

Microbiology Laboratory: Primer for NICU

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Disclosures

- This is not an all-inclusive look at the laboratory, but rather to help you understand how laboratory processes may affect antibiotic selection and use
- To really understand your laboratory, ask your microbiology team questions to clarify how their workflow is setup

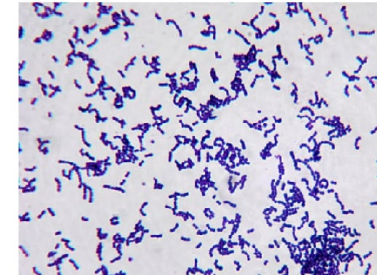
Blood cultures collected, now what?



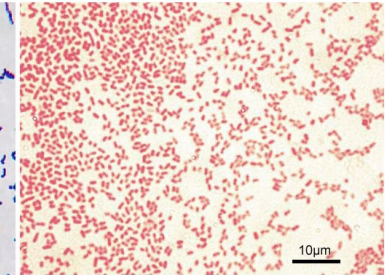
Placed in incubator



Gram Stain, streaked on agar plates

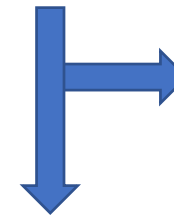


Gram Positive Bacteria

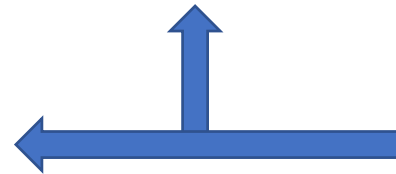


Gram Negative Bacteria

MALDI-TOF Rapid Identification



Sensitivity Panel +/- Identification



Rapid PCR testing panel for identification +/- resistance

Rapid PCR Identification System

Verigene Platform

Gram-Positive Blood Culture Test (BC-GP)

Species	Genus
<i>Staphylococcus aureus</i>	<i>Staphylococcus</i> spp.
<i>Staphylococcus epidermidis</i>	<i>Streptococcus</i> spp.
<i>Staphylococcus lugdunensis</i>	<i>Micrococcus</i> spp. ⁺
<i>Streptococcus agalactiae</i>	<i>Listeria</i> spp.
<i>Streptococcus pneumoniae</i>	Resistance
<i>Streptococcus pyogenes</i>	
<i>Enterococcus faecalis</i>	
<i>Enterococcus faecium</i>	
Group	
<i>Streptococcus anginosus</i>	

Gram-Negative Blood Culture Test (BC-GN)

Species	Resistance
<i>Escherichia coli</i> *	CTX-M (ESBL)
<i>Klebsiella pneumoniae</i>	IMP (carbapenemase)
<i>Klebsiella oxytoca</i>	KPC (carbapenemase)
<i>Pseudomonas aeruginosa</i>	NDM (carbapenemase)
<i>Serratia marcescens</i> **	OXA (carbapenemase)
	VIM (carbapenemase)
Genus	
<i>Acinetobacter</i> spp.	
<i>Citrobacter</i> spp.	
<i>Enterobacter</i> spp.	
<i>Proteus</i> spp.	

Biofire Film Array

Gram Positive Bacteria

<i>Enterococcus faecalis</i>	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.
<i>Enterococcus faecium</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus agalactiae</i> (Group B)
<i>Listeria monocytogenes</i>	<i>Staphylococcus epidermidis</i>	<i>Streptococcus pneumoniae</i>
	<i>Staphylococcus lugdunensis</i>	<i>Streptococcus pyogenes</i> (Group A)

Gram Negative Bacteria

<i>Acinetobacter calcoaceticus-baumannii</i> complex	<i>Enterobacteriales</i>
<i>Bacteroides fragilis</i>	<i>Enterobacter cloacae</i> complex
<i>Haemophilus influenzae</i>	<i>Escherichia coli</i>
<i>Neisseria meningitidis</i> (encapsulated)	<i>Klebsiella aerogenes</i>
<i>Pseudomonas aeruginosa</i>	<i>Klebsiella oxytoca</i>
<i>Stenotrophomonas maltophilia</i>	<i>Klebsiella pneumoniae</i> group
	<i>Proteus</i> spp.
	<i>Salmonella</i> spp.
	<i>Serratia marcescens</i>

Yeast

<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Cryptococcus neoformans/gattii</i>
<i>Candida auris</i>	<i>Candida parapsilosis</i>	
<i>Candida glabrata</i>	<i>Candida tropicalis</i>	

Rapid PCR Identification System

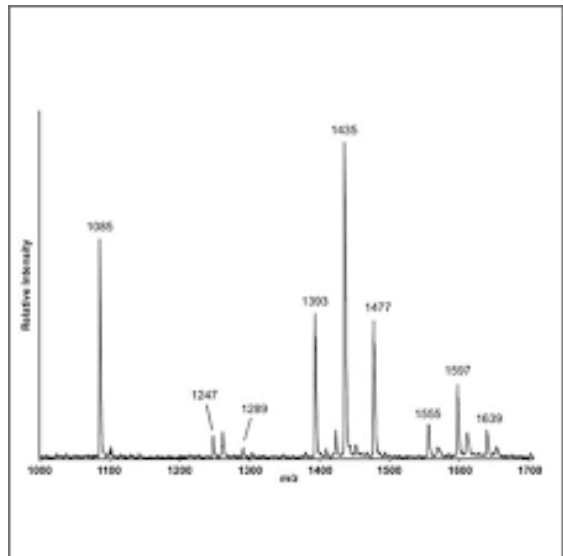
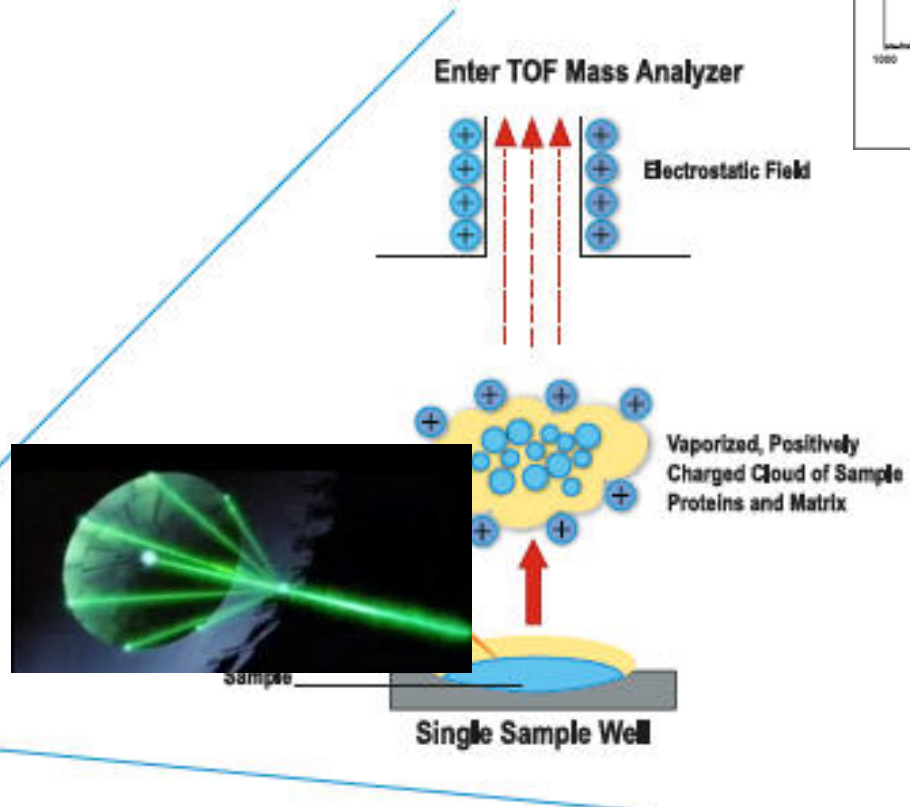
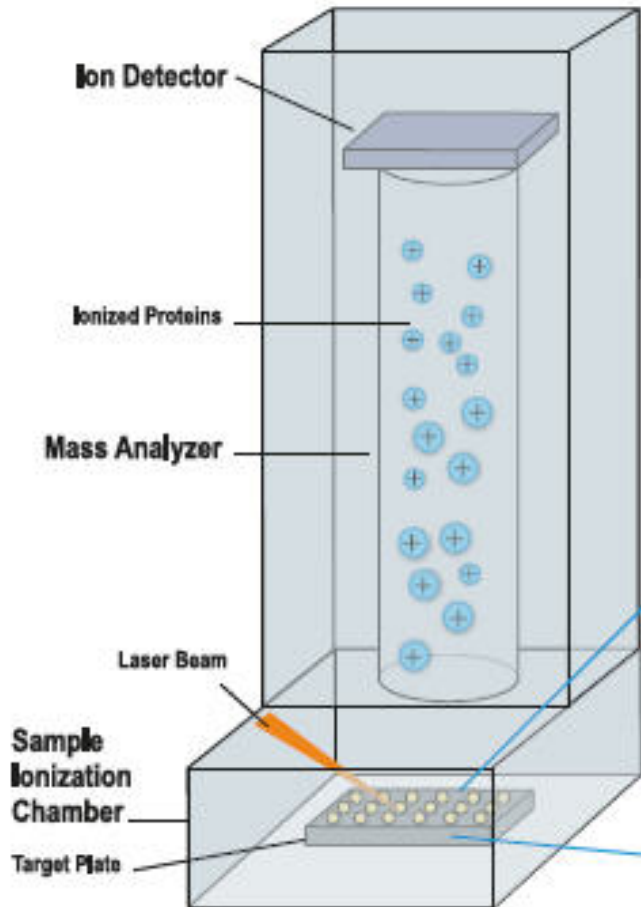
Verigene Platform

Resistance	Resistance
<i>mecA</i> (methicillin)*	CTX-M (ESBL)
<i>vanA</i> (vancomycin)**	IMP (carbapenemase)
<i>vanB</i> (vancomycin)**	KPC (carbapenemase)
	NDM (carbapenemase)
	OXA (carbapenemase)
	VIM (carbapenemase)

Biofire Film Array

Antimicrobial Resistance Genes				
CTX-M	KPC	<i>mecA/C</i>	NDM	<i>vanA/B</i>
IMP	<i>mcr-1</i>	<i>mecA/C</i> and MREJ (MRSA)	OXA-48-like	VIM

MALDI-TOF



Automated Systems Identification/Sensitivity

- Use biochemical reactions to generate colorimetric changes
- The machine then reads the pattern of the biochemical reactions and matches to an organism based on a data base
- Run time is ~16-24hrs
- For sensitivity testing it looks at turbidity of the various antibiotics 16-24 hrs
- This is then given an interpretation based on standards which is then assigned an MIC
- Often multiple panels which is decided on by your microbiology generally in consultation with ID

Standard Antibiotic Susceptibility Test Reporting

- the rules for which govern the analysis of various organisms and the drug susceptibility testing pathway
 - Your lab will have a standard process for determining which antibiotics will be reported
 - Some of the information will be derived directly from the Clinical and Laboratory Standards Institute
 - <http://em100.edaptivedocs.net/Login.aspx> - can be accessed for free here
 - Some of it will be based on local drug availability
 - Some of it will be based on expert guidance (which drugs to test on multidrug resistant organisms)

Enterobacteriaceae

Antibiotics

Non-CSF

CSF

Amikacin

Ampicillin

Ampicillin/Sulbactam

Pip/Tazobactam

Cefazolin

Cefoxitin

Ceftazidime

Ceftriaxone

Gentamicin

Tobramycin

Levofloxacin

Ciprofloxacin

Trimethoprim/Sulfa

Nitrofuratoin

**Report Any Antibiotic that tests I
or R**

***If Gentamicin &/or Tobramycin =
I, R:***

Amikacin

X

X

X^{1A, 1B}

X

X

X

X

X

X

X (urine only)²

X

X

X

X

X

X

X

X

X - on request

X⁷ - on request

X (if OK per CLSI)

X

Enterobacteriaceae			
If Cefazolin = R			
Cefepime (EUCAST/CLSI hybrid interpretation)	X (ONLY routinely reported for non-KEP bugs) ^{3A,3B}		X (ONLY routinely reported for non-KEP bugs) ^{3A,3B}
If Ceftriaxone = I, R:			
Pip/Tazobactam	X ⁴		Not reported
Meropenem	X ⁸		X ⁸
If ESBL Pos(+)			
Report detection of ESBL	X ⁵		X ⁵
Cephalosporin = Report as tested (CLSI rec)	X		X
Fosomycin (urine-E. coli only)	X ⁶		
ESBL from blood cultures - test Ertapenem	X		
If Meropenem R⁸			
Colistin	X		X
Ceftazidime-Avibactam	X		X
Meropenem-Vaborbactam	X (upon request only - until future)		X (upon request only - ur
If CRE			
Carba-R PCR (KPC, NDM, OXA, VIM, IMP)	X		X

<i>Streptococcus</i> , Beta-hemolytic		
Antibiotics	Sterile Sites	Non-sterile sites, except GrpB screens
Ampicillin	X	Do not test, report with footnote ²
Ceftraxione		
Clindamycin*	X	
Erythromycin*	X	
Levofloxacin*		
Penicillin	X	Do not test, report with footnote ²
Tetracycline*		
Vancomycin	X	
Inducible Clindamycin Resistance	X ¹	
<i>If PCN R</i>		
Tetracycline*		
Vancomycin		X
Clindamycin (non-urine?)		X
Inducible Clindamycin R		X
*Not reported for CSF sources		

Cascade Reporting

- A strategy of differentially reporting antimicrobial susceptibility patterns
- Selective reporting of narrower spectrum drugs, lower toxicity drugs, or less expensive drugs
- Generally, at least two classes of drugs are reported

CONTENTS OF THE CARD

Antimicrobial	Code	Concentration §	Calling Range		FDA Indications for Use
			≤	≥	
Amikacin	AN	8, 16, 64	2	64	CSAGNB**
Amoxicillin/Clavulanic Acid	AMC	4/2, 16/8, 32/16	2/1	32/16	CSAGNB**
Ampicillin	AM	4, 8, 32	2	32	CSAGNB**
Cefazolin	CZ	4, 16, 64	4	64	CSAGNB**
Cefoxitin	FOX	8, 16, 32	4	64	CSAGNB**
Ceftazidime	CAZ	0.25, 1, 2, 8, 32	0.12	64	N/A**
Ceftriaxone	CRO	0.12, 0.25, 1, 4, 16	0.25	64	N/A**
Chloramphenicol	C	4, 16, 32	2	64	N/A**
Ciprofloxacin	CIP	0.5, 2, 4	0.25	4	CSAGNB**
Colistin	CS	4, 16, 32	0.5	16	N/A**
Ertapenem	ETP	0.03, 0.12, 0.5, 2	0.12	8	N/A**
Fosfomycin	FOS	8, 16, 32	16	256	N/A**
Gentamicin	GM	4, 16, 32	1	16	CSAGNB**
Meropenem	MEM	0.5, 2, 6, 12	0.25	16	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>Acinetobacter</i> spp., <i>C. freundii</i> , <i>E. cloacae</i> , <i>K. oxytoca</i> , <i>M. morgani</i> , <i>P. vulgaris</i> , <i>S. marcescens</i> , <i>A. hydrophila</i> , <i>C. diversus</i> , <i>H. alvei</i> , <i>P. multocida</i> , <i>Salmonella</i> spp., <i>Shigella</i> spp.
Nitrofurantoin	FT	16, 32, 64	16	512	CSAGNB**
Piperacillin/Tazobactam	TZP	2/4, 8/4, 24/4, 32/4, 32/8, 48/8	4/4	128/4	<i>A. baumannii</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>C. koseri</i> , <i>M. morgani</i> , <i>P. mirabilis</i> , <i>P. vulgaris</i> , <i>Pv. rettgeri</i> , <i>Pv. stuartii</i> , <i>S. enterica</i>
Trimethoprim/Sulfamethoxazole	SXT	1/19, 4/76, 16/304	20 (1/19)	320 (16/304)	<i>Klebsiella</i> spp., <i>Enterobacter</i> spp., <i>M. morgani</i> , <i>P. vulgaris</i> , <i>P. mirabilis</i> , <i>S. sonnei</i> , <i>S. flexneri</i> , Eco(+ETEC)**, <i>C. sakazakii</i>

Antibiogram

- MALDI-TOF, Rapid diagnostic PCR testing

Gram-Negative Organisms (Non-Urine Isolates)	No. of isolates tested	Beta-lactams						Fluoroquinolones		Aminoglycosides		Other
		Ampicillin	Ceftriaxone	Ceftazidime	Cefepime	Pip/tazo	Meropenem	Ciprofloxacin	Levofloxacin	Gentamicin	Amikacin	TMP-SMX
<i>Acinetobacter baumannii</i> complex	25**	-	12	68	68	72	76	76	76	80	-	72
<i>Citrobacter koseri</i>	32	-	97	97	NR	99	100	100	100	100	100	97
<i>E. coli</i>	414	39	81	90	NR	91	100	74	74	89	99	66
<i>Enterobacter cloacae</i> complex	119	-	85	89	100	86	99	99	99	100	100	93
<i>Klebsiella (Enterobacter) aerogenes</i>	94	-	63	64	100	64	99	97	96	100	100	100
<i>Klebsiella oxytoca</i>	36	-	89	94	NR	97	100	97	100	92	100	86
<i>Klebsiella pneumoniae</i>	206	-	80	81	NR	88	96	83	84	88	98	81
<i>Morganella morganii</i>	46	-	83	83	NR	93	98	83	85	76	100	65
<i>Proteus mirabilis</i>	160	78	94	100	NR	98	100	86	86	91	100	76
<i>Pseudomonas aeruginosa</i>	289	-	-	88	87	85	88	91	79	93	100	-
<i>Serratia marcescens</i>	78	-	95	99	NR	-	99	97	97	100	99	100

Questions?

- Comments?