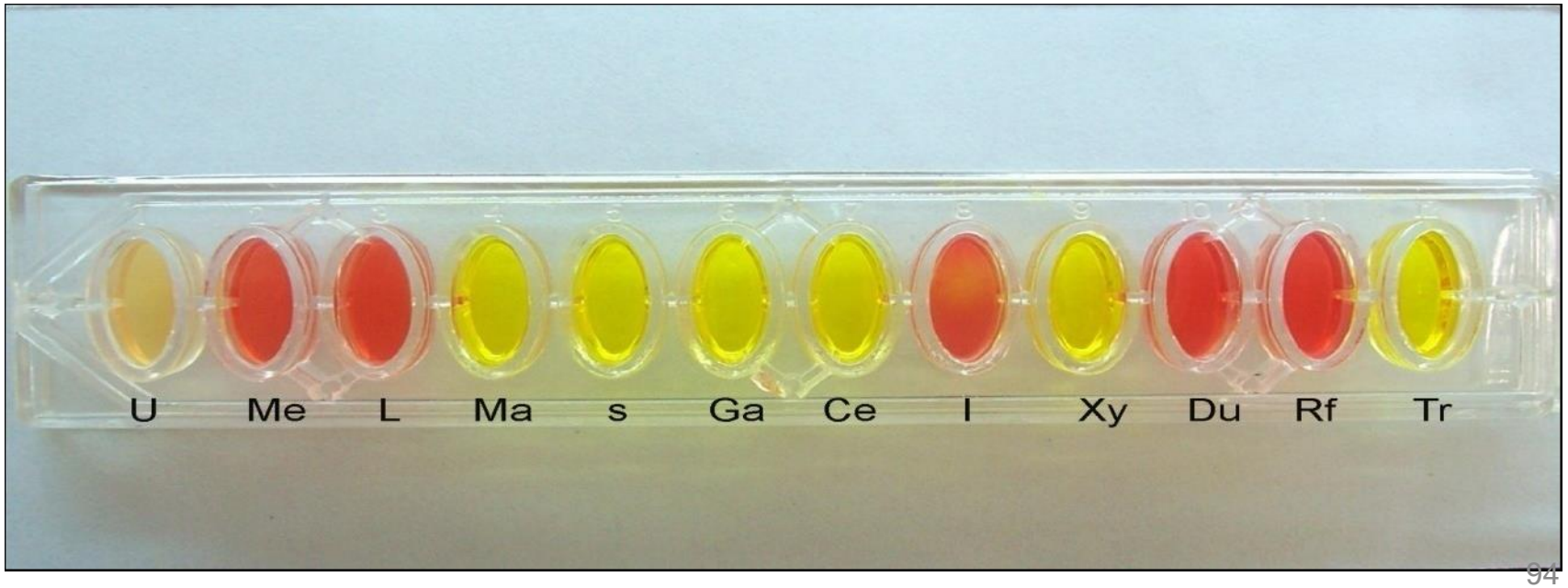
Biochemical characterization

- Biochemical identification of *Candida* spp. is based on assimilation and fermentation of carbohydrates.
- Many manual and automated techniques like VITEK system



Other conventional methods for speciation of *Candida*