Rapid Diagnostics and AMR

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Disclosures

- Scientific Advisory Boards:
  - Roche Molecular, Quidel, Cepheid, DNAe, IDbyDNA, Luminex
- Clinical Trials:
  - T2 Biosystems, Hologic

Rapid Diagnostics and AMR

- Rapid detection of pathogens from positive blood culture or colony
- Rapid susceptibility testing
- Direct detection from blood
  - T2 Biosystems
- Xpert Carba-R test for carbapenem-resistant bacteria

Rapid Techniques for Identification of MDRO

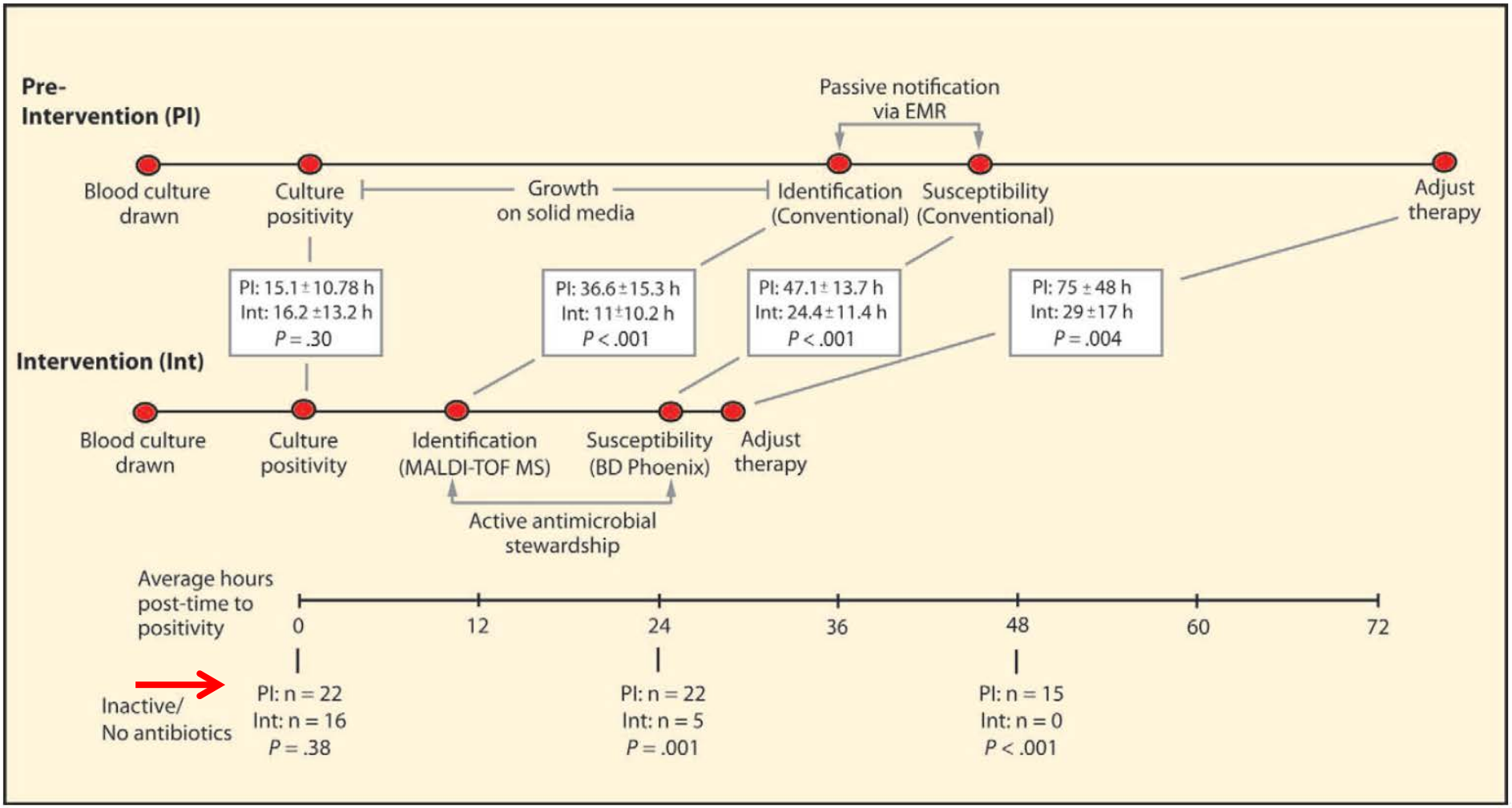
- How fast is rapid
  - Less than an hour, hours, days, better than what we are currently doing
- Location of testing
  - Central laboratory, rapid response laboratory
- Type of testing
  - From an isolate, positive blood culture bottle, primary specimen
- Goal: change clinical management, support stewardship program

MALDI-TOF MS

Matrix-assisted laser desorption ionization-time of flight mass spectrometry

- Rapid method to identify bacteria and fungi
  - From a colony, positive blood culture bottle
  - Genus and/or species level
- Based on the protein signature of the organism
  - Database is key
- Testing is very rapid, expensive instrument, inexpensive testing
- Mixed cultures are a problem
- No susceptibility results

MALDI-TOF: Blood culture positive for GNR



Identification: 37 hrs vs 11 hrs; Susceptibility 47 hrs vs 24 hrs;
Adjust therapy 75 hrs vs 29 hrs

Perez KK et al. Arch Pathol Lab Med 2013:137:1247

Table 2. Length of Stay and Cost Outcomes in Survivors[a]

Outcome	Preintervention Cohort (n = 100)	Intervention Cohort (n = 101)	*P*
Hospital length of stay	11.9 ± 9.3	9.3 ± 7.6	.01
Hospital length of stay after BSI onset	9.9 ± 7.1	8.1 ± 6.4	.01
ICU length of stay	7.3 ± 8.5	6.3 ± 8.7	.05
ICU length of stay after BSI onset	6.1 ± 6	4.9 ± 6.7	.09
Total hospital costs	$45 709 ± $61 806	$26 162 ± $28 996	.009
MS DRG weight	2.7 ± 2.4	±1.9	54

Rapid identification and susceptibility testing with antimicrobial stewardship program improved time to optimal therapy, reduced LOS and total costs

Perez KK et al. Arch Pathol Lab Med 2013:137:1247

Cost Analysis of Implementing Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Plus Real-Time Antimicrobial Stewardship Intervention for Bloodstream Infections

Twisha S. Patel,[a,b] Rola Kaakeh,[b] Jerod L. Nagel,[a,b] Duane W. Newton,[c] James G. Stevenson[b]

TABLE 4 Clinical outcomes for preintervention group compared to intervention group

Parameter	Preintervention (n = 247)	Intervention (n = 233)	Relative risk reduction (%)	P value
30-day mortality	52 (21)[a]	28 (12)[a]	43	<0.01
Hospital LOS[c] (days)	14.2 ± 16.7[b]	13.0 ± 16.5[b]		0.44

[a]Data represent number (percent) of patients.

[b]Data represent mean ± standard deviation.

[c]LOS, length of stay (length of time of hospitalization blood culture positivity to discharge).

Cost: Preintervention: $45,019, postintervention $42,589 (NS), savings to the health system ~$2 million annually

Patel TS et al. JCM 2016;55:60-67

FilmArray Blood Culture ID Panel
FDA Cleared, Positive Blood Culture, ~1hr

Gram + Bacteria

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

Gram - Bacteria

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

Fungi

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

Antibiotic Resistance

mecA
Van A/B
KPC

Verigene Gram Positive Test
FDA Cleared, Positive Blood Culture, ~2hrs

Species

S. aureus
S. epidermidis
S. lugdunensis
Streptococcus anginosus Group
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes
Enterococcus faecalis
Enterococcus faecium

Genus

Staphylococcus spp.
Streptococcus spp.
Listeria spp.

Resistance

mecA (methicillin)
vanA (vancomycin)
vanB (vancomycin)

Verigene Gram Negative Test
FDA Cleared, Positive Blood Culture, ~2hrs

Species

Escherichia coli
Klebsiella pneumoniae
Klebsiella oxytoca
Pseudomonas aeruginosa

Genus

Acinetobacter spp.
Citrobacter spp.
Enterobacter spp.
Proteus spp.

Resistance

CTX-M (ESBL)
IMP (carbapenemase)
KPC (carbapenemase)
NDM (carbapenemase)
OXA (carbapenemase)
VIM (carbapenemase)

Prospective randomized trial to assess benefit of rapid highly multiplexed PCR-based test in identifying bacteria and yeast from positive blood cultures

- FilmArray: >20 bacteria and yeast, resistance genes *mecA*, *van A/B,* and bla_{KPC}
- Role of stewardship program evaluated

Banerjee R et al CID 2015; 61:1071-80

Study Design

- 3 arms to the study
  - SOC – Maldi-TOF MS from colonies isolated from positive BC
  - PCR - Rapid multiplex PCR with templated comments
  - PCR/AS - Rapid multiplex PCR, templated comments and real-time stewardship
- FilmArray run 24/7, as soon as bottle positive
- Results of PCR called to the team and put in EMR
- PCR/AS stewardship team (ID/pharmacist) called 24/7

Banerjee R et al CID 2015; 61:1071-80

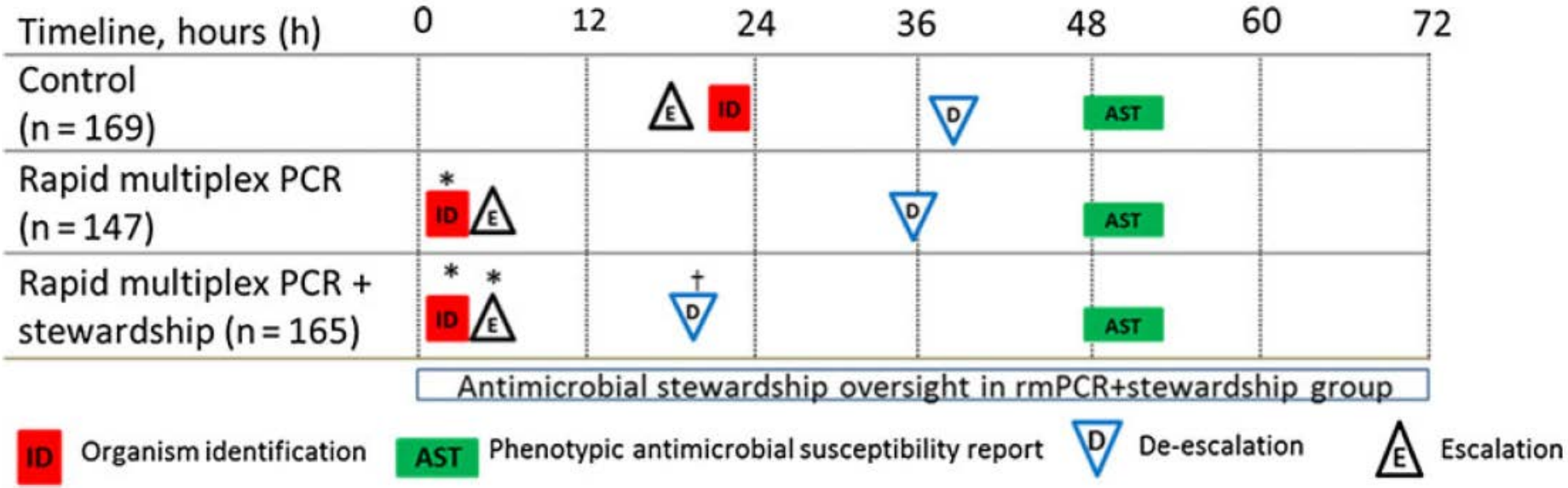
Time from blood culture bottle gram stain to organism ID

Intervention arm: 1.3 hours, SOC: 22.3 hours ($p < 0.001$)

Table 3. Antibiotic Utilization Among All Study Subjects in the First 96 Hours Following Enrollment

Outcome	Control	Rapid Multiplex PCR	Rapid Multiplex PCR + Stewardship	*P* Value Comparing 3 Groups
Duration of therapy[a], h				
Vancomycin				
All patients (n = 357)	44 (22–72)	42 (21–93)	42 (19–90)	.92
Organisms not requiring vancomycin[b] (n = 169)	8.2 (0–26)	0 (0–16)	0 (0–3)[c]	.032
Vancomycin-susceptible enterococci (n = 32)	20 (1–59)	70 (48–88)[c]	82 (40–96)[c]	.037
Methicillin-susceptible *Staphylococcus aureus* (n = 42)	23 (20–53)	11 (0–26)	8 (0–44)	.2
Nafcillin, oxacillin, or cefazolin (n = 50)	42 (24–57)	71 (51–79)[c]	85 (42–92)[c]	.035
Piperacillin-tazobactam (n = 214)	56 (39–82)	44 (27–74)[c]	45 (19–78)[c]	.012
Cefepime (n = 181)	55 (28–96)	71 (43–96)	58 (32–96)	.56
Antibiotic modifications				
Time to first appropriate de-escalation[d] (n = 344)	34 (21–55)	38 (22–66)	21 (7–37)[c,e]	<.0001
Time to first appropriate escalation[f] (n = 122)	24 (3–67)	6 (2–36)	5 (2–22)[c]	.04
Time to administration of active antibiotics (n = 123)[g]	11 (2–51)	6 (2–31)	4 (2–20)	.55
Contaminated blood cultures not treated or treated for <24 h, No. (%)[h]	47 (75)	49 (89)[c]	57 (92)[c]	.015

Banerjee R et al CID 2015; 61:1071-80



Groups did not differ in mortality, length of stay, cost
Study was not powered for these outcomes

81% of organisms isolated were detected by FilmArray

Low % of KPC producing organisms

Designing large multi-center study

Banerjee R et al CID 2015; 61:1071-80

Survey to Assess Interpretation of FilmArray BCID Results

- 156 physicians responded (41%), 56% IM, 20% FM
- Only 60% of physicians adjusted antibiotics based on FilmArray BCID results

TABLE 5 Knowledge questions with corresponding proportion of correct responses by specialty

Competency assessed in question	*n*	% (no.) by specialty				*P* value
		Overall	Family medicine	Internal medicine	Other	
Organism identification based on BCID results						
Escherichia coli detected	119	60 (71)	38 (45)	70 (83)	45 (54)	0.02
Coagulase-negative *Staphylococcus* species detected	118	71 (84)	50 (59)	80 (94)	70 (83)	0.03
Methicillin-resistant *Staphylococcus aureus* detected	116	81 (94)	75 (87)	84 (97)	80 (93)	0.60
Appropriate treatment based on clinical history and BCID results						
Discontinue therapy, contaminated blood culture, coagulase-negative *Staphylococcus* species detected	113	76 (86)	65 (73)	80 (90)	76 (86)	0.37
Need to initiate therapy, vancomycin-resistant *Enterococcus* detected	110	86 (95)	87 (96)	88 (97)	82 (90)	0.71
De-escalate therapy indicated, *S. aureus* (methicillin susceptible) detected	102	53 (54)	33 (34)	61 (62)	52 (53)	0.10
De-escalate therapy indicated, *Streptococcus agalactiae* detected	98	52 (51)	60 (59)	52 (51)	46 (45)	0.63

Donner et al. JCM 2017;55:1496

Genotypic Resistance Testing

- Gram positive bacteria
  - Genotypic susceptibility testing can provide important and complete information
  - *mecA, vanA/B*
- Gram negative pathogens
  - Unlikely that genotypic data will be complete, positive result is helpful, not necessarily a negative result
  - Will need to be modified regularly
  - Not clear how efficiently this will be done from a regulatory perspective

Accelerate Pheno System

- Fully automated system
- Rapid identification from a positive blood culture
  - 1-2 hour, using FISH probes
- Susceptibility testing
  - ~7 hours, monitoring growth patterns and intensity
- Working from a positive blood culture

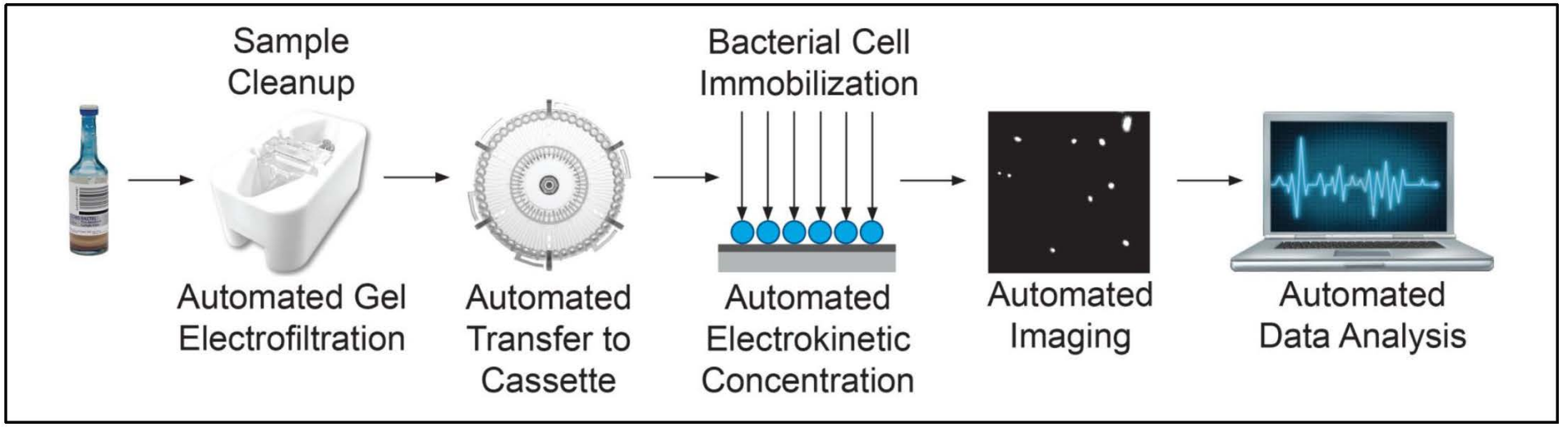
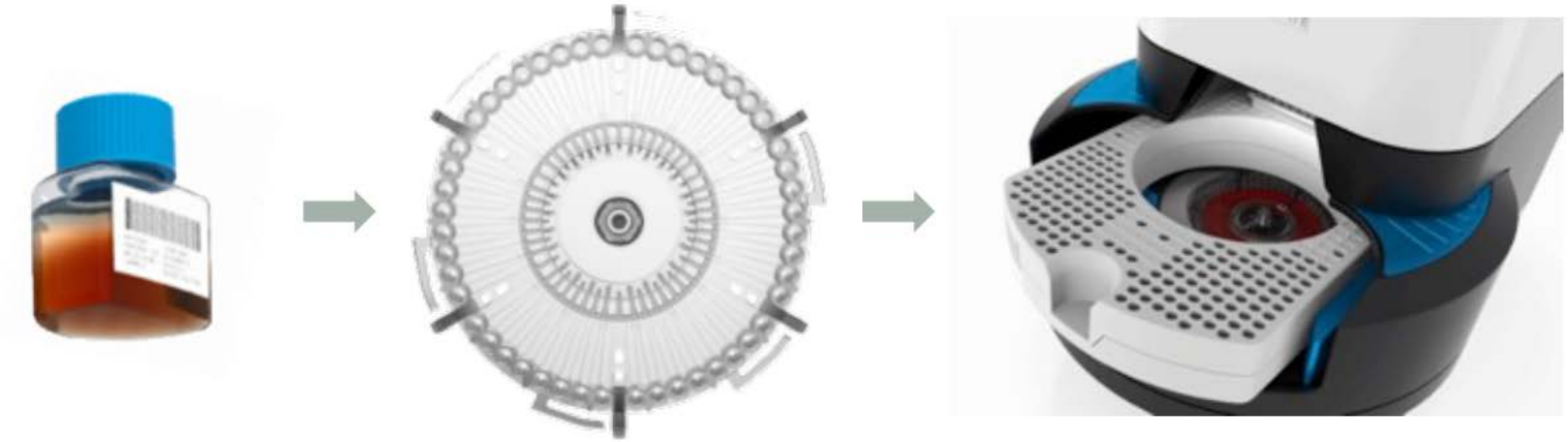


Figure 1. Accelerate ID/AST Technology process flow.



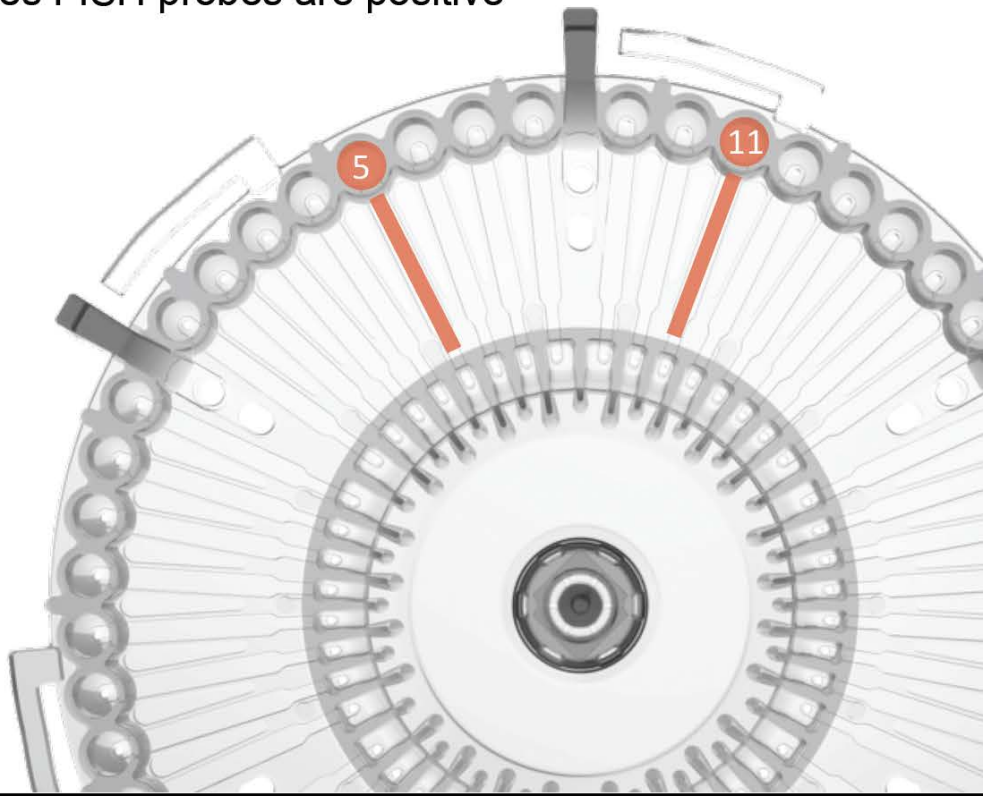
Price CS. Satellite Symposium ASM 2015

Polymicrobial Detection

Example: *E. coli* and *C. koseri* in same sample.
Flow channel 5 & 11 species FISH probes are positive

- Universal probes signal bacteria and yeast
- E. coli flowcell (5) is positive.
- Citrobacter spp. flowcell (11) is also positive.

Polymicrobial infection is detected by comparing results from each flow channel.



n use only. Not for use in diagnostic procedures.

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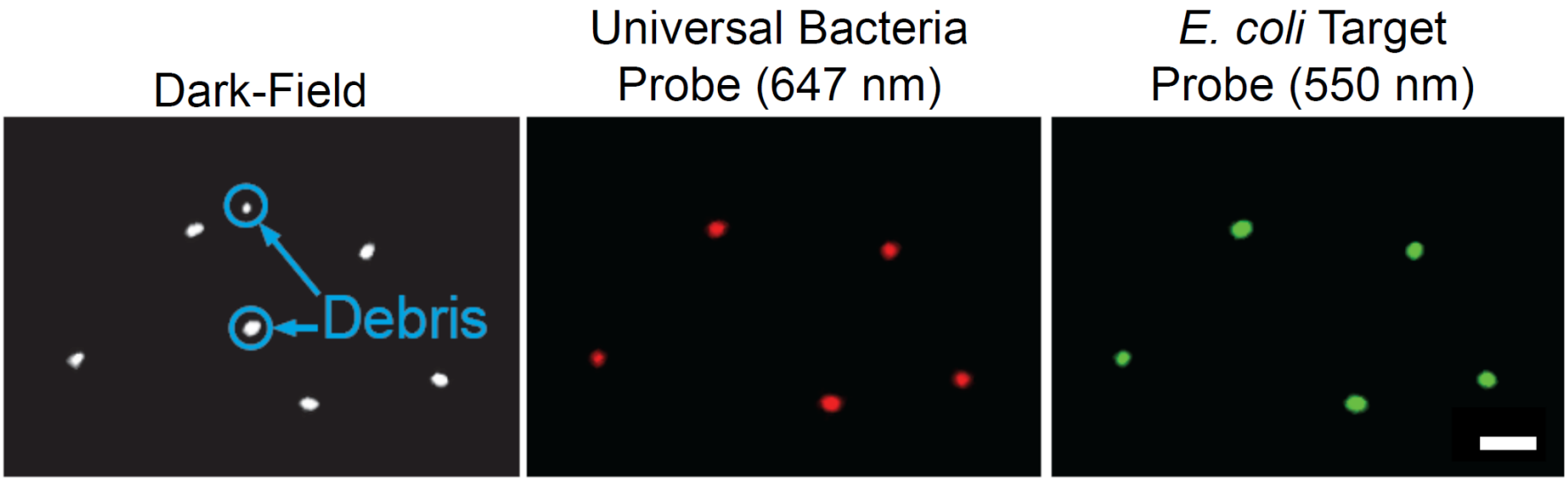
1-Hour FISH Identification

- *E. coli* Sample in *E. coli* Test Channel



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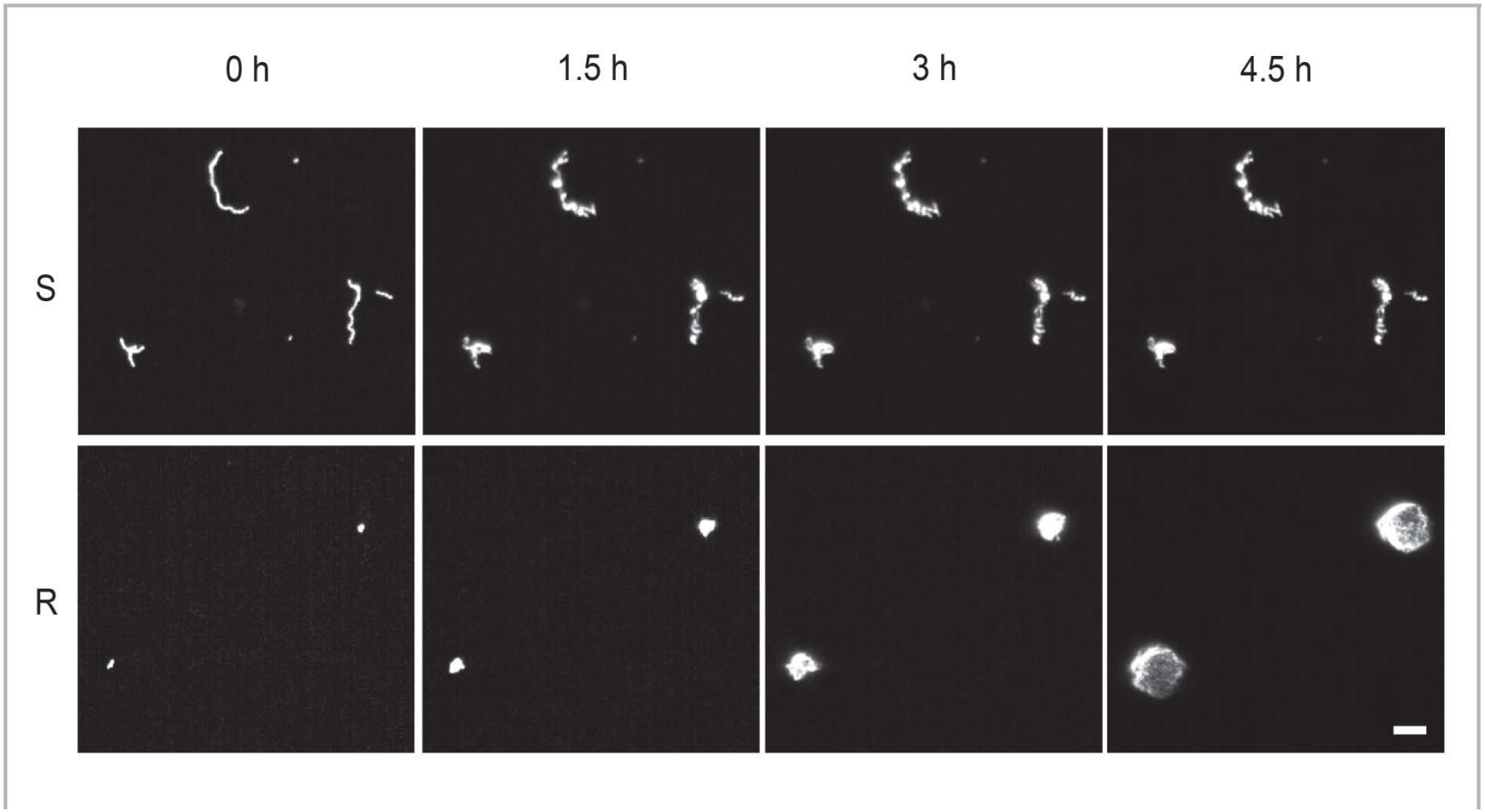
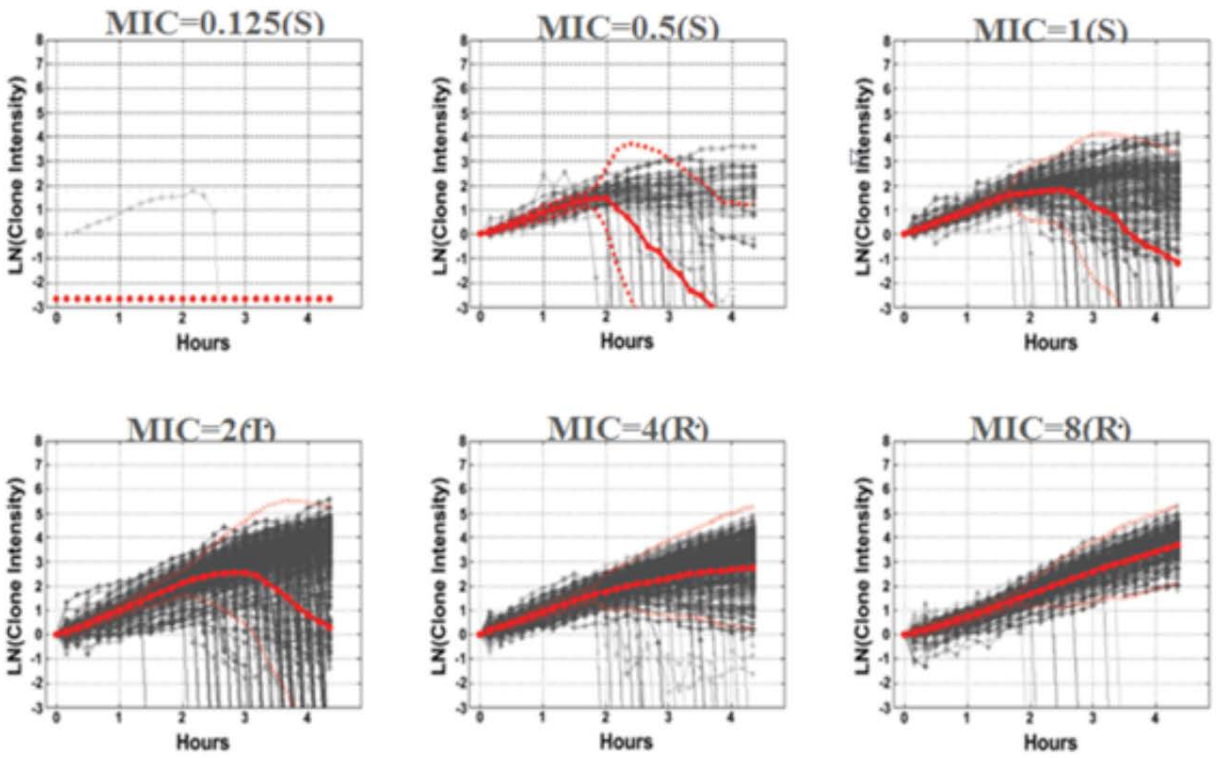
Time lapsed images of susceptible (S) and resistant (R) *S. pneumoniae* isolates growing in penicillin. Susceptible bacteria exhibit slow elongated growth compared to resistant bacteria



Gamage D et al. ICAAC 2014, Poster D-873, Washington DC

Phenotypic features such as morphological growth patterns, intensity, and growth rate are tracked and a MIC is calculated using multivariate logistic regression.

***P. aeruginosa* with 2 µg/mL ciprofloxacin**



Each grey line represents the growth of an individual cell

Average

2 σ

Not for use in diagnostic procedures.

Price CS. Satellite Symposium ASM 2015

Accelerate Pheno System

- 115 blood stream infections (BSI) with gram negative pathogens
- Pheno System identification
  - 88.7% (102/115) of all BSI
  - 97.1% (102/105) of bacteria on the panel
- AST results
  - 91.3% (95/104) in which GNR was identified by Pheno System
  - 96.4% category agreement (Vitek 2 and Etest)
  - 1.4% minor, 2.3% major and 1.0% very major errors

Marschal M et al. JCM 2017;55:2116

TABLE 2 Performance characteristics of the Accelerate Pheno system for organism identification (after adjudication of discrepant results)

Organism	Sensitivity[a]		Specificity[b]	
	No. detected/ no. tested	%	No. detected/ no. tested	%
Gram positives				
Coagulase-negative *Staphylococcus* spp.	52/52	100	169/172	98.3
Enterococcus faecalis	15/17	88.2	215/215	100
Enterococcus faecium	3/5	60	227/227	100
Staphylococcus aureus	18/19	94.7	200/202	99
Staphylococcus lugdunensis	0/0	NA[c]	228/228	100
Streptococcus spp.	21/21	100	205/210	97.6
Total	109/114	95.6	1,244/1,254	99.1
Gram negatives				
Acinetobacter baumannii	3/3	100	229/229	100
Citrobacter spp.	2/2	100	230/230	100
Enterobacter spp.	11/13	84.6	215/216	99.5
Escherichia coli	30/31	96.8	201/201	100
Klebsiella spp.	20/21	95.2	211/211	100
Proteus spp.	3/3	100	229/229	100
Pseudomonas aeruginosa	9/9	100	223/223	100
Serratia marcescens	3/3	100	229/229	100
Total	81/85	95.3	1,767/1,768	99.9
Yeast				
Candida albicans	2/2	100	229/229	100
Candida glabrata	3/3	100	224/229	97.8
Total	5/5	100	453/458	98.9
Overall[d]	195/204	95.6	3,464/3,480	99.5

Charnot-Katsikas A et al. JCM 2018;56: e01166-17

TABLE 3 Summary of very major and major errors in antimicrobial susceptibility

Antibiotic	No. of VMEs[a]					No. of MEs[b]				
	Total	Resolved to AXDX	Resolved to SOC	Resolved to neither	Unresolved	Total	Resolved to AXDX	Resolved to SOC	Resolved to neither	Unresolved
Gram-positive organisms										
Erythromycin	2	2	0	0	0	0	0	0	0	0
Linezolid	1	1	0	0	0	0	0	0	0	0
Gram-negative organisms										
Ampicillin-sulbactam	1	0	0	1	0	0	0	0	0	0
Cefepime	0	0	0	0	0	1	0	0	1	0
Ceftazidime	1	0	0	1	0	1	0	1	0	0
Ciprofloxacin	1	1	0	0	0	0	0	0	0	0
Ertapenem	1	0	0	1	0	1	0	1	0	0
Resistance phenotype tests										
Cefoxitin (methicillin resistance)	1	0	1	0	0	1	1	0	0	0
Total	8	4	1	3	0	4	1	2	1	0

[a]VME, very major error.
[b]ME, major error.

Charnot-Katsikas A et al. JCM 2018;56: e01166-17

Accelerate Pheno System

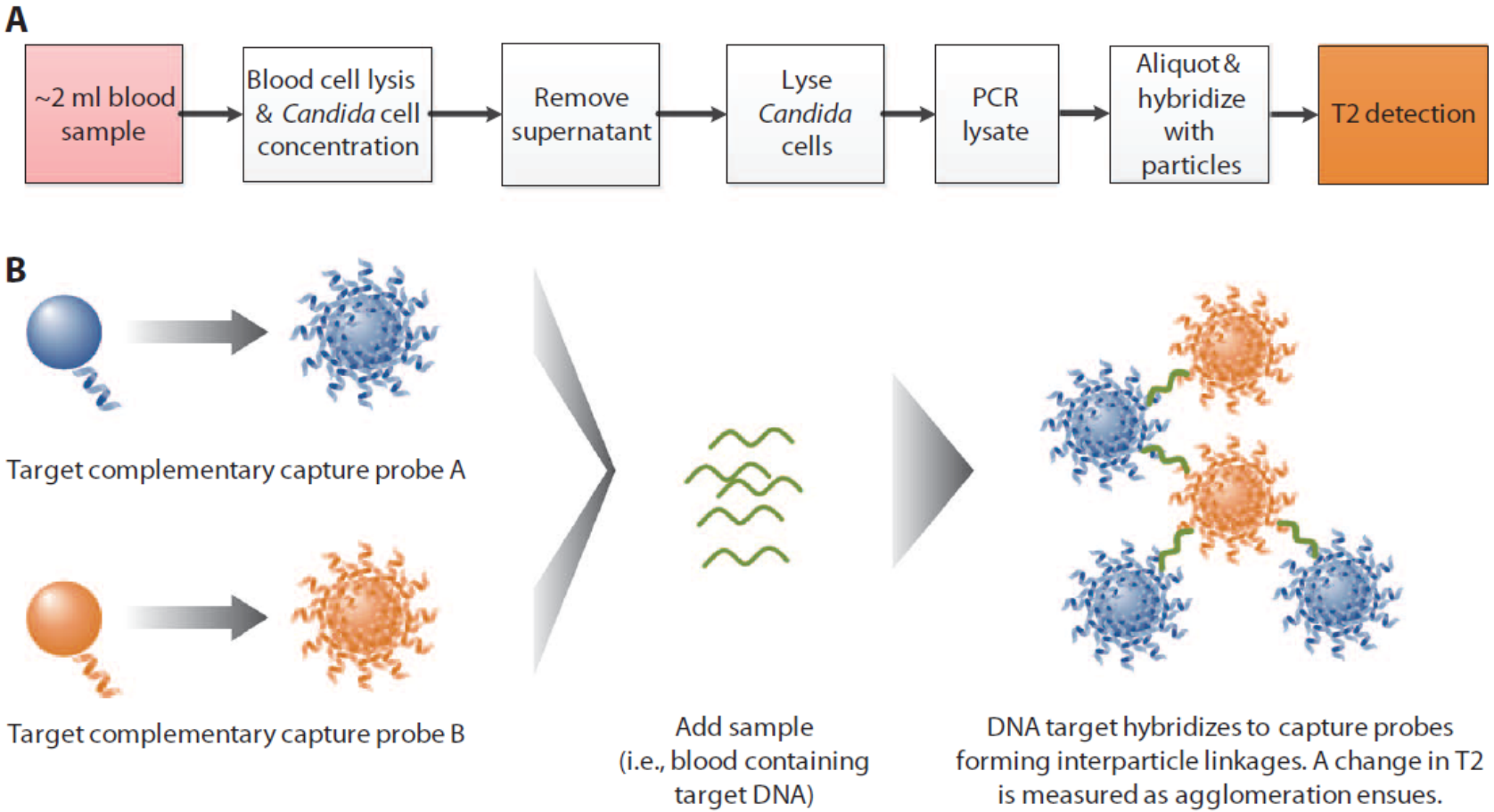
- Rapid, sensitive and accurate if organism is on the panel
- Not complete data
  - Continued improvement to the system and instrumentation (currently one test per instrument)
- Add-on test in the clinical laboratory
- Outcomes data to fully understand the value
- Incremental progress not transformative

Direct Detection of Pathogens from Blood

The graveyard of direct from sample tests

- SeptiFast: real-time PCR (Roche)
  - Direct from blood test for detecting bacteria
  - Sensitivity ~80%
  - Specificity issues, ubiquitous bacteria and molds
  - Learned a lot about contamination of reagents with bacterial DNA
- PCR/EIS-MS (Abbott)
  - PCR electrospray ionization-mass spectrometry
  - Direct from blood for detecting bacteria

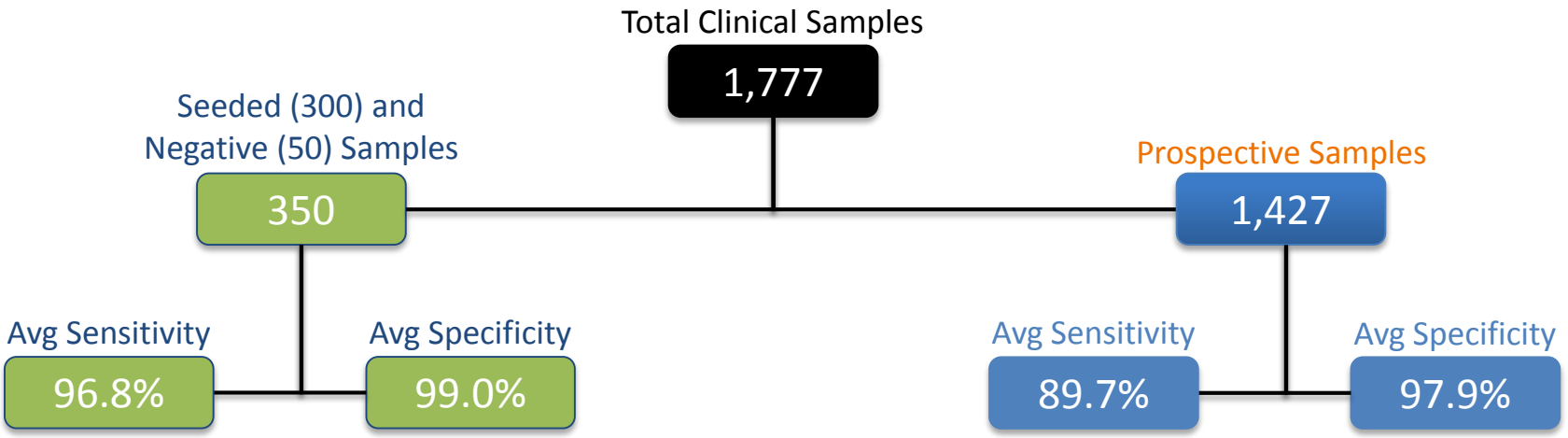
T2 Magnetic Resonance (T2MR)



Time to result: 3-5 hours

Neely LA, et al. Science Translational Medicine 2013;5, 182ra54

T2Bacteria Pivotal Clinical Trial



Species	Seeded Sensitivity	Seeded Specificity
A. baumannii	97.5% (39/40)	99.7% (299/300)
E. coli	90.9% (20/22)	97.3% (292/300)
E. faecium	100.0% (40/40)	100.0% (300/300)
K. pneumoniae	100.0% (40/40)	99.3% (298/300)
P. aeruginosa	97.4% (38/39)	97.7% (293/300)
S. aureus	92.3% (36/39)	100.0% (300/300)

7 T2-/BC+

18 T2+/BC-

Species	Prospective Sensitivity	Prospective Specificity
A. baumannii	- (0/0)	99.7% (1414/1427)
E. coli	90.9% (10/11)	95.0% (1345/1416)
E. faecium	100.0% (1/1)	99.4% (1417/1426)
K. pneumoniae	100.0% (6/6)	98.5% (1399/1421)
P. aeruginosa	100.0% (5/5)	97.7% (1389/1422)
S. aureus	81.3% (13/16)	98.0% (1383/1411)

4/39 T2-/BC+

176 T2+/BC-

T2Bacteria Pivotal Clinical Study Data Provided by T2: ***T2Bacteria Panel data submitted to FDA. Performance characteristics have not been established.***

Preliminary Analysis of Discordant Results T2Bacteria+/Blood Culture-

Evaluated looking at additional blood culture results obtained +/- 14 days of the paired T2 / blood culture draw.

36% of the T2+/BC- results were concordant with species identification from other cultures.

Further adjudication is in progress

Bacteria	Percentage of T2+/BC- results with other Positive Cultures
A. baumannii	0/13
E. coli	23/70 (33%)
E. faecium	4/9 (44%)
K. pneumoniae	8/21 (38%)
P. aeruginosa	7/32 (22%)
S. aureus	21/28 (75%)
Total	63/173 (36%)

T2Bacteria Pivotal Clinical Study Data Provided by T2: ***T2Bacteria Panel data submitted to FDA. Performance characteristics have not been established.***

T2 Biosystem

- Rapid result (with the need for culture) with a sensitivity of ~90%, specificity 98-99%
  - Will results change management?
- Incomplete data
  - *Candida auris* in development
  - Expansion of bacterial panel underway
- No susceptibility results
- Outcomes studies are needed to assess the clinical impact

Xpert Carba-R Assay to Detect Carbapenem-Resistant Bacteria

- Cartridge detects five classes of carbapenem resistance genes (91 in total):
  - *bla*$_{KPC}$
  - *bla*$_{NDM}$
  - *bla*$_{VIM}$
  - *bla*$_{OXA-48}$
  - *bla*$_{IMP-1}$
- Samples types: carbapenem non-susceptible colonies, rectal and perirectal swabs
- Time to result: 48 minutes

Xpert Carba-R Spectrum of Detection

ALL KPCs 1-19

IMP-1 subgroup

- IMP 1
- IMP 3
- IMP 5
- IMP 6
- IMP 10
- IMP1 5
- IMP 25
- IMP 29
- IMP 30
- IMP 40

ALL VIMS 1-40

ALL NDMs 1-12

OXA-48 like

- OXA-162
- OXA-163
- OXA-181
- OXA-204
- OXA-232
- OXA-244
- OXA-245
- OXA-247
- OXA-370

Courtesy of Cepheid

TABLE 2 Performance of CDC surveillance and UCLA CRE definitions for identification of CP-CRE with and without ancillary carbapenemase testing using Xpert Carba-R or CIM assay[a]

Definition and ancillary test used	Sensitivity		Specificity	
	%	95% CI	%	95% CI
CDC CP-CRE				
No ancillary testing	98.9	93.2–99.9	6.1	1.1–21.6
With Xpert Carba-R	99.9	94.9–99.9	100	19.8–100
With CIM[b]	97.6	92.5–99.4	100	19.8–100
UCLA CRE				
No ancillary testing	100	95.0–100	24.2	11.7–42.6
With Xpert Carba-R	100	95.0–100	100	87.0–100
With CIM	97.7	91.4–99.6	100	87.0–100

[a]The CDC definition is ertapenem, meropenem, and/or imipenem resistant. The UCLA definition is intermediate or resistant to imipenem and/or meropenem, with the exception of *Proteus/Providencia/Morganella*, where only meropenem is considered. $n = 125$ isolates of *Enterobacteriaceae*; analysis with CIM ancillary testing excludes the 3 indeterminate results.

[b]The CIM ancillary test method consisted of incubating a meropenem disk in a TSB suspension of organisms for 2 h, followed by 18 h of incubation of disk diffusion plate.

CDC defined CRE to assist labs to identify carbapenemase producing CRE (CP-CRE)

125 isolates of *Enterobacteriaceae*

CIM – carbapenem inactivation method – phenotypic screen (over night)

Miller SA et al. JCM 2017;55:1827

Table 2. Performance characteristics of methods evaluated for the detection of CP-GNB.

Method	Overall percent agreement, %	PPA, %	NPA, %
CIM	95.7	99.3	86.0
HardyCHROM CRE	73.0	99.3	0.0
bioMérieux chromID CARBA	79.9	99.3	25.0
Xpert Carba-R	95.8[a]/100.0[b]	94.2[a]/100.0[b]	100.0[a]/100.0[b]

[a] Calculation based on all carbapenemase genes, including those not detected by the Xpert Carba-R assay (e.g., bla_{SME}, bla_{IMI}).
[b] Calculations based only on carbapenemase genes detectable by the Xpert Carba-R assay (bla_{KPC}, bla_{NDM}, bla_{IMP}, $bla_{OXA\text{-}48\text{-}like}$, and bla_{VIM}).

189 isolates of *Enterobacteriaceae, P. aeruginosa, A. baumannii* complex

CIM – carbapenem inactivation method – phenotypic screen (over night)

HardyCHROM CRE and chromID CARBA – chromogenic agars

Screen with CIM followed by Xpert Carba-R: accurate method for detecting and characterizing CP-GNB including *Enterobacteriaceae, P. aeruginosa, A. baumannii* complex

McMullen AR et al. Clin Chem 2017;63:723

Summary

- Array of rapid methods available
- Manage incomplete data
  - Clinicians need support and expert advice
  - Role of the Stewardship Team
- Essential to identify tests that provide value
- Need outcomes data to support use of these tests
  - May differ for each institution, patient population