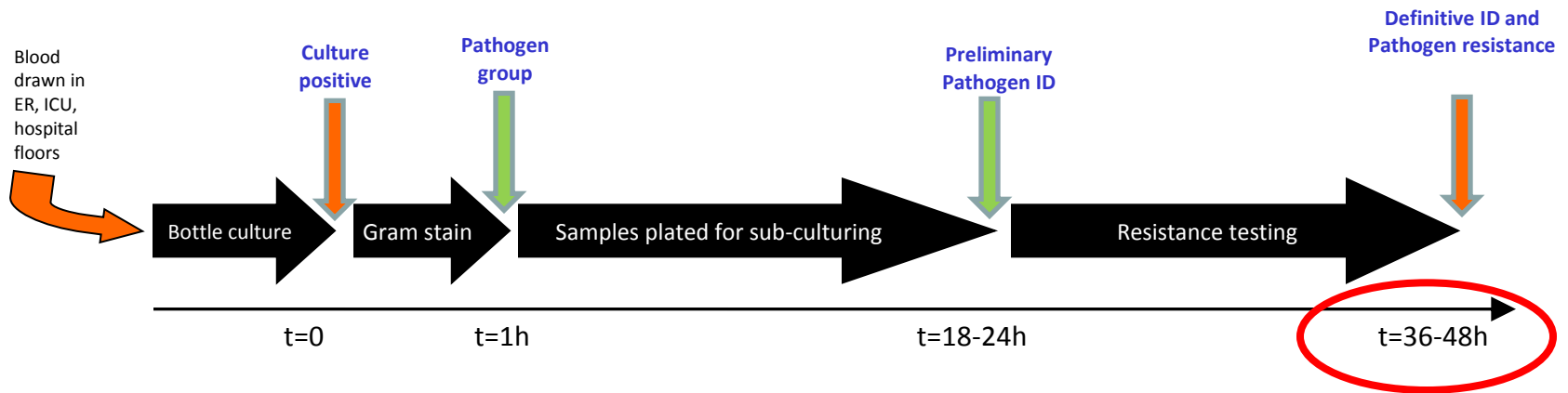


Blood Culture Panels

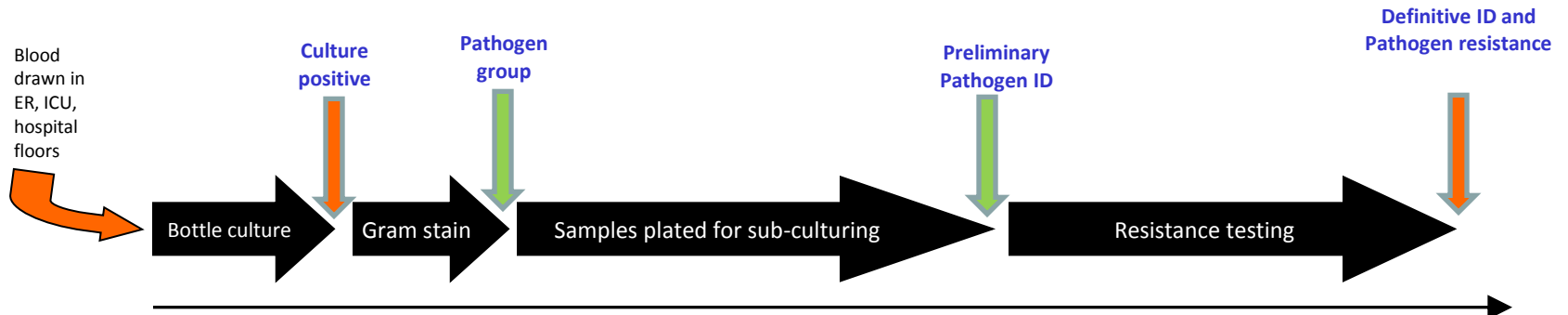
From Bottle to Result

- Blood culture challenges
- Testing options
- Outcomes

Traditional Blood Culture Workflow



Traditional Blood Culture Workflow



t: JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 495-497
 0095-1137/03/\$08.00+0 DOI: 10.1128/JCM.41.1.495-497.2003
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Vol. 41, No. 1

Detection and Treatment of Bloodstream Infection: Laboratory Reporting and Antimicrobial Management

Erik L. Munson,¹ Daniel J. Diekema,^{1,2} Susan E. Beekmann,¹
 Kimberle C. Chapin,³ and Gary V. Doern^{1*}

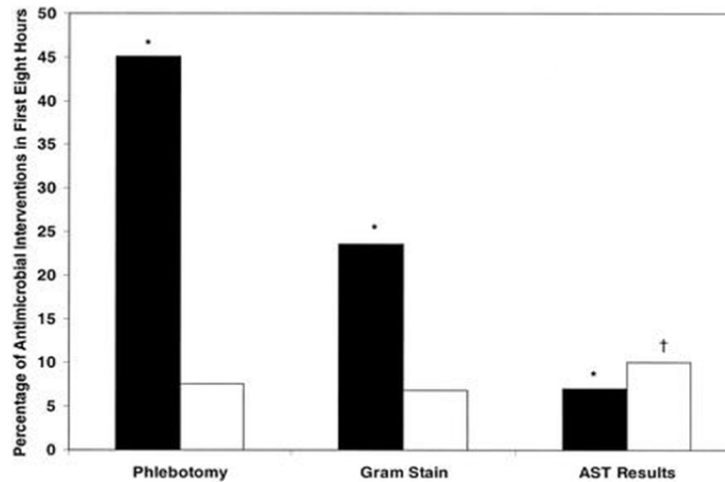


FIG. 1. Percentage of all antimicrobial interventions occurring within the first 8 h (initiations, solid bars; discontinuations, open bars) after each event of interest.

Empirical use of antibiotics and adjustment of empirical antibiotic therapies in a university hospital: a prospective observational studyJulian Mettler¹, Mathew Simcock^{1,2}, Pedram Sendi^{1,2}, Andreas F Widmer¹, Roland Bingisser³, Manuel Battegay¹, Ursula Fluckiger¹ and Stefano Bassetti^{*1,4}**Table 3: Reasons for inadequacy of empirical and adjusted antibiotic therapies**

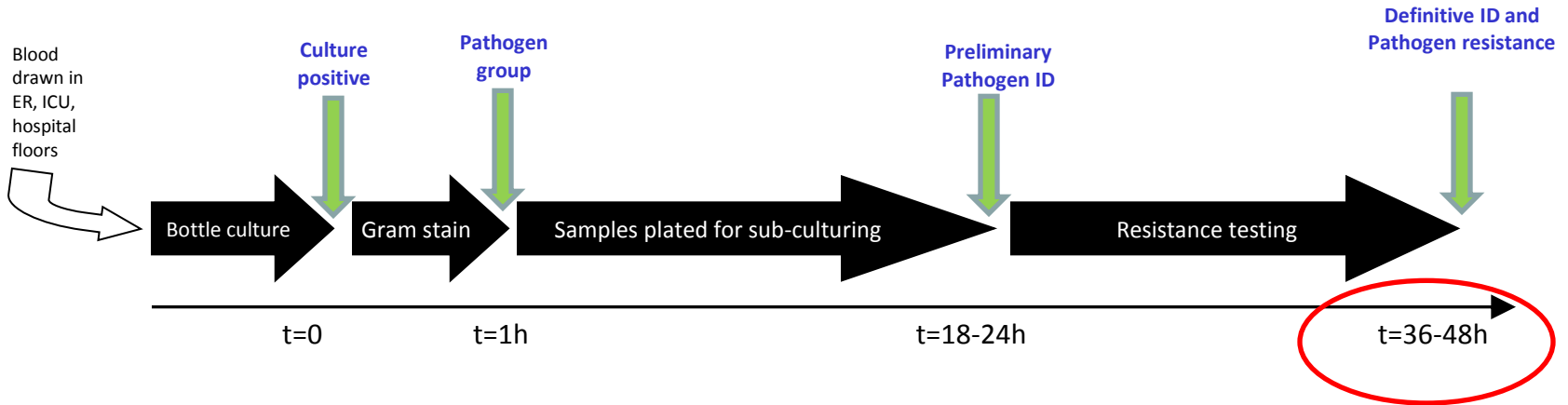
Reason for inadequacy ^a	Number of patients	%
Empirical antibiotic therapies	539	
Inadequate empirical therapy	121	100%
Spectrum too broad	29	24.0%
Inadequate duration	28	23.1%
Insufficient dosage	27	22.3%
Spectrum too narrow	22	18.2%
Wrong spectrum/inadequate use ^b	21	17.4%
Excessive dosage	3	2.5%
Adjusted antibiotic therapies	168	
Inadequate adjusted therapy	46	100%
Spectrum too broad	24	52.2%
Wrong spectrum/inadequate use ^c	12	26.1%
Insufficient dosage	7	15.2%
Inadequate duration	2	4.4%
Excessive dosage	1	2.2%
Spectrum too narrow	1	2.2%

^a More than one reason may apply for each patient.

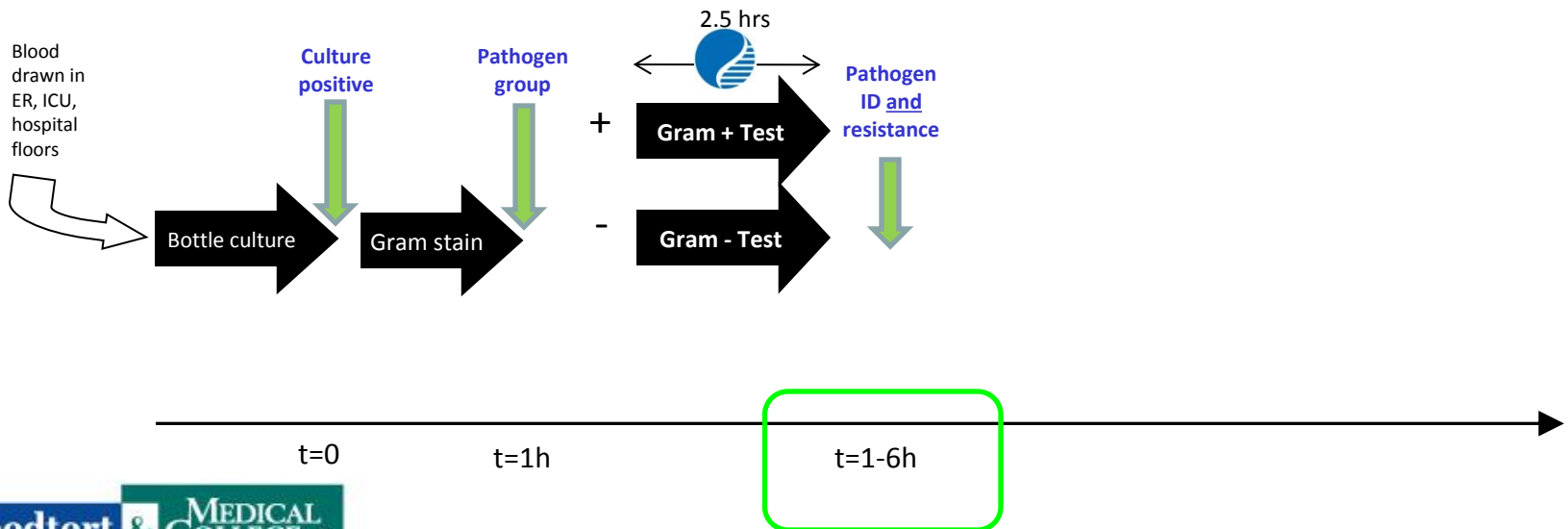
^b If antibiotic given covered completely different spectrum than expected bacteria would require or no antibiotic therapy was warranted.

^c If bacteriological results had shown that identified bacteria were resistant to antibiotics used, or antibiotic covered completely different spectrum than expected bacteria would require or no antibiotic therapy was warranted.

Traditional Blood Culture Workflow



Workflow with Rapid Molecular Based testing



Testing Platforms

- Positive Blood Culture bottles
 - PNA FISH
 - Fluorescently labeled probes attach to rRNA
 - Read with fluorescent microscope
 - Verigene BC-GP and BC-GN
 - Target organisms DNA is captured on a DNA microarray
 - BioFire FilmArray BCID
 - PCR
 - iCUBATE
 - PCR
 - GeneXpert
 - PCR
 - Targets only MRSA/MSSA

Testing Platforms

- Direct Whole Blood
 - Roche Septifast
 - 19 bacteria
 - 6 fungi
 - Magicplex
 - 73 Gram positive
 - 12 Gram negative
 - 3 resistance markers
 - 6 fungi

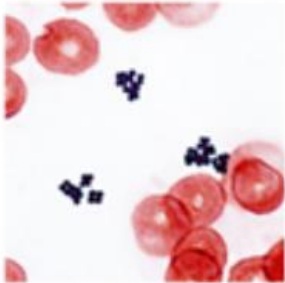
PNA FISH

- Manual “staining” process
- 5 minute hands on time - 20 minute incubation
- > 95% Sensitivity and Specificity
- Separate assays
 - Staphylococcus
 - S. aureus vs. coagulase negative Staph
 - Enterococcus
 - E. faecium vs E. faecalis
 - Gram negative
 - E. coli, K. pneumonia, P. aeruginosa
 - Candida
 - C albicans, C parapsilosis, C. glabrata

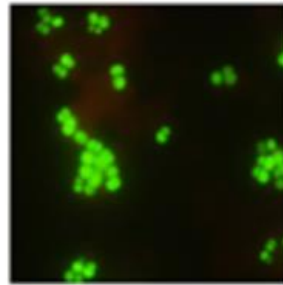
PNA FISH

- Staphylococcus

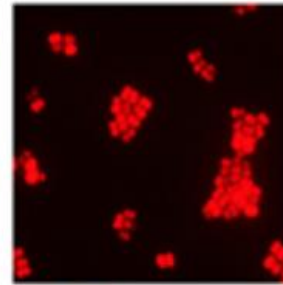
Gram Stain



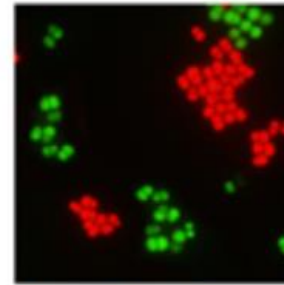
AdvanDx QuickFISH[®] (20 Minute Pathogen ID)



S. aureus



CoNS



Mixed Positive



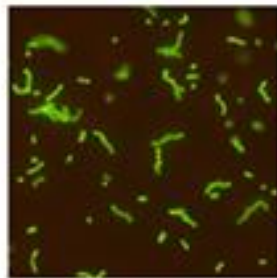
Negative

- Enterococcus

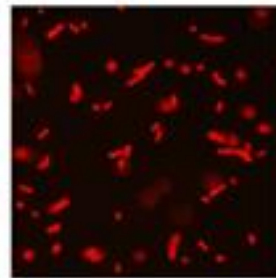
Gram Stain



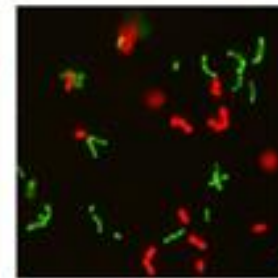
AdvanDx QuickFISH[®] (20 Minute Pathogen ID)



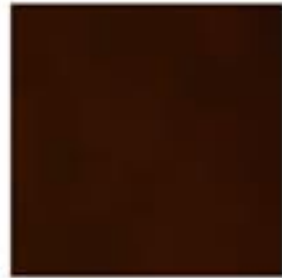
E. faecalis



E. faecium (or OE)



Mixed Positive

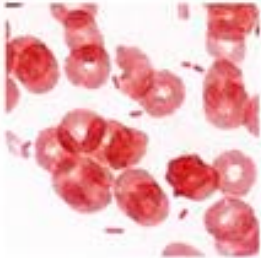


Negative

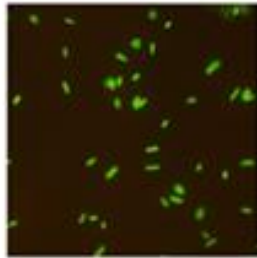
PNA FISH

- Gram negative

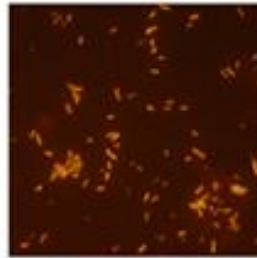
Gram Stain



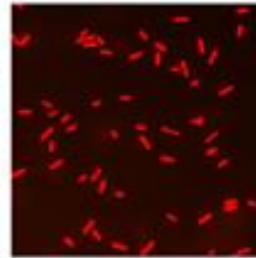
AdvanDx QuickFISH[®] (20 Minute Pathogen ID)



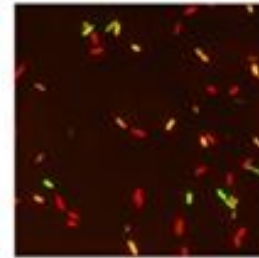
E. coli



K. pneumoniae



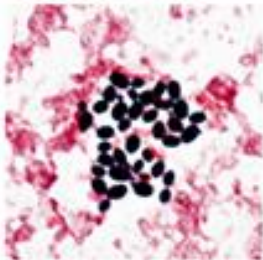
P. aeruginosa



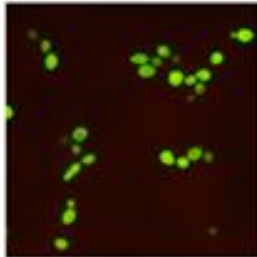
Mixed Positive

- Candida

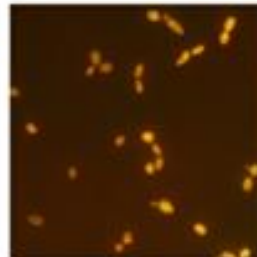
Gram Stain



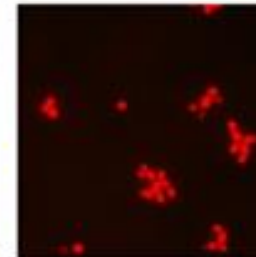
AdvanDx QuickFISH[®] (20 Minute Pathogen ID)



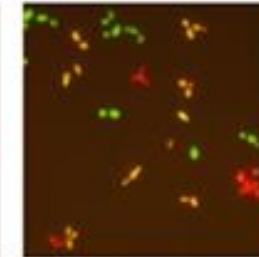
C. albicans



C. parapsilosis



C. glabrata



Mixed Positive

Verigene

- DNA probe microarray
- No amplification
- 2-5 minute hands on time – 2.5 hour incubation
- > 90% Sensitivity and Specificity
 - Decreased sensitivity with mixed cultures
- Separate assays
 - Gram positive
 - Gram negative



Blood Culture

Gram Positive Targets:

Species level identification (9)

- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus lugdunensis*
- *Streptococcus anginosus* group
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*
- *Enterococcus faecalis*
- *Enterococcus faecium*

Genus level identification

- *Staphylococcus* sp.
- *Streptococcus* sp.
- *Listeria* sp.

Resistant Markers

- Methicillin – *mecA*
- Vancomycin – *vanA* and *vanB*

Blood Culture

Gram Negative Targets:

Species level identification (4)

- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Klebsiella oxytoca*
- *Pseudomonas aeruginosa*

Genus level identification (4)

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

Resistant Markers (6)

- CTX-M (bla_{CTX-M})
- KPC (bla_{KPC})
- NDM (bla_{NDM})
- VIM (bla_{VIM})
- IMP (bla_{IMP})
- OXA (bla_{OXA})

VERIGENE BC-GP

PERFORMANCE OVERVIEW

Verigene BC-GP Performance vs. Reference Methods (n=1,426)

		Sensitivity (%)	Specificity (%)
Species	<i>S. aureus</i>	99.1	100
	<i>S. epidermidis</i>	93.1	98.9
	<i>S. lugdunensis</i>	95	100
	<i>S. agalactiae</i>	98.6	100
	Strep anginosus Group	100	99.8
	<i>S. pneumoniae</i>	100	99.6
	<i>S. pyogenes</i>	95.8	100
	<i>E. faecalis</i>	96.9	99.9
	<i>E. faecium</i>	97.1	100
Genus	Staphylococcus spp.	98	99.4
	Streptococcus spp.	93.6	99.6
	Listeria spp.	100	100
Resistance	mecA— <i>S. aureus</i>	97.5	98.8
	mecA— <i>S. epidermidis</i>	92	81.5
	vanA	94.2	99.8
	vanB	100	100

VERIGENE BC-GN

PERFORMANCE OVERVIEW

Verigene BC-GN Performance vs. Reference Methods (n=1,412)			
		Sensitivity (%)	Specificity (%)
Species	<i>Escherichia coli</i>	99.8	99.4
	<i>Klebsiella pneumoniae</i>	93.1	100
	<i>Klebsiella oxytoca</i>	92.2	99.6
	<i>Pseudomonas aeruginosa</i>	97.6	100
Genus	Acinetobacter spp.	98.2	99.9
	Citrobacter spp.	100	99.9
	Enterobacter spp.	97.6	99.4
	Proteus spp.	100	99.9
Resistance	CTX-M (bla _{CTX-M})	98.7	99.9
	KPC (bla _{KPC})	100	100
	NDM (bla _{NDM})	100	100
	VIM (bla _{VIM})	100	100
	IMP (bla _{IMP})	100	100
	OXA (bla _{OXA})	95.3	99.9

Please see Verigene BC-GN Package Insert for details on results.

BioFire FilmArray BCID

- Nested PCR
 - Contamination may be a concern
- 2 minute hands on time – 1 hour incubation
- > 90% Sensitivity and Specificity
 - Decreased with mixed cultures
- Combined assay for 27 targets



BioFire

Gram positive

- *Enterococcus*
- *Listeria monocytogenes*
- *Staphylococcus sp.*
- *Staphylococcus aureus*
- *Streptococcus sp.*
- *Streptococcus agalactiae*
- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*

Gram negative

- *Acinetobacter baumannii*/*Haemophilus influenza*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Enterobacteriaceae*
- *Enterobacter cloacae complex*
- *Escherichia coli*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Proteus sp.*
- *Serratia marcescens*

BioFire

Yeast

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

Resistance markers

- *mecA*
- *vanA/vanB*
- KPC

BioFire

Clinical sensitivity and specificity of the BIOFIRE® FILMARRAY® Blood Culture Identification Panel

Analyte	Prospective and Seeded Blood Cultures ^a	
	Sensitivity/PPA ^b	Specificity/NPA ^c
Gram-Positive		
<i>Enterococcus</i> ^d	97.7%	99.8%
<i>Listeria monocytogenes</i>	100%	100%
<i>Staphylococcus</i> ^d	98.5%	99.1%
<i>Staphylococcus aureus</i>	98.4%	99.8%
<i>Streptococcus</i> ^d	97.5%	99.8%
<i>Streptococcus agalactiae</i> (Group B)	100%	100%
<i>Streptococcus pneumoniae</i>	97.3%	99.9%
<i>Streptococcus pyogenes</i> (Group A)	100% ^d	99.9%

BioFire

Clinical sensitivity and specificity of the BIOFIRE® FILMARRAY® Blood Culture Identification Panel

Gram-Negative		
<i>Acinetobacter baumannii</i>	100%	99.8%
<i>Enterobacteriaceae</i> ^d	98.4%	99.8%
<i>Enterobacter cloacae</i> complex	97.4%	99.9%
<i>Escherichia coli</i>	98.0%	99.8%
<i>Klebsiella oxytoca</i>	92.2%	99.9%
<i>Klebsiella pneumoniae</i>	97.1%	99.6%
<i>Proteus</i> ^d	100%	100%
<i>Serratia marcescens</i>	98.7%	99.9%
<i>Haemophilus influenzae</i>	100%	100%
<i>Neisseria meningitidis</i>	100%	100%
<i>Pseudomonas aeruginosa</i>	98.1%	99.9%

BioFire

Clinical sensitivity and specificity of the BIOFIRE® FILMARRAY® Blood Culture Identification Panel

Yeast		
<i>Candida albicans</i>	100%	99.8%
<i>Candida glabrata</i>	100%	99.9%
<i>Candida krusei</i>	100%	100%
<i>Candida parapsilosis</i>	96.7%	99.9%
<i>Candida tropicalis</i>	100%	100%

iCubate

- Amplicon rescue PCR
 - Contamination a concern
- 3 minute hands on time – 4-5 hour incubation
- > 95% Sensitivity and Specificity



iCubate

Bacteria

- *Staphylococcus aureus*
 - *Staphylococcus epidermidis*
 - *Streptococcus pneumonia*
 - *Enterococcus faecalis*
 - *Enterococcus faecium*
-
- Gram negative panel currently under FDA review

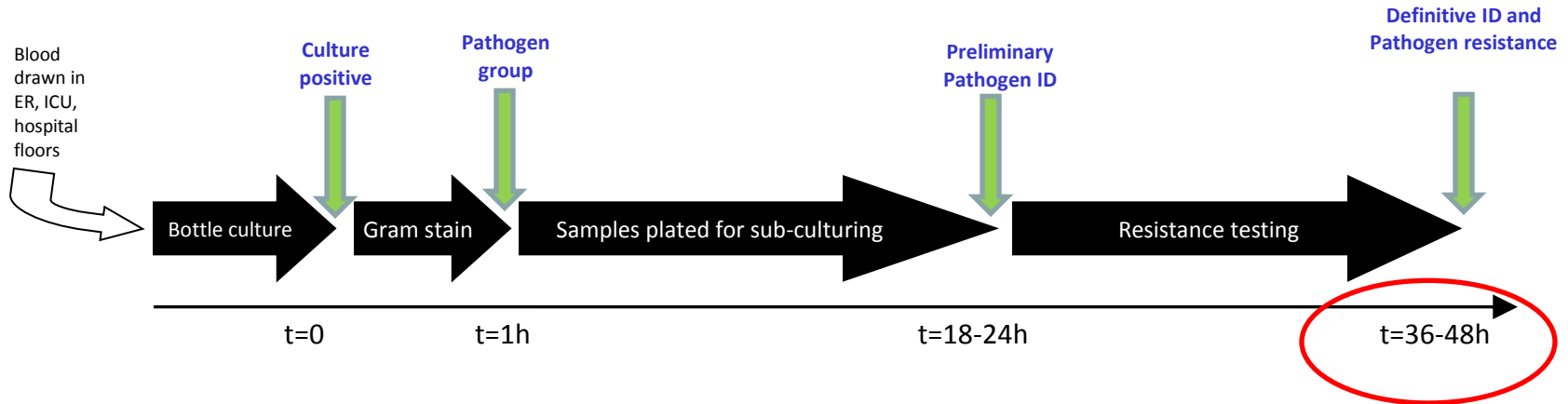
Resistance markers

- *mecA*
- *vanA/vanB*

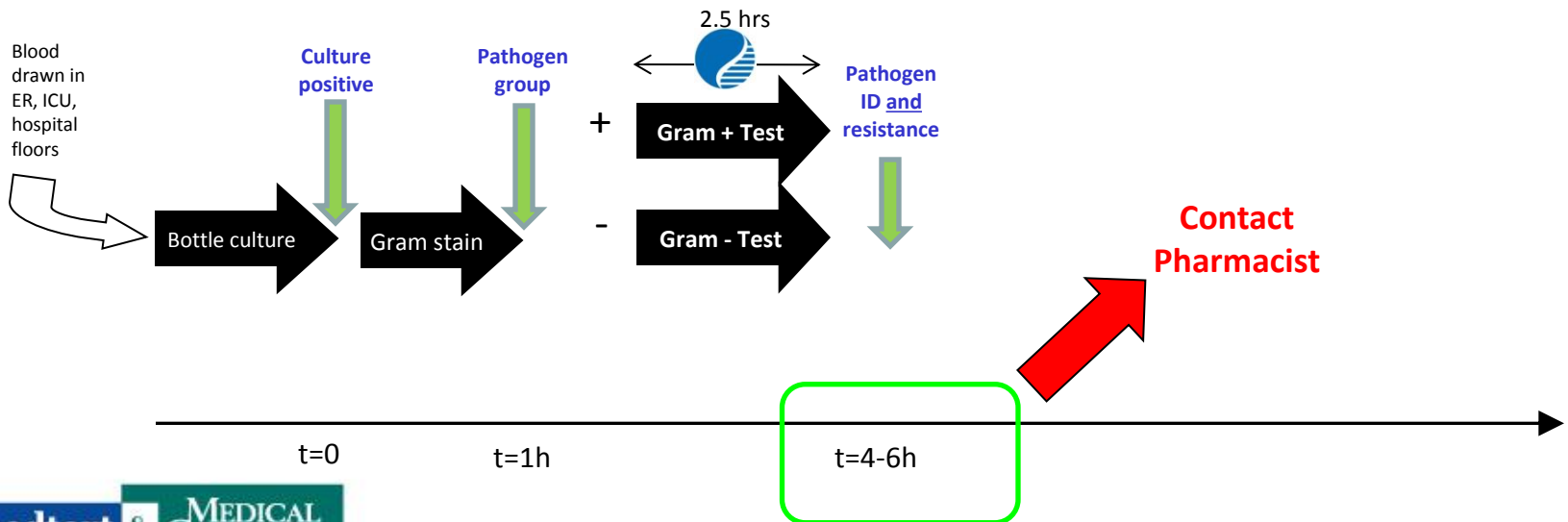
The Verigene[®] System from Nanosphere – Our experience

- Utilized at Academic Medical Center since Oct 2012
- Implemented in Community Hospitals in Jan 2016

Traditional Blood Culture Workflow



Workflow with Rapid Molecular Based testing



Performance

Verigene/Conventional ID correlation

<i>E. coli</i>	14
<i>Staph epidermidis</i>	14
MRSA	2
<i>Staph aureus</i> (MSSA)	7
Group B Streptococcus	3
<i>Streptococcus pneumonia</i>	7
<i>Pseudomonas aeruginosa</i>	1
<i>Proteus mirabilis</i>	3
<i>Klebsiella pneumonia</i>	3
<i>Staph lugdunensis</i>	1
<i>Klebsiella oxytoca</i>	1
<i>Enterobacter cloacae</i>	1
<i>Enterococcus faecalis</i>	1
<i>Enterococcus faecium</i>	1

- 2 CTX-M (ESBL)
- 1 van B (VRE)

Performance

Gram negative bacilli - no Verigene ID

<i>Elizabethkingia</i>	4
<i>Serratia marcescens</i>	1
<i>Argobacterium</i>	1

Gram positive cocci - no Verigene ID

Micrococcus	12
Anaerobic GPC	1
<i>Staph epidermidis</i>	1
<i>Streptococcus</i> sp	2

Performance

Gram negative bacilli - no Verigene ID	
<i>Elizabethkingia</i>	4
<i>Serratia marcescens</i>	1
<i>Argobacterium</i>	1

Gram positive cocci - no Verigene ID	
Micrococcus	12
Anaerobic GPC	1
<i>Staph epidermidis</i>	1
<i>Streptococcus</i> sp	2

Streptococcus sp.	
viridian group Streptococcus	1

Staphylococcus sp.	
<i>S. hominis</i>	5
<i>S. capitis</i>	3
<i>S. haemolyticus</i>	1
coagulase-negative Staphylococcus	4

CLINICAL VALUE OF RAPID BLOOD CULTURE TESTING

- Based on several studies using other available test methods, rapid blood culture testing has been shown to:
 - **Reduce mortality:** Each hour that appropriate antimicrobial treatment is delayed, a sepsis patient's mortality rate increases by 7.6%¹
 - **Reduce hospitalization costs:**
 - Implementing rapid results reporting for *S. aureus* blood cultures can lead to an average 6.2-day reduction in length of stay and a \$21,387 reduction in costs per *S. aureus*-infected patient²
 - Implementing rapid results reporting for gram-negative blood cultures can lead to an average 2-day reduction in length of stay and a \$19,500 reduction in costs per gram-negative infected patient³
 - **Improve antimicrobial stewardship:** Rapid *mecA* reporting for patients with *S. aureus* bacteremia results in a 25.4-hour reduction in the time to optimal antimicrobial therapy⁴
 - **Reduce the impact of contaminants:** patients with false-positive blood culture results triggered by contaminants such as *S. epidermidis* have hospitalization costs \$8,750 higher than true negative blood culture patients⁵

¹Kumar et al. 2006. *Crit Care Med*, 34:1589-96.

²Bauer et al. 2010. *Clin Infect Dis*, 51:1074-80

³Perez et al . 2013. *Arch Pathol Lab Med*, 137: 1247-54.

⁴Carver et al. 2008. *J Clin Microbiol*, 46:2381-83.

⁵Zwang and Albert 2006. *J Hosp Med*, 1:272-76.

Blood Culture Outcomes

Measure: Time to Targeted Therapy (hours)	Pre-Intervention	Intervention	p-value
Overall	61.1 (n=87)	35.4 (n=46)	< 0.01
MSSA	63.4 (n=46)	36.7 (n=14)	< 0.01
MRSA	43.77 (n=4)	43.5 (n=4)	0.91
<i>E. faecalis</i>	68.54 (n=18)	20.13 (n=5)	<0.01
<i>Streptococcus</i> spp.	52.4 (n=19)	36.4 (n=23)	0.01

Blood Culture Outcomes

Measure	Pre-Intervention	Intervention	p-value
Length of stay, median (days)	9.1	7.2	0.04
Mortality (%)	9.1	9.2	0.98
Pharmacy costs, median (\$)	822	425	0.11
Total direct variable costs, median (\$)	17,530	10,290	0.04

Blood Culture Outcomes

Reference	Decrease in time to optimal therapy	Decrease in patients length of stay
Beal, S. et. al. ^a	42 hr	
Ward, C. et al ^e	29 hr	
Beal, S. et. ^b	19 hr	
Nguyen, D. et.al ^d	3 days	3 days
Ledeboer ^c	20 hr	1.7 days
Jacobson ^f	16hr	1.1 days (ICU 1.6 days)

A. J. Clin Microbio 2013 51: 3988-3992.

B. Proc (Bayl Univ Med Cent). 2015 28(2): 139-143.

C. Ledebor, N., and Revolinski, S. Unpublished Data

D. J. Clin. Microbiol. 2010 48: 785-790.

E. Eur J Clin Mrocbiol Infect Dis. 2015 34: 487-496

F. Jacobson, E., et.al. Unpublished Data

Implementation

- Consideration points
 - Cost
 - Pharmacy coverage
 - Antimicrobial Stewardship Program
 - Technical expertise
 - Gram stains
 - Staffing
 - Can test be run on all shifts
 - Space
 - Common blood culture isolates

