Ferrara, 16 giugno 2016

FAST MICROBIOLOGY

Applicazioni cliniche

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RIGHT USE OF FAST-MICROBIOLOGY

The rapid molecular diagnostics strategy

Especially considering multidrug-resistant (MDR) Gram-negative bacteria, the rapid and accurate detection of antibiotic resistance determinants offers the potential to transform clinical practice by:

1) informing early appropriate and targeted antibiotic therapy;
2) shortening the duration and narrowing the spectrum of activity of empirical antibiotic regimens, thus limiting the selection of antibiotic-resistant bacteria;
3) improving clinical outcomes;
4) streamlining development of new antimicrobials by quickly identifying patients infected with antibiotic-resistant bacteria and enriching participant enrollment in clinical trials
5) Improving surveillance and epidemiological studies of transmissible diseases

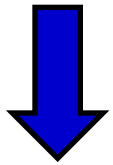
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 9, 2010 VOL. 363 NO. 11

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

With a single test, Xpert MTB/RIF identified 98% of patients with smear-positive and culture-positive tuberculosis (including more than 70% of patients with smear-negative and culture positive disease) and correctly identified 98% of bacteria that were resistant to rifampin



PROMPT ACCESS TO APPROPRIATE TREATMENT

SHORTENING THE DURATION OF HOSPITALIZATION

REDUCING THE OVERALL DRUG EXPOSURE

DECENTRALIZATION OF MOLECULAR DIAGNOSIS,
since it potentially can be used at the point of treatment

IMPROVED EPIDEMIOLOGICAL STUDIES

Fast and low-cost decentralized surveillance of transmission of tuberculosis based on strain-specific PCRs tailored from whole genome sequencing data: a pilot study

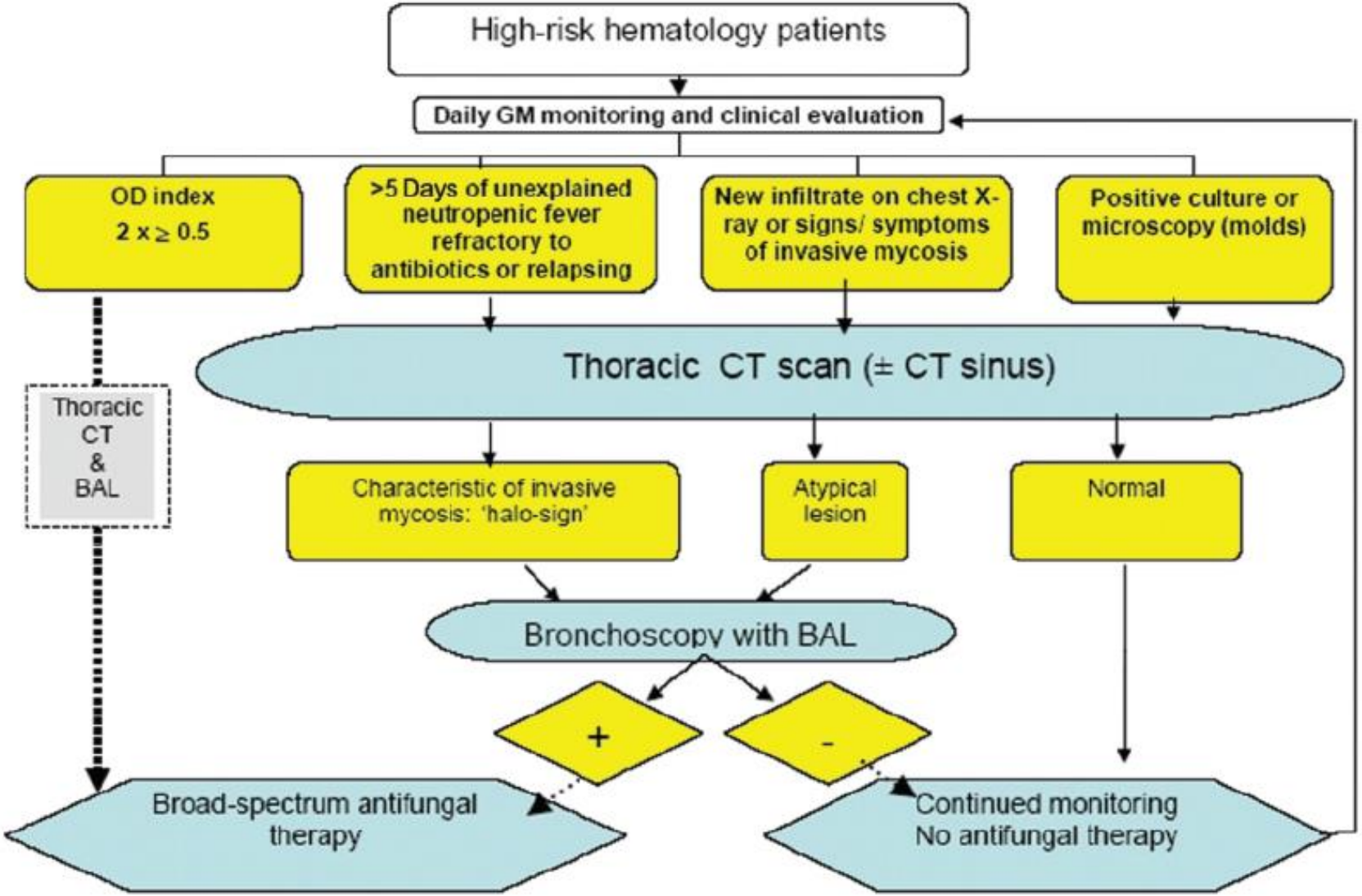
Pérez-Lago L et al, Clin Microbiol Infect 2015; 21: 249.e1-249.e9

Making possible to reconcile the high resolution offered by Whole Genomic Sequencing assay with the speed and ease of implementation of simple PCR-based strategies, could realize the «perfect tool» to provide an accurate picture of the transmission dynamics of M tuberculosis.

An allele-specific PCR targeting strain-specific single nucleotide polymorphisms, (TRAP) identified from WGS gives a substantial change to track actively transmitted Mycobacterium tuberculosis strains

Galactomannan and Computed Tomography–Based Preemptive Antifungal Therapy in Neutropenic Patients at High Risk for Invasive Fungal Infection: A Prospective Feasibility Study

Maertens J et al, Clin Infect Dis, 2005



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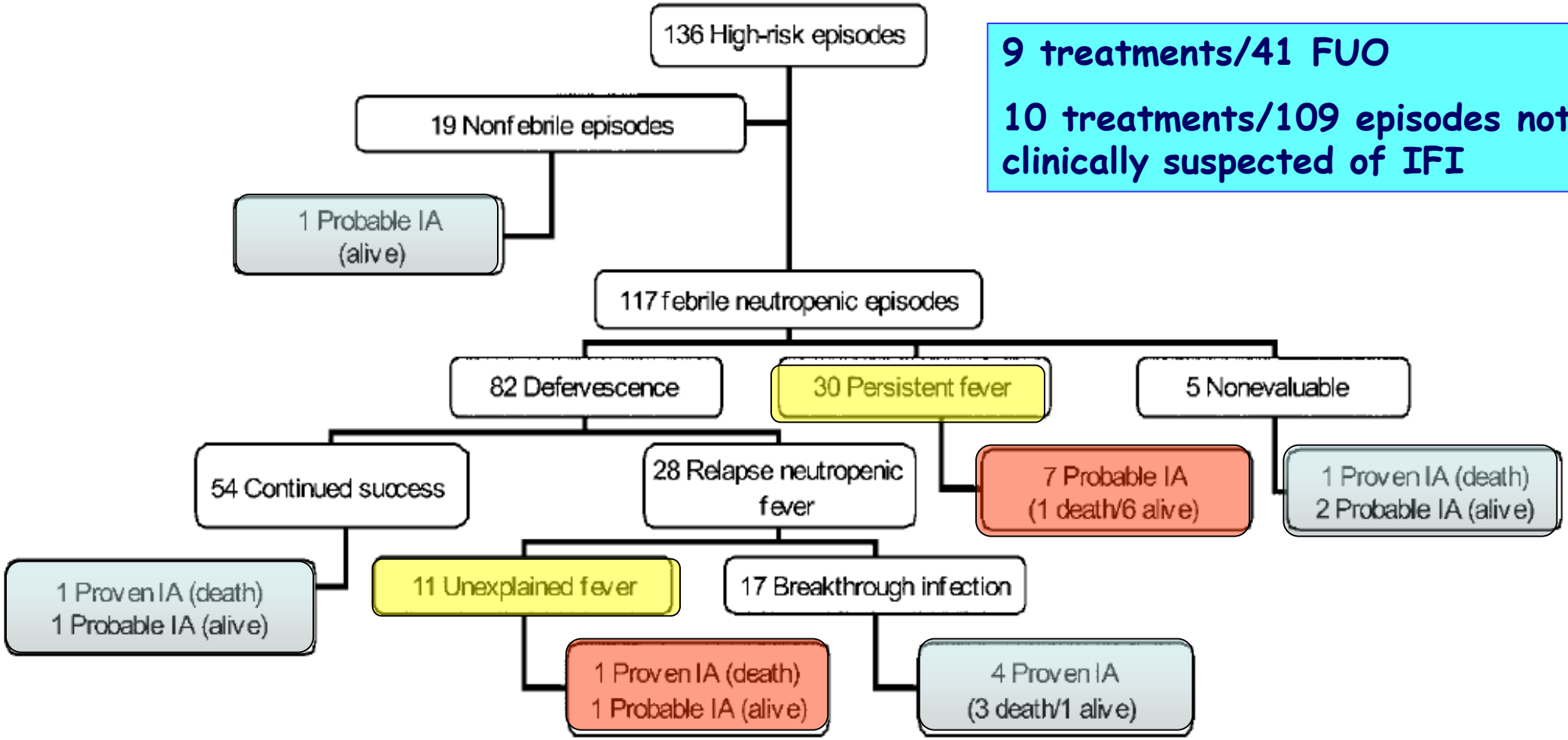
Maertens J et al, Clin Infect Dis, 2005;41:1242-50



9 treatments/41 FUO

10 treatments/109 episodes not clinically suspected of IFI

Direct comparison of galactomannan performance in concurrent serum and bronchoalveolar lavage samples in immunocompromised patients at risk for invasive pulmonary aspergillosis

Boch T et al, Mycoses, 2016, 59, 80–85

Table 3 Diagnostic performance of BAL GM and serum GM for proven/probable ($n = 26$) and no IPA ($n = 8$).

Test method	Sensitivity, % ($n = 26$)	Specificity, % ($n = 8$)	PPV, %	NPV, %	DOR (95% CI)
BAL GM	85	88	96	64	38.5 (3.7–404.2)
Serum GM	23	88	88	26	2.1 (0.2–20.7)

A systematic review and meta-analysis of diagnostic accuracy of serum 1,3-b-D-glucan for invasive fungal infection: Focus on cutoff levels

He S et al, J Microbiol Immunol Infection 2014

β-D-glucan (BDG) is a cell wall constituent of Candida species and other fungi.

The sensitivity and specificity of serum BDG testing for diagnosing invasive candidiasis have ranged from 57% to 97% and 56% to 93%, respectively

IN THE PRESENT META-ANALYSIS, REGARDING 28 STUDIES, AVERAGE SENSITIVITY AND SPECIFICITY RESULT 78% AND 81%

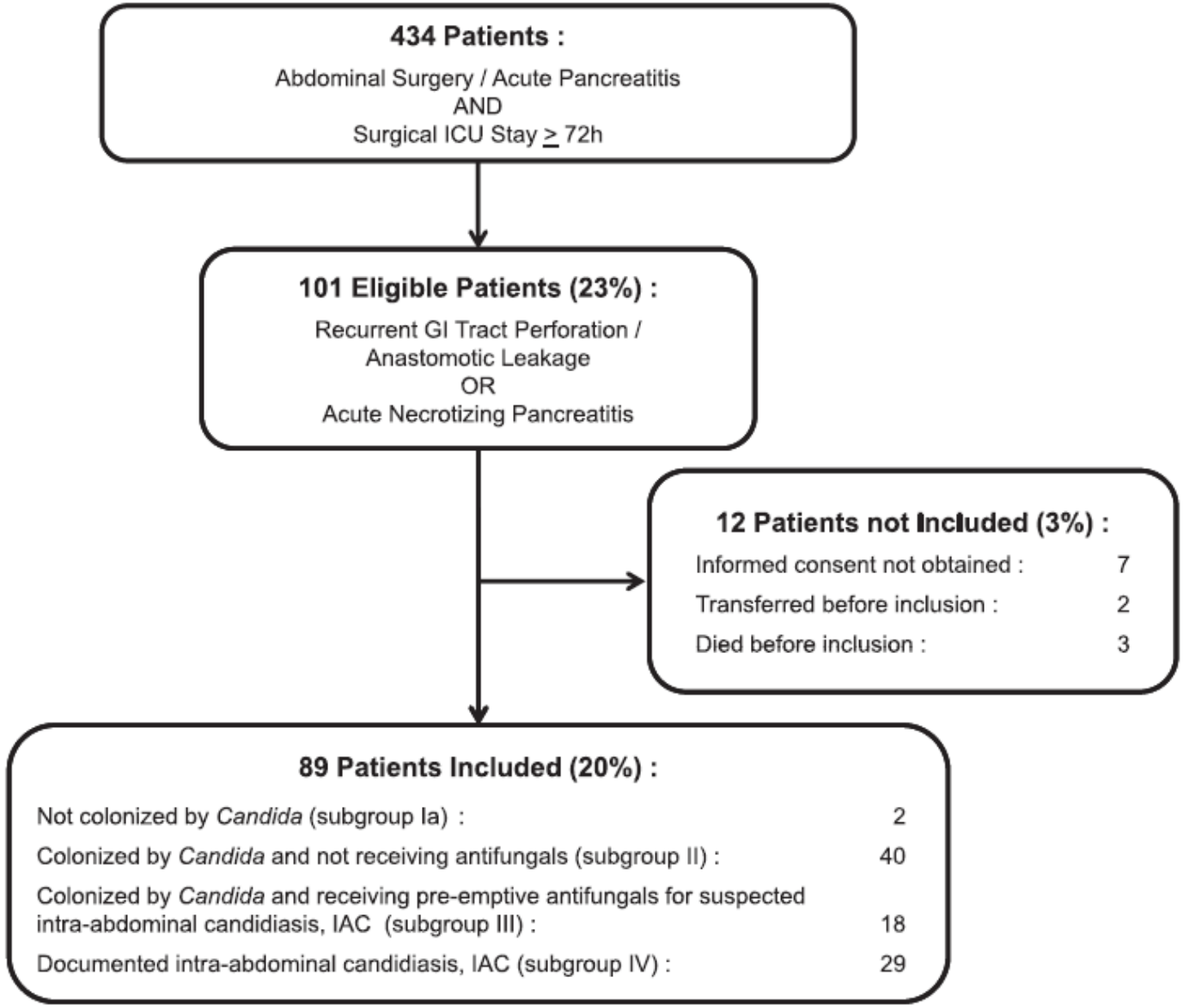
The cutoff value of BDG at 80 pg/mL had the best diagnostic accuracy

Optimal results are achieved if 2 consecutive tests are positive

The major uncertainties for BDG detection are specificity and false-positivity, particularly among high-risk populations

b-Glucan Antigenemia Anticipates Diagnosis of Blood Culture–Negative Intra-abdominal Candidiasis

Tissot F et al, Am J Respir Crit Care Med 2013;188: 1100–1109



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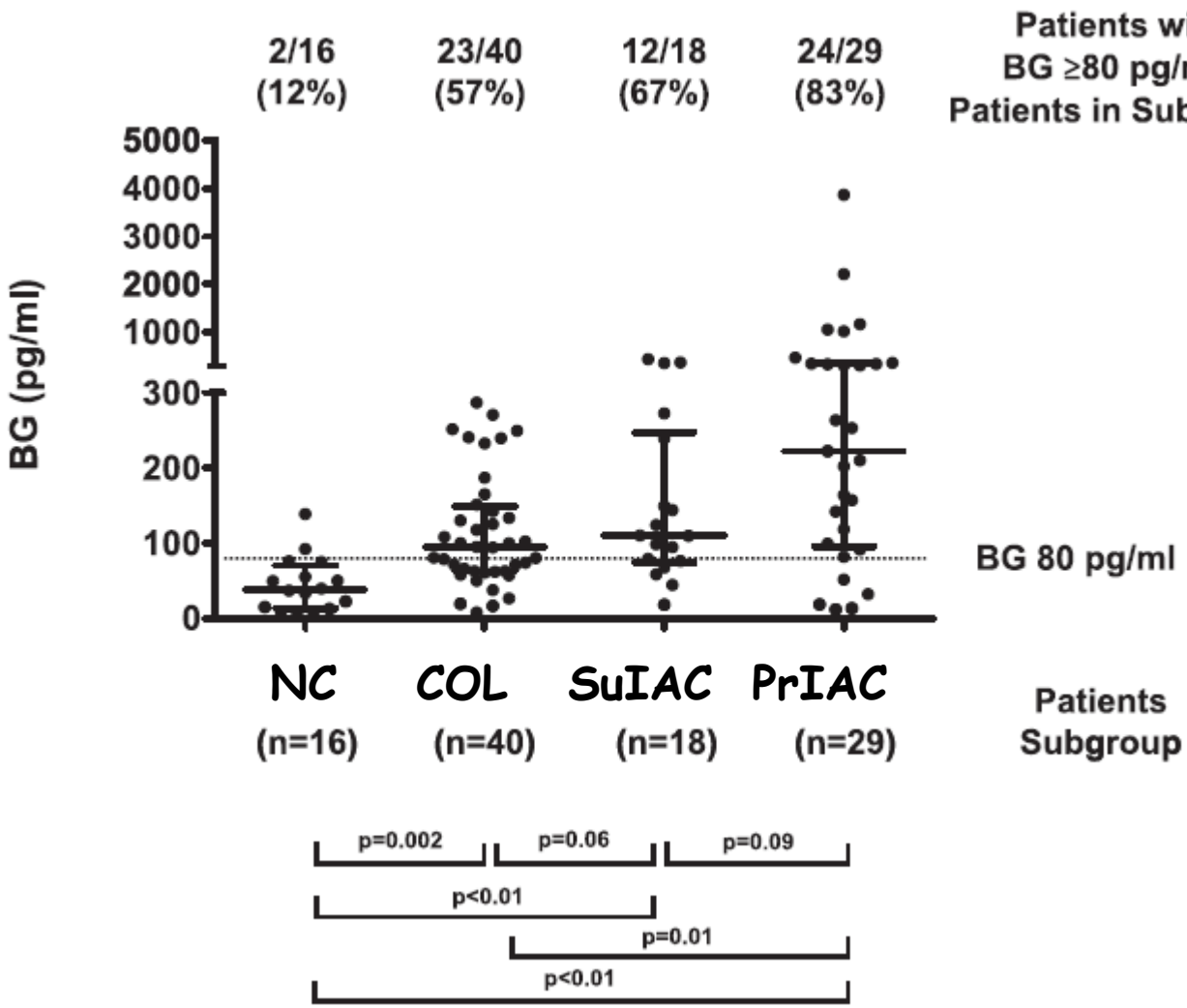


Median BDG

COLONIZED 99 pg/ml (8-440)

IAC 254 pg/ml (46-8557)

β-D-glucan (BDG) : open questions.

THE BEST CUT OFF?

(need for different cut offs?)

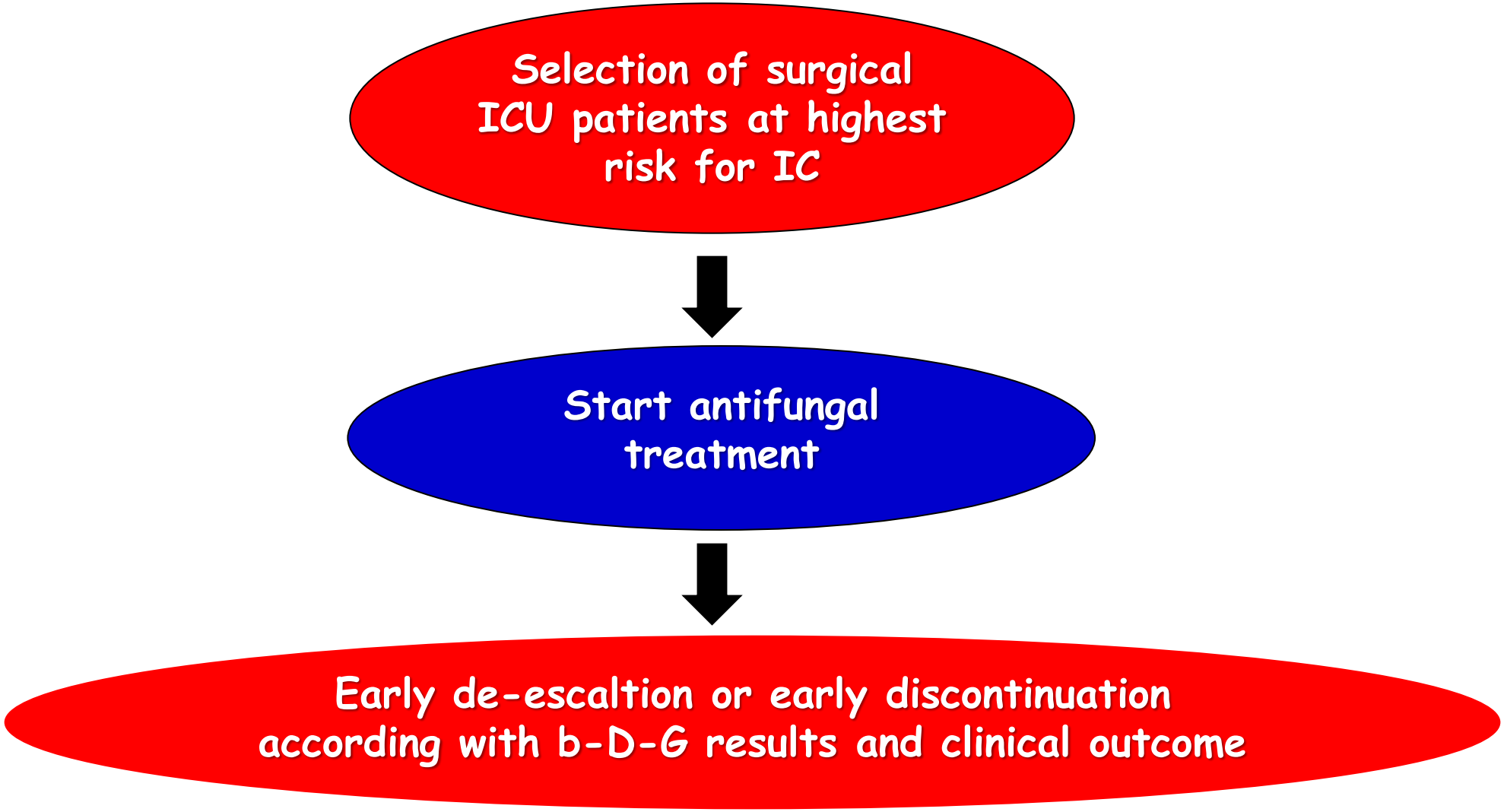
SINGLE OF SERIAL DETERMINATIONS?

TO MAKE OR TO EXCLUDE DIAGNOSIS OF IC?

ALONE or INCLUDED IN A NEW SCORE?

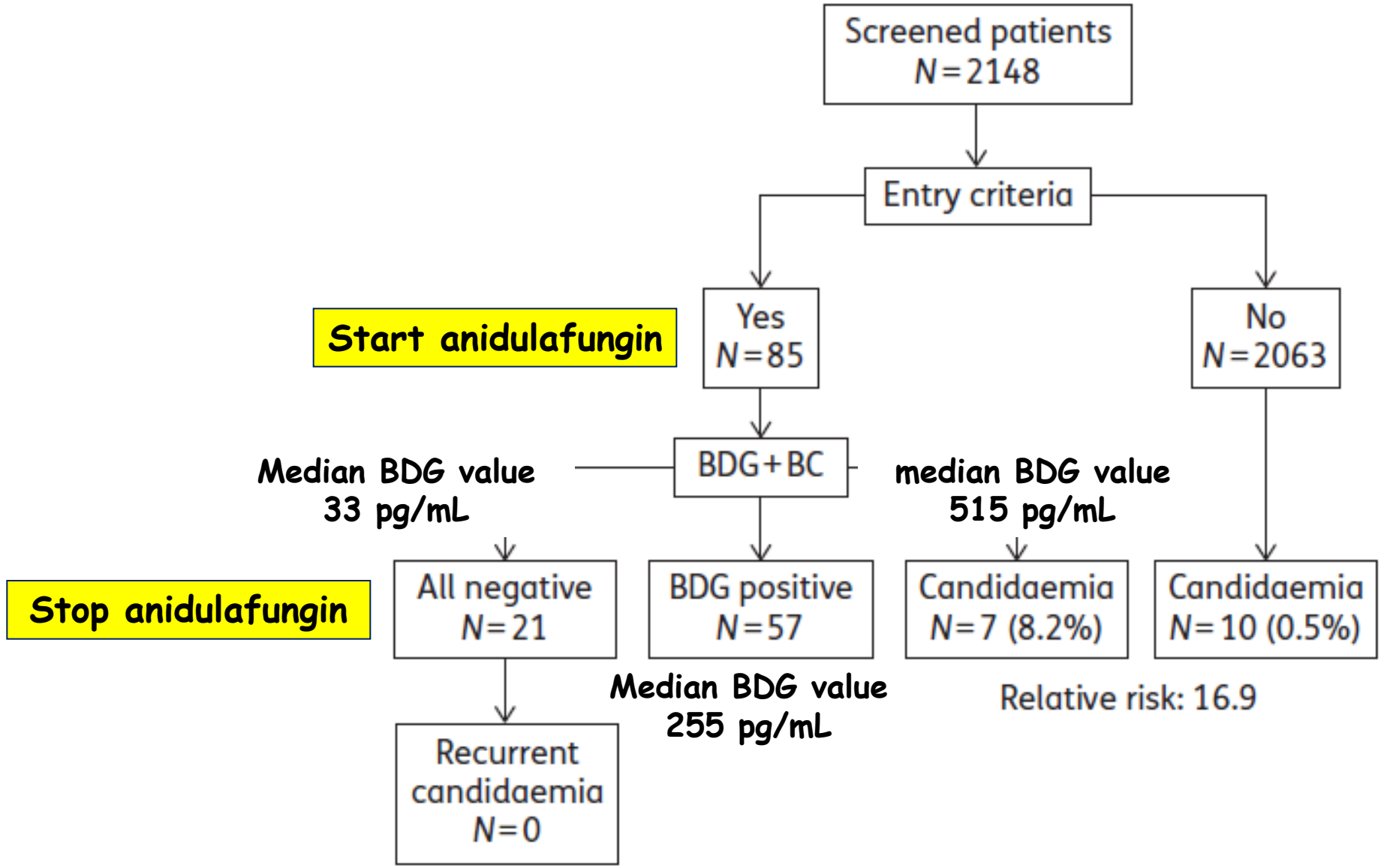
THE PROBLEM OF THE TURNROUND

Antifungal stewardship – a proposal



Discontinuation of empirical antifungal therapy in ICU patients using 1,3-b-D-glucan

Nucci M et al, J Antimicrob Chemother Advance Access published June 10, 2016



T2 Magnetic Resonance Assay for the Rapid Diagnosis of Candidemia in Whole Blood: A Clinical Trial

Mylonakis E et al, Clin Infect Dis 2015;60:892–9

T2Dx lyses the red blood cells, concentrates the pathogen cells and cellular debris, lyses the Candida cells by mechanical bead beating, amplifies Candida DNA using a thermostable polymerase and pan-Candida primers for the intervening transcribed spacer 2 region within the Candida ribosomal DNA operon, and finally, detects amplified product by amplicon-induced agglomeration of supermagnetic particles and T2MR measurement.

Blood specimens were collected from 1801 hospitalized patients who had a blood culture ordered for routine standard of care; 250 of them were manually supplemented with concentrations from <1 to 100 colony forming units /mL for 5 different Candida species

The mean time to species identification was …

4.4 ± 1.0 hours for T2MR and

129.9 ± 26.3 hours for the blood cultures (P < .001)

overall specificity 99.4% (95% CI 99.1%–99.6%)

overall sensitivity 91.1% (95% CI, 86.9%–94.2%)

T2Candida Provides Rapid and Accurate Species Identification in Pediatric Cases of Candidemia.

Hamula CL et al, Am J Clin Pathol March 2016;0:1-4

	T2Candida Result, No.			
Blood Culture Result	*Candida albicans/ Candida tropicalis*	*Candida parapsilosis*	*Candida krusei/ Candida glabrata*	Negative
C parapsilosis	0	7	0	0
C albicans	4	0	0	0
C glabrata	0	0	3	0
C tropicalis	1	0	0	0
Negative	0	0	0	9

The T2Candida panel provided the appropriate result for each specimen compared with blood culture–based species identification and correctly identified 15 positive and nine negative results in 3 to 5 hours

Impact of Rapid Organism Identification via Matrix-Assisted Laser Desorption / Ionization Time-of-Flight Combined With Antimicrobial Stewardship Team Intervention in Adult Patients With Bacteremia and Candidemia.

Huang AM et al Clin Infect Dis 2013; 57: 1237-45

A pre–post quasi-experimental study was conducted to analyze the impact of MALDI-TOF with AST intervention in patients with bloodstream infections. The AST provided evidence based antibiotic recommendations after receiving real-time notification following blood culture Gram stain, organism identification, and antimicrobial susceptibilities. Outcomes were compared to a historic control group.

A total of 501 patients with bacteremia or candidemia were included in the final analysis: 245 patients in the intervention group and 256 patients in the pre-intervention group

AMS team intervention

Intervention	Timing of Intervention Recommendation: Gram Stain	Organism Identification	Antimicrobial Susceptibility	Total (%)
Narrowed coverage to target the isolated organism	2	22	48	72 (34.3)
Discontinued therapy targeting organisms not isolated	5	44	19	68 (32.4)
Initiated or broadened coverage	39	5	9	53 (25.2)
Other	8	4	5	17 (8.1)
Total (%)	54 (25.7)	75 (35.7)	81 (38.6)	210 (100)
Interventions accepted (%)	49 (90.7)	62 (82.7)	78 (96.3)	189 (90.0)

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Outcomes

Outcome	Total		
	Preintervention (n = 256)	Intervention (n = 245)	*P* Value
Clinical outcomes			
30-day all-cause mortality	52 (20.3)	31 (12.7)	.021
Time to microbiological clearance, d	3.3 ± 4.8	3.3 ± 5.7	.928
Length of hospitalization, d[a]	14.2 ± 20.6	11.4 ± 12.9	.066
Length of ICU stay, d[a]	14.9 ± 24.2	8.3 ± 9.0	.014
Recurrence of same BSI	15 (5.9)	5 (2.0)	.038
30-day readmission with same BSI	9 (3.5)	4 (1.6)	.262
Treatment-related outcomes			
Time to effective therapy, h	30.1 ± 67.7	20.4 ± 20.7	.021
Time to optimal therapy, h	90.3 ± 75.4	47.3 ± 121.5	<.001

MALDI-TOF MS and ASP

Study	Organisms	Population	Antibiotic use	Patient outcome
Perez et al APLM 2013	Gram-neg	201 pts with BSI (100 pre-intervention, 101 intervention)	Time to antibiotic optimization 46-h reduction (P =.004); Reduced time to active treatment (P < .001	LOS (11.9 d vs 9.3 d; P = .01) Hospital costs per pt (P = .009)
Huang et al CID 2013	Aerobic Gram-pos, Gram-neg and yeast	501 pts with BSI (256 pre-intervention, 245 intervention)	Time to effective therapy of 30.1 vs 20.4 h (P = .021) Optimal antibiotic therapy (90.3 vs 47.3 h; P < .001)	2.8-day decrease in mean LOS (P = .07) **Reduced mortality (20.3% vs 14.5%; P= .02)**
Clerc et al CID 2013	Gram-neg	202 pts with BSI	Greater percentage of patients with ID consultation compared with Gram stain results alone (35.1% vs 20.8%; P = NR)	NR
Wenzler et al. ECCMID 2014	*A. baumannii*	109 pts with BSI and/or pneumonia (66 pre-intervention, 53 intervention)	Time to effective therapy (77.7 h vs 36.6 h; P < .0001)	**Increase in clinical cure (15% vs 34%; P = .016)**
Perez et al J Infect 2014	XDR Gram-neg	265 pts with BSI (112 pre-intervention, 153 intervention)	Time to optimal antibiotic therapy (80.9 h vs 23 h; P < .001)	**Reduced mortality (21% vs 8.9%; P = .01)**
Carreno et al ICAAC 2014	Gram-neg Gram-pos	104 pts with BSI (78 pre and 26 post test)	NR	Time to sepsis resolution 4 vs. 3 days (P=0.08)

Impact of Antimicrobial Stewardship Intervention on Coagulase- Negative *Staphylococcus* Blood Cultures in Conjunction with Rapid Diagnostic Testing.

Nagel JL et al, J. Clin. Microbiol. 2014, 52:2849

single-center, quasi-experimental study. Adult patients with a CoNS blood culture identified via MALDI-TOF over a 3-month period were compared to a historical control group with CoNS identified by conventional methods . Patients were divided into 4 categories:

Pts with CoNS BSI before/after implementation of MADLI-TOF plus AST intervention
Pts with CoNS contamination before/after MADLI-TOF plus AST intervention

During the preintervention study period, prescribers were immediately notified of positive Gram stain results from blood cultures. The AST did not intervene for positive bacterial cultures in real time but AST reviewed daily reports from Monday through Friday for all patients receiving restricted antimicrobials and recommended therapy changes on the basis of institutional guidelines and clinical judgment.

All stewardship activities, except for the addition of real-time alerts for positive blood cultures during the intervention period, remained unchanged during the study time frame.

Impact of Antimicrobial Stewardship Intervention on Coagulase- Negative *Staphylococcus* Blood Cultures in Conjunction with Rapid Diagnostic Testing.

Nagel JL et al, J. Clin. Microbiol. 2014, 52:2849

Gram positive cocci in clusters:
Initiate broad spectrum gram-positive antimicrobial therapy (vancomycin, alternative: daptomycin). Draw repeat blood cultures prior to initiating antimicrobials if contamination suspected.

If repeat cultures positive:
Continue gram-positive antimicrobial.
Discontinue unnecessary gram-negative coverage.
*consider antimicrobial de-escalation if isolate is susceptible and patient not critically ill

If repeat cultures negative + hemodynamically stable or no source identified:
Discontinue gram-positive antimicrobial.
Discontinue unnecessary gram-negative coverage.

If repeat cultures negative + [hemodynamically unstable or source identified]:
Continue gram-positive antimicrobial, consider obtaining ID consult. Discontinue unnecessary gram-negative coverage.

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OUTCOMES – CoNS bacteremia

Characteristic	Preintervention group ($n = 46$)	AST intervention group ($n = 32$)	P value
Time to organism identification[a] (h)	83.4 ± 29.5	57.0 ± 32.3	<0.001
Time to effective therapy[a] (h)	37.7 ± 40.1	23.0 ± 10.7	0.064
Time to optimal therapy[a] (h)	58.7 ± 56.4	34.4 ± 29.9	0.030
No. (%) of patients with 30-day all-cause mortality	10 (21.7)	1 (3.1)	0.023
Length of hospitalization[a,b] (days)	14 ± 22	15 ± 14	0.954
Length of ICU stay[a,b] (days)	28 ± 33	11 ± 11	0.188
No. (%) of patients with recurrent bacteremia	6 (13.0)	0 (0.0)	0.076

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Nagel JL et al, J. Clin. Microbiol. 2014, 52:2849

OUTCOMES – CoNS contamination

Characteristic	Preintervention group ($n = 83$)	AST intervention group ($n = 85$)	P value
Duration of CoNS antibiotic therapy[a] (days)	4.4 ± 4.2	3.0 ± 1.6	0.015
Vancomycin utilization[a] (g)	4.8 ± 6.3	3.0 ± 3.9	0.038
Daptomycin utilization[a] (g)	2.88	0	0.243
No. of vancomycin serum assays obtained[a]	2.0 ± 2.2	0.9 ± 1.4	<0.001
No. (%) of patients with 30-day all-cause mortality	9 (10.8)	10 (11.8)	>0.99
Length of hospitalization[a] (days)	14.6 ± 22.9	15.8 ± 18.6	0.7
No. (%) of patients with recurrent bacteremia	3 (3.6)	2 (2.4)	0.68

Rapid Testing Using the Verigene Gram-Negative Blood Culture Nucleic Acid Test in Combination with Antimicrobial Stewardship Intervention against Gram-Negative Bacteremia.

Bork JT et al Antimicrob Agents Chemother. 2015 ;59:1588-95

Multiplex, automated molecular diagnostic test for identification of eight Gram-neg organisms and resistance markers from blood culture with a turnaround time of approximately 2 h.
A theoretical evaluation of time to effective and optimal antibiotic was performed, comparing actual antibiotic administration times from chart review ("control") to theoretical administration times based on BC-GN reporting and antimicrobial stewardship team review ("intervention").

132 first episodes of Gram- BSI were included
59% ICU pts

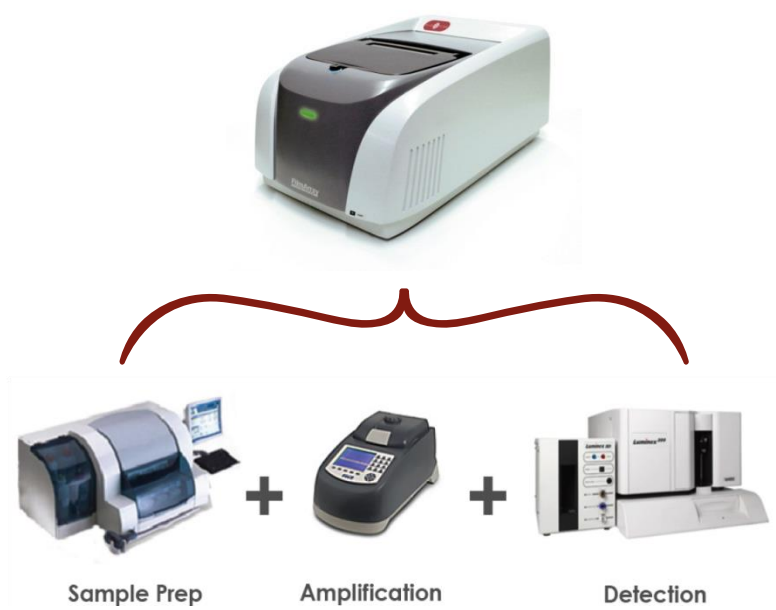
39% primary
26% urine
10% lung
9% CVC related
8% intra-abdominal

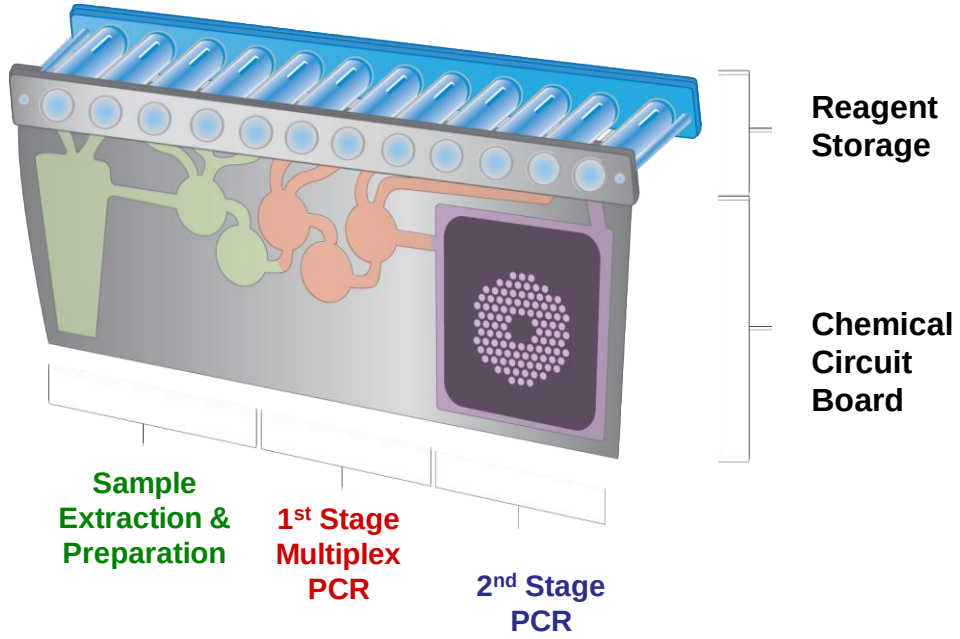
Correct identification by VERIGENE	95.6%
Sensitivity	97.1%
Specificity	99.5%

TIME to EFFECTIVE ATB	3.7 h earlier	p <.001
Allowing 12 h for ATB changes to be made based on Verigene		
TIME to OPTIMAL ATB	18.3 h earlier	p <.001

FilmArray: Advanced Syndromic Screening for the Diagnosis of Infections

- **The FilmArray PCR-based device integrates sample preparation, amplification, detection and analysis into one simple system that requires 2 min of hands-on time and has a total run time of about 1 hour**



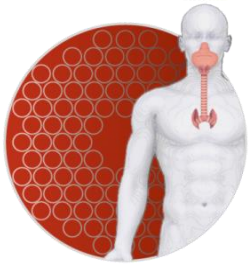


FilmArray: Complete and Certified Offer

4 CE-IVD / FDA cleared panels

83 pathogens identification

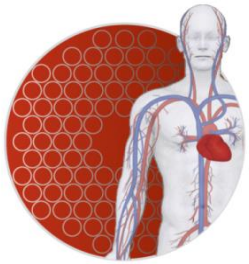
Respiratory Panel



20
targets

- 3 bacteria
- 17 viruses

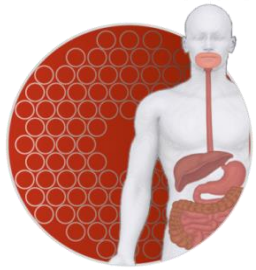
Blood Culture Identification Panel



27
targets

- 19 bacteria
- 5 yeast
- **3** resistance genes

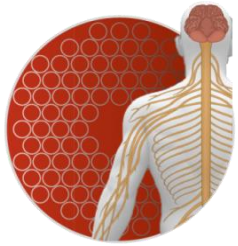
Gastrointestinal Panel



22
targets

- 13 bacteria
- 5 viruses
- 4 parasites

Meningitis Encephalitis Panel



14
targets

- 6 bacteria
- 7 viruses
- 1 fungus

FilmArray: Blood Culture Identification Panel

27 pathogens

Gram + Bacteria:

Enterococcus spp.
L. monocytogenes
Staphylococcus
S. aureus
Streptococcus spp.
S. agalactiae (Group B)
S. pyogenes (Group A)
S. pneumoniae

Antibiotic Resistance:

mecA
Van A/B
KPC

Gram - Bacteria :

A. baumannii
Enterobacteriaceae
Enterobacter cloacae
Complex
E. coli
H. influenzae
K. oxytoca
K. pneumoniae
N. meningitidis
P. aeruginosa
Proteus
S. marcescens

Fungi:

C. albicans
C. glabrata
C. krusei
C. parapsiolosis
C. tropicalis

Sample: Positive Blood culture

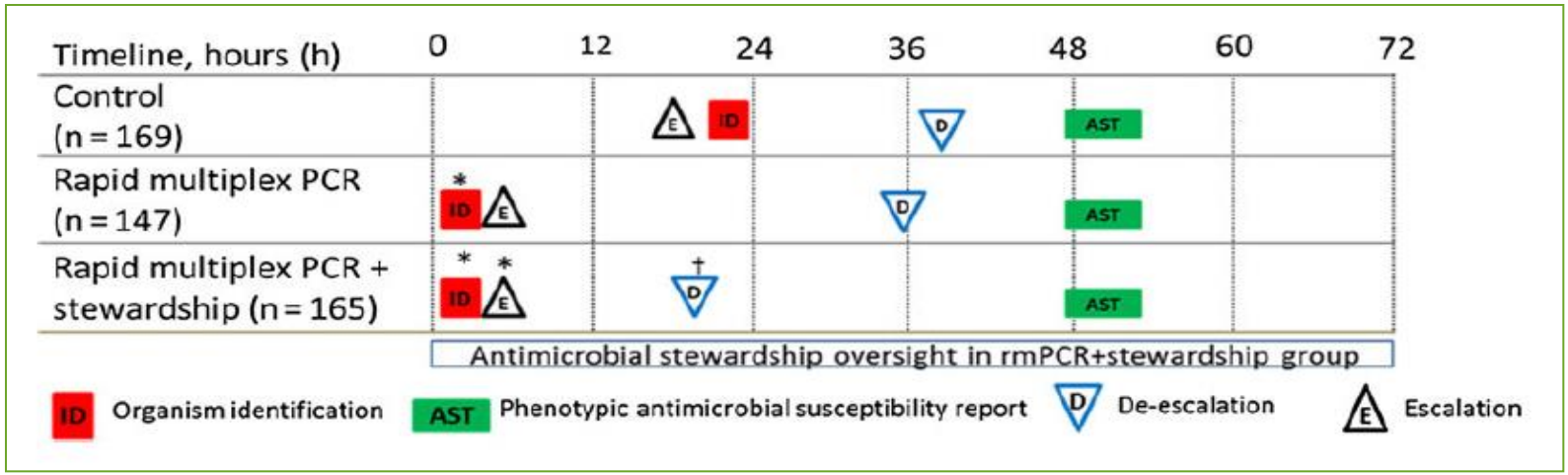
CLINICAL INFECTIOUS DISEASES
2015;61(7):1071-1080

Randomized Trial of Rapid Multiplex Polymerase Chain Reaction-Based Blood Culture Identification and Susceptibility Testing.

Banerjee R, Teng CB, Cunningham SA, Ihde SM, Steckelberg JM, Moriarty JP, Shah ND, Mandrekar JN, Patel R.

INT1

INT2



- **Median time from Gram stain result to organism identification was shorter in both intervention groups (both 1.3 hours) *versus* the control group (22 hours) (P < .0001)**
- **Time from GS result to appropriate antimicrobial de-escalation and escalation was significantly shorter in INT 2 and INT *versus* control group respectively**