Methodology Working Group

June 16, 2015

Methodology ad hoc groups

- Direct AST
  Romney Humphries
- Anaerobe
  Darcie Roe-Carpenter
- Broth Microdilution
  Bill Brasso
- Polymyxins
  John Turnidge
- Tables 1 & 2 clean-up
  Mary York
- Molecular Results Reporting
  Cathy Petti & Thomas Kirn
- Alternate Disk Potency
  Laura Koeth
- Intrinsic Resistance
  Barb Zimmer
- Atypical Staph
  Romney Humphries
- Surrogate Testing
  Jim Jorgensen

Methodology WG Agenda
June 16, 2015

1. BMD ad hoc WG – Bill Brasso
2. Report from the Disk Mass ad hoc WG
3. Report from Tables 1 & 2 cleanup ad hoc WG
4. Report from Anaerobe ad hoc WG
5. Report from the Molecular Results Reporting ad hoc WG
6. Update on ISO documents for microbiology
7. Report from the Intrinsic Resistance WG
8. Testing for Oxacillin Resistance in *S. pseudointermedius*
9. Atypical Staph aureus ad hoc WG
10. Direct AST ad hoc WG
11. Surrogate Testing ad hoc WG – informational update

BMD ad hoc WG Update

– Bill Brasso

Report from the Disk Mass ad hoc WG

– Laura Koeth

Working Group Participants

- Chair: Laura M. Koeth, LSI
- Sousan Altaie, FDA
- Patricia Bradford, AstraZeneca
- Maria Karlsson, CDC
- Erika Matuschek, EUCAST
- Gregory Stone, AstraZeneca
- Sue Thomson, Mast

Working Group Objectives

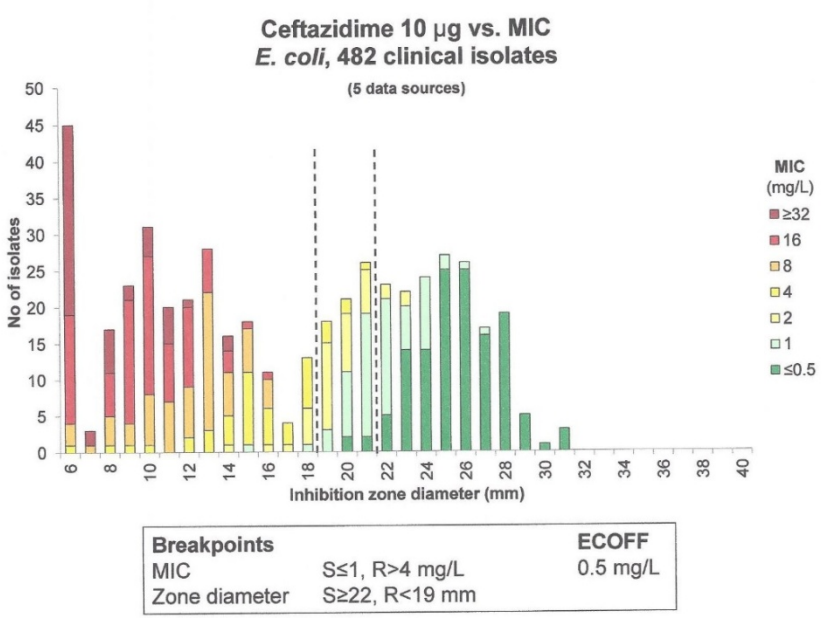
- Review list of agents that vary in disk mass between CLSI and EUCAST and set a priority by drug for evaluation by the working group
- Collect any available MIC/disk data for the two disks for each agent and compare agreement rates for relevant pathogens
- If it is determined that smaller mass disk results provide higher category agreement rates and reduced errors compared to broth microdilution, the disk mass working group will consider steps necessary to change disk mass and anticipate potential issues.

Disks to consider:

Agent	Disk Mass (mcg)		Relevant Bacteria
	CLSI	EUCAST	
Cefotaxime	30	5	Enterobacteriaceae, Beta strep, Haemophilus
Ceftaroline	30	5	Enterobactericeae, Staph, Beta strep & S. pneumoniae
Ceftazidime	30	10	Enterobacteriaceae, P. aeruginosa
Linezolid	30	10	Staph, Beta strep & S. pneumoniae, Enterococcus
Netilmicin	30	10	Enterobacteriaceae, P. aeruginosa, Staph
Nitrofurantoin	300	100	Enterobacteriaceae, Staph, Enterococcus
Penicillin	10	1	Staph, Enterococcus
Piperacillin	100	30	Enterobactericeae, P. aeruginosa,
Piperacilllin/ Tazobactam	100/10	30/6	Enterobactericeae, P. aeruginosa, Haemophilus
Vancomycin	5	30	Beta strep & S. pneumoniae, Enterococcus

Sources of Disk MIC Data

- CLSI (old agenda materials) – CLSI has archives and can assist in locating the initial disk breakpoint disk-MIC scatterplots
  - EUCAST – Histograms by zone & MIC by organisms species or group (http://www.eucast.org/ast_of_bacteria/calibration_and_validation/)
  - Literature
  - Pharma
  - Surveillance Studies
  - Other



Consideration regarding change in disk mass

- Would a M23 based study be required or would EUCAST data and possibly additional data be acceptable?
- If M23 studies are required, how will studies be funded?
- What is impact on USA drug labels and sponsors and on disk manufacturers?
- Other?

Report from Tables 1 & 2 clean-up ad hoc WG

– Mary York

Anaerobe Working Group

- Darcie Roe-Carpenter
- Audrey Schuetz
- Joanne-Dzink-Fox
- Nilda Jacobus
- Hanna Wexler
- Diane Citron
- Steve Jenkins
- Laura Koeth
- Karen (Kitty) Anderson
- Cindy Knapp
- Meredith Hackel
- Maria Karlsson

Meeting Summary

- Epidemiologic cutoff values (ECV) for vancomycin and *C. difficile*
  - Old versus New Data
  - Data by Ribotype (new vs wildtype)
  - Bring back in January
- Collecting data for ECV for other gram-positive species
- Draft Antibiogram Manuscript Review
  - Christine Hastey, Ph.D.
- Agar vs Broth data update
- M11-A8 document revisions in progress – wait to publish until agar/broth issue resolved
- *E. lenta*
  - Wording proposed for M100 QC tables
  - Wording proposed for M100 Appendix C QC strains

Text and Tables Revisions

M100

Table 5D MIC Quality Control Ranges for Anaerobes (Agar Dilution Method) pg 168, and

5E pg 170 Table 5D MIC Quality Control Ranges for Anaerobes (Broth Microdilution Method)

Footnote to the organism

MIC variability with some agents has been reported with *Eggerthella lenta* (*E. lentum*) ATCC 43055; therefore, QC ranges may not have been established for all antimicrobial agents with this organism.

M100 Appendix C, Quality Control Strains for Antimicrobial Susceptibility Tests pg 204

Added to "Other column" for *E. lenta*

MIC variability with some agents has been reported with *Eggerthella lenta* (*E. lentum*) ATCC 43055. Therefore, QC ranges may not have been established for all antimicrobial agents with this organism and is not required to include in M23 QC Tier 2 studies if MIC result variability is documented in early drug development studies (ie M23 QC Tier1).

Votes required?

Molecular Detection of Antimicrobial Resistance Ad Hoc Working Group

June 2015

Molecular Detection of Antimicrobial Resistance Ad Hoc Working Group Group Members

- Karen Carroll
- Paul Edelstein
- Ferric Fang
- Thomas Kirn co-chair
- Cathy Petti co-chair
- Ribhi Shawar
- Yi-Wei Tang
- Simon Walker
- Neil Woodford

Purpose

- Provide guidance to laboratories that employ molecular methods to predict antibiotic resistance phenotypes for clinical applications

Discussion

- Scope
  - Define molecular
    - Proteins, Nucleic Acids, other non-phenotypic methods
  - FDA cleared assays, LDTs (NGS, etc)
  - Methods vs practical guidance to guide discrepant resolution
- Delivery
  - Separate document, M100, etc
  - Text vs Tables

Discussion

- Scope
  - Define molecular
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  - Separate document, M100, etc
  - Text vs Tables

S. aureus

Indication	Target	Method	Specimen Type	Discordant Result: Genotype or Predicted Phenotype	Discordant Result: Observed Phenotype	Suggestions for Resolution and Possible Reasons for Observed Discrepancy*	Report as:	Footnotes
Detection of methicillin resistance in *S. aureus*	PBP2a	Latex agglutination	Colony	PBP2a positive	cefoxitin S	Confirm isolate identification, repeat latex agglutination and AST and consider *mecA* colony NAT if available.	If discrepancy is not resolved by suggested testing, report as methicillin R	1-2
				PBP2a negative	cefoxitin R	Confirm isolate identification, repeat latex agglutination and AST.	If discrepancy is not resolved by suggested testing, report as methicillin R	1
	mecA	NAT, microarray hybridization, ISH	Colony	*mecA* detected	cefoxitin S	Confirm isolate identification, repeat *mecA* colony NAT and AST.	If discrepancy is not resolved by suggested testing, report as methicillin R	2
				mecA not detected	cefoxitin R	Confirm isolate identification and repeat *mecA* colony NAT and AST.	If discrepancy is not resolved by suggested testing, report as methicillin R	3
			Nasal Swab/Direct specimen	*mecA not* detected	cefoxitin R *S. aureus* isolated	Confirm isolate identification and repeat AST.	If discrepancy is not resolved by suggested testing, report methicillin R *S. aureus* in culture and MRSA not detected by molecular test	3
				mecA detected	cefoxitin S or no *S. aureus* isolated in culture	If possible, confirm isolate identification, repeat AST and consider *mecA* colony NAT if available.	If discrepancy is not resolved by suggested testing, report as MRSA detected by molecular test but culture negative for MRSA	2, 4-6
			Blood culture broth	*mecA not* detected	cefoxitin R *S. aureus* isolated	Confirm isolate identification and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin R	3, 7
				mecA detected	cefoxitin S *S. aureus* isolated	Confirm isolate identification, repeat AST and consider *mecA* colony NAT. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggesting testing, report as methicillin R	2, 8-9
	SCCmec-orfX junctional regions ONLY	NAT	Nasal Swab/Direct Specimen	SCCmec detected	cefoxitin S or no *S. aureus* isolated in culture	If possible, confirm isolate identification, repeat AST and consider *mecA* colony NAT.	If discrepancy is not resolved by suggested testing, report as MRSA detected by molecular test but culture negative for MRSA	*2, 10-11*
				SCCmec *not* detected	cefoxitin R *S. aureus* isolated in culture	Confirm isolate identification and repeat AST.	If discrepancy is not resolved by suggested testing, report methicillin R *S. aureus* in culture and MRSA not detected by molecular test	*12*
			Blood culture broth	SCCmec detected	cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAT. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	*2, 10-11*
				SCCmec *not* detected	cefoxitin R	Confirm isolate identification and repeat AST. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	7, 12
	SCC mec-orfX junctional regions AND *mec A* and/or other targets	NAT	Nasal Swab/Direct Specimen	SCCmec AND mecA or other target detected	cefoxitin S or no *S. aureus* isolated in culture	If possible, confirm isolate identification, repeat AST and consider *mecA* colony NAT.	If discrepancy is not resolved by suggested testing, report as MRSA detected by molecular test but culture negative for MRSA	2, 6
				SCCmec AND mecA or other target *not* detected	cefoxitin R *S. aureus* isolated in culture	Confirm isolate identification and repeat AST.	If discrepancy is not resolved by suggested testing, report methicillin R *S. aureus* in culture and MRSA not detected by molecular test	3, 12
			Blood culture broth	SCCmec AND mecA or other target detected	cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAT. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	2
				SCCmec AND mecA or other target *not* detected	cefoxitin R	Confirm isolate identification and repeat AST. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	3, 12

*In addition the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets

1. False positive and false negative PBP2a latex bead agglutination results have been observed (J Clin Microbiol. 2005 Sep;43(9):4541-4).
2. Rare *mecA* positive *S. aureus* isolates will test susceptible to cefoxitin (Curr Microbiol. 2007 Dec;55(6):473-9; J Clin Microbiol. 2005 Aug;43(8):3818-23)
3. *mecC* or *mecA* variant gene mediated methicillin resistance may not be detected by *mecA* PCR (Antimicrob Agents Chemother. 2011 Aug;55(8):3765-73; Lancet Infect Dis. 2011 Aug;11(8):595-603).
4. The presence of *mecA* positive CoNS and MSSA may result in falsely positive MRSA molecular results (J Clin Microbiol. 2008 Oct;46(10):3285-90; Antimicrob Agents Chemother. 2008 Dec;52(12):4407-19).
5. Strains harboring unstable SSCmec insertions may lose *mecA* during culture (J Clin Microbiol. 2010 Oct;48(10):3525-31).
6. The sensitivity of molecular methods is generally higher than culture while the specificity is lower.
7. Occasional false negative *mecA* results have been reported for direct blood culture molecular assays (J Clin Microbiol. 2013 Dec;51(12):3988-92).
8. For ISH assays with a cefoxitin induction step, false positive *mecA* results should be rare (J Clin Microbiol. 2014 Nov;52(11):3928-32).
9. In polymicrobial cultures, the presence of *mecA* cannot be attributed to a specific isolate.
10. Laboratories using molecular tests that only detect SCCmec-orfX junctional region targets may consider adding a disclaimer to the report stating the proportion of false positives related to *mecA* dropouts observed in isolates from the patient population served..
11. Strains harboring a SCCmec remnant lacking the *mecA* gene (*mecA* dropout) or mutant *mecA* allele may test positive in assays that only target SCCmec-orfX junctional regions (J Clin Microbiol. 2011 Apr;49(4):1240-4).
12. Multiple SCCmec types exist; depending on the design of the assay, some SCCmec variants may not be detected (Clin Microbiol Infect. 2007 Mar;13(3):222-35).

S. aureus

Target	Method	Specimen Type	Phenotype		Reasons for Observed Discrepancy	Report as:	Footnotes
PBP2a	Latex agglutination	Colony	PBP2a positive	cefoxitin S	Confirm isolate identification, repeat latex agglutination and AST and consider *mecA* colony NAT if available.	If discrepancy is not resolved by suggested testing, report as methicillin R	1-2
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mecA	NAT, microarray hybridization, ISH	Colony	*mecA* detected	cefoxitin S	Confirm isolate identification, repeat *mecA* colony NAT and AST.	If discrepancy is not resolved by suggested testing, report as methicillin R	2
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		Blood culture broth	*mecA not* detected	cefoxitin R *S. aureus* isolated	Confirm isolate identification and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin R	3, 7
			mecA detected	cefoxitin S *S. aureus* isolated	Confirm isolate identification, repeat AST and consider *mecA* colony NAT. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggesting testing, report as methicillin R	2, 8-9
						If discrepancy is not resolved by	

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S. aureus

					isolates individually.		
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S. aureus

					mixed culture, test isolates individually	suggested testing, report as methicillin R	
SCC mec-orfX junctional regions AND *mec A* and/or other targets	NAT	Nasal Swab/Direct Specimen	SCCmec AND mecA or other target detected	cefoxitin S or no *S. aureus* isolated in culture	If possible, confirm isolate identification, repeat AST and consider *mecA* colony NAT.	If discrepancy is not resolved by suggested testing, report as MRSA detected by molecular test but culture negative for MRSA	2, 6
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		Blood culture broth	SCCmec AND mecA or other target detected	cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAT. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	2
			SCCmec AND mecA or other target *not* detected	cefoxitin R	Confirm isolate identification and repeat AST. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	3, 12

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12. Multiple SCCmec types exist; depending on the design of the assay, some SCCmec variants may not be detected (Clin Microbiol Infect. 2007 Mar;13(3):222-35).

Enterococci

Indication	Target	Method	Specimen Type	Discordant Result		Suggestions for Resolution and Possible Reasons for Observed Discrepancy*	Report as	Footnotes
				Genotype or Predicted Phenotype	Observed Phenotype			
Detection of vancomycin resistant enterococci	*vanA*, *vanB*	Real-time PCR in batched platform	Perianal and rectal swabs for surveillance	*vanA* or/and *vanB* detected	Vancomycin S	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. *vanA* may be present in nonenterococcal species. *vanB* gene has been found in several commensal nonenterococcoal bacteria which may lead to misclassification of vancomycin susceptible enterococci as resistant [a].	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* and/or *vanB* detected and culture negative for VRE	
				vanA and/or *vanB* *not* detected	Vancomycin R	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. Constitutive low-level vancomycin resistance can be detected phenotypically (2-32µg/ml) from the presence of vanC, an intrinsic resistance characteristic of *E. gallinarum* (vanC1) and *E. casseliflavus* (vanC2-4)[f] .	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* and/or *vanB* not detected and culture positive for VRE	
	vanA	Real-time PCR in integrated and random-access	Rectal swab for surveillance	van A detected	Vancomycin S	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. *vanA* may be present in nonenterococcal species.	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* detected and culture negative for VRE	
				vanA not detected	Vancomycin R	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. Targeting *vanA* only may miss regional *vanB*-carrying VRE [b]. Constitutive low-level vancomycin resistance can be detected phenotypically (2-32µg/ml) from the presence of vanC, an intrinsic resistance characteristic of *E. gallinarum* (vanC1) and *E. casseliflavus* (vanC2-4)[f] .	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* not detected and culture positive for VRE	
	vanA, *vanB*	NAAT and/or array technology	Signal Positive blood culture for diagnosis	*vanA* or/and *vanB* detected	Vancomycin S	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. If mixed culture, test isolates individually. Vancomycin-variable *E. faecium* isolates have been recently revealed in Canada. They carry wildtype *vanA*, but initially test as vancomycin-susceptible with culture based method. They are able to convert to a resistant phenotype during vancomycin treatment [d,e].	If discrepancy is not resolved by suggested testing report as vancomycin R	
				vanA or/and *vanB* *not* detected	Vancomycin R	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat susceptibility test. If mixed culture, test isolates individually. Constitutive low-level vancomycin resistance can be detected phenotypically (2-32µg/ml) from the presence of vanC, an intrinsic resistance characteristic of *E. gallinarum* (vanC1) and *E. casseliflavus* (vanC2-4)[f].	If discrepancy is not resolved by repeat testing, report as vancomycin R	

*In addition to the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

References

[a] Ballard SA et al., Comparison of three PCR primer sets for identification of *vanB* gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by *vanB*-containing anaerobic bacilli. Antimicrob Agents Chemother 2005;49:77-81

[b] Nebreda T et al. Hospital dissemination of a clonal complex 17 *vanB*2-containing *Enterococcus faecium*. J Antimicrob Chemother 2007; 59:806-7

[c] Deck MK et al. Rapid detection of *Enterococcus* spp. direct from blood culture bottles using Enterococcus QuickFISH method: a multicenter investigation. Diagn Microbiol Infect Dis. 2014; 78:338-42

[d] Gagnon S et al. 2011. *vanA*-containing *Enterococcus faecium* susceptible to vancomycin and teicoplanin because of major nucleotide deletions in Tn1546. J Antimicrob Chemother 66:2758–2762.

[e] Thaker MN et al. 2015. Vancomycin-variable enterococci can give rise to constitutive resistance during antibiotic therapy. Antimicrob Agents Chemother 59:1405–1410.

Enterococci

Method	Specimen Type	Discordant Result		Suggestions for Resolution and Possible Reasons for Observed Discrepancy*	Report as
		Genotype or Predicted Phenotype	Observed Phenotype		
Real-time PCR in batched platform	Perianal and rectal swabs for surveillance	*vanA* or/and *vanB* detected	Vancomycin S	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. *vanA* may be present in nonenterococcal species. *vanB* gene has been found in several commensal nonenterococcoal bacteria which may lead to misclassification of vancomycin susceptible enterococci as resistant [a].	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* and/or *vanB* detected and culture negative for VRE
		vanA and/or *vanB* *not* detected	Vancomycin R	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. Constitutive low-level vancomycin resistance can be detected phenotypically (2-32µg/ml) from the presence of vanC, an intrinsic resistance characteristic of *E. gallinarum* (vanC1) and *E. casseliflavus* (vanC2-4)[f] .	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* and/or *vanB* not detected and culture positive for VRE
				Confirm isolate identification as *E. faecalis* or *E.*	If discrepancy is not

*In addition to the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

References

[a] Ballard SA et al., Comparison of three PCR primer sets for identification of *vanB* gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by *vanB*-containing anaerobic bacilli. Antimicrob Agents Chemother 2005;49:77-81

[b] Nebreda T et al. Hospital dissemination of a clonal complex 17 *vanB*2-containing *Enterococcus faecium*. J Antimicrob Chemother 2007; 59:806-7

[c] Deck MK et al. Rapid detection of *Enterococcus* spp. direct from blood culture bottles using Enterococcus QuickFISH method: a multicenter investigation. Diagn Microbiol Infect Dis. 2014; 78:338-42

[d] Gagnon S et al. 2011. *vanA*-containing *Enterococcus faecium* susceptible to vancomycin and teicoplanin because of major nucleotide deletions in Tn1546. J Antimicrob Chemother 66:2758–2762.

[e] Thaker MN et al. 2015. Vancomycin-variable enterococci can give rise to constitutive resistance during antibiotic therapy. Antimicrob Agents Chemother 59:1405–1410.

Enterococci

Real-time PCR in integrated and random-access	Rectal swab for surveillance	van A detected	Vancomycin S	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. *vanA* may be present in nonenterococcal species.	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* detected and culture negative for VRE	
		vanA not detected	Vancomycin R	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. Targeting *vanA* only may miss regional *vanB*-carrying VRE [b]. Constitutive low-level vancomycin resistance can be detected phenotypically (2-32µg/ml) from the presence of vanC, an intrinsic resistance characteristic of *E. gallinarum* (vanC1) and *E. casseliflavus* (vanC2-4)[f] .	If discrepancy is not resolved by suggested testing, report molecular test as *vanA* not detected and culture positive for VRE	

*In addition to the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

References

[a] Ballard SA et al., Comparison of three PCR primer sets for identification of *vanB* gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by *vanB*-containing anaerobic bacilli. Antimicrob Agents Chemother 2005;49:77-81

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Enterococci

					casseliflavus (vanC2-4)[f].		
vanA, *vanB*	NAAT and/or array technology	Signal Positive blood culture for diagnosis	*vanA* or/and *vanB* detected	Vancomycin S	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat AST. If mixed culture, test isolates individually. Vancomycin-variable *E. faecium* isolates have been recently revealed in Canada. They carry wildtype *vanA*, but initially test as vancomycin-susceptible with culture based method. They are able to convert to a resistant phenotype during vancomycin treatment [d,e].	If discrepancy is not resolved by suggested testing report as vancomycin R	
			vanA or/and *vanB* *not* detected	Vancomycin R	Confirm isolate identification as *E. faecalis* or *E. faecium* and repeat susceptibility test. If mixed culture, test isolates individually. Constitutive low-level vancomycin resistance can be detected phenotypically (2-32µg/ml) from the presence of vanC, an intrinsic resistance characteristic of *E. gallinarum* (vanC1) and *E. casseliflavus* (vanC2-4)[f].	If discrepancy is not resolved by repeat testing, report as vancomycin R	

*In addition to the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

References

[a] Ballard SA et al., Comparison of three PCR primer sets for identification of *vanB* gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by *vanB*-containing anaerobic bacilli. Antimicrob Agents Chemother 2005;49:77-81

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Enterobacteriaceae

Indication	Molecular Target	Method	Specimen Type	Discordant Result		Suggestions for Resolution and Possible Reasons for Observed Discrepancy*	Report as:	Footnotes
				Molecular Target Result	Observed Phenotype			
Detection of Extended Spectrum β-Lactam resistance in *Enterobacteriaceae* (in an isolate susceptible to all carbapenems)	CTX-M, SHV, TEM	NAAT, Microarray	Colony, blood culture	Detection of any tested target	ceftriaxone S cefotaxime S ceftazidime S cefepime S	Repeat molecular and phenotypic tests; if blood culture, check for mixed culture; if mixed, test isolates individually and report as found	If discrepancy not resolved, report all cephs R and refer to reference laboratory	1-11
				CTX-M detected	ceftriaxone R cefotaxime R ceftazidime R or S cefepime R or S	Expected phenotype for some CTX-M strains; check cefepime using non-automated method if S	Report as found, including manual cefepime result	1-11
				No detection of tested targets	ceftriaxone R cefotaxime R ceftazidime R cefepime R or S	Likely non-tested broad spectrum β-lactamase (e.g. AmpC, carbapenemase or other ESBL); repeat molecular tests; check cefepime using non-automated method if S	Report as found, including manual cefepime result	1-11
Detection of Carbapenem resistance in *Enterobacteriaceae*	KPC, OXA-48-like, VIM, NDM or IMP	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target	meropenem S imipenem S doripenem S ertapenem R or S	Repeat molecular and phenotypic tests; if blood culture, check for mixed culture; if mixed, test isolates individually and report as found; perform CarbaNP	If discrepancy is not resolved by suggested testing and/or CarbaNP test is positive, report all carbapenems as R and refer to reference laboratory	1-4, 12-14
				No detection of tested carbapenemase targets	ertapenem R, other carbapenems S	Likely ESBL/AmpC and porin alteration, especially for *Enterobacter*; perform CarbaNP; carbapenemase unlikely if negative, rare carbapenemases, e.g. GES-types, are still possible	If CarbaNP positive, report all carbapenems as R and refer to reference laboratory; Otherwise report as found on phenotypic testing	1-4, 12-14
				No detection of tested carbapenemase targets	meropenem, imipenem or doripenem R	Possible other carbapenemase; if blood culture, check for mixed culture; if mixed, test isolates individually and report as found ; repeat molecular and susceptibility tests, including CarbaNP	If CarbaNP positive, report all carbapenems as R and refer to reference laboratory; Otherwise report as found on phenotypic testing	1-4, 12-15

*In addition the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

Footnotes

1. Multiple beta-lactamases may be carried by individual bacterial isolates. Most carbapenemase-producing bacteria are resistant to 3rd and 4th gen cephalosporins, although bacteria with OXA-48 enzymes may not be unless they co-produce an ESBL or AmpC enzyme.
2. Molecular assays can detect the presence of specific beta-lactamase genes but cannot exclude the presence of other beta-lactamase genes or resistance mechanisms, or novel variants with changes in primer / probe annealing sites. Therefore phenotypic resistance should always be reported.
3. Isolates with phenotypic susceptibility despite the presence of a resistance determinant may indicate the potential for resistance to emerge during therapy.
4. These are provisional guidelines based on general principles; however, the performance characteristics of many individual RUO assays are presently unknown.
5. Susceptibility of TEM/SHV-carrying strains to beta-lactam/inhibitor combinations is variable.
6. Susceptibility of ESBL-carrying strains to cefepime is variable.
7. Susceptibility of ESBL-carrying strains to beta-lactam/inhibitor combinations is variable.
8. Some strains carrying CTX-M ESBLs remain susceptible to ceftazidime.
9. Some strains carrying TEM/SHV-derived ESBLs remain susceptible to cefotaxime/ceftriaxone.
10. Some molecular assays for *ampC* may not reliably distinguish between chromosomal and plasmid-encoded genes in some bacterial species.
11. Most strains with de-repressed AmpC expression remain susceptible to cefepime.
12. These recommendations are based on carbapenem breakpoints in M100-S26.
13. The susceptibility to other carbapenems of ertapenem-resistant strains with ESBL or AmpC enzymes and reduced porin expression that do not contain carbapenemase genes or express carbapenemase activity may be reported as measured in phenotypic susceptibility assays.
14. Rapid tests for carbapenemase activity (e.g., Carba NP) may not detect OXA-48-like and some other carbapenemases.
15. Some isolates of Enterobacteriaceae, in particular but not exclusively *Morganella, Proteus* spp. and *Providencia* spp., may exhibit intrinsic low-level resistance to imipenem on a non-carbapenemase-mediated basis.

Enterobacteriaceae

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Methods WG Recommendations

- That the ad hoc group consider expanding the documents to include recommendations to clinical labs as to how results of testing (particularly for the presence of genes) should be reported to clinicians
- Work to date has focused primarily on approaches that labs should consider to address discordant phenotypic/genotypic findings
- This will be a large increase in scope for M100 and will require considerable interaction with the Outreach Committee
- Robin Patel has indicated that she would be very willing to share the approaches taken at Mayo with the ad hoc WG, as they have considerable experience in approaches to reporting and interpretation of molecular findings

Update on ISO Documents for Microbiology

– Barb Zimmer

- ISO 20776-1 (Clinical laboratory testing and *in vitro* diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases)
  - Currently undergoing periodic review until June 1. If it is determined that it needs to be revised, there will be a project team formed. Each country nominates participants.
- ISO 16782 (Antimicrobial susceptibility testing – Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing.
  - Approved in April 2015 to skip final ISO voting and move directly to publication. It is anticipated that this will be prior to October.

Report from the Intrinsic Resistance ad hoc WG

– Barb Zimmer

Intrinsic Resistance Working Group
Fosfomycin and *P. aeruginosa*
Should it be in intrinsic resistance tables?

- Dyan Luper (Recording Secretary), Jeff Alder, Rafael Canton, German Esparza, Sandy Richter, Susan Sharp, Carole Shubert, Paul Schreckenberger, Tom Thomson
- Item for Vote: Removal of "R" in Appendix B-2 Fosfomycin with *P. aeruginosa*
- Approved unanimously by email vote of IR Working Group
- Approved 9/0/1 by Methods WG

Intrinsic Resistance Working Group

Fosfomycin and *P. aeruginosa* – should it be in intrinsic resistance tables?

- No CLSI breakpoints for this drug/bug.
- The initial discussion came from a pharmacist at Scripps in San Diego, who sent to CLSI the Lu and Reffert references (in agenda materials)
  - Lu et al. Used other BPs, but showed isolates with lower MICs.
  - Reffert et al. "Fosfomycin susceptibility (of PSA) dependent on local antibiogram"
- Our EUCAST colleagues also do not have a breakpoint, nor is this listed as intrinsically resistant in their tables. Their ECOFF data show a range of MICs (see agenda materials).
- Falagas et al (see agenda materials). Literature Review, including combination therapy. "Fosfomycin could have a role as therapeutic option against MDR *P. aerguinosa*"

Report from the Direct AST ad hoc WG

– Romney Humphries

Ad Hoc WG Members

- Romney Humphries (chair)
- April Abbott (recording secretary, on phone)
- Mel Weinstein (absent)
- Barb Zimmer (interim recording secretary)
- Thomas Kirn
- Lauri Thrupp
- Ben Turng (on phone)
- Dyan Luper
- Bill Brasso (absent)

Goal

- Define a standardized methodology for performing AST directly from blood culture broths
  - Important for treatment of sepsis
  - Important for antimicrobial stewardship
  - Provide diagnostic manufacturers with a “reference” method by which to compare future studies/development

Notes on Goals

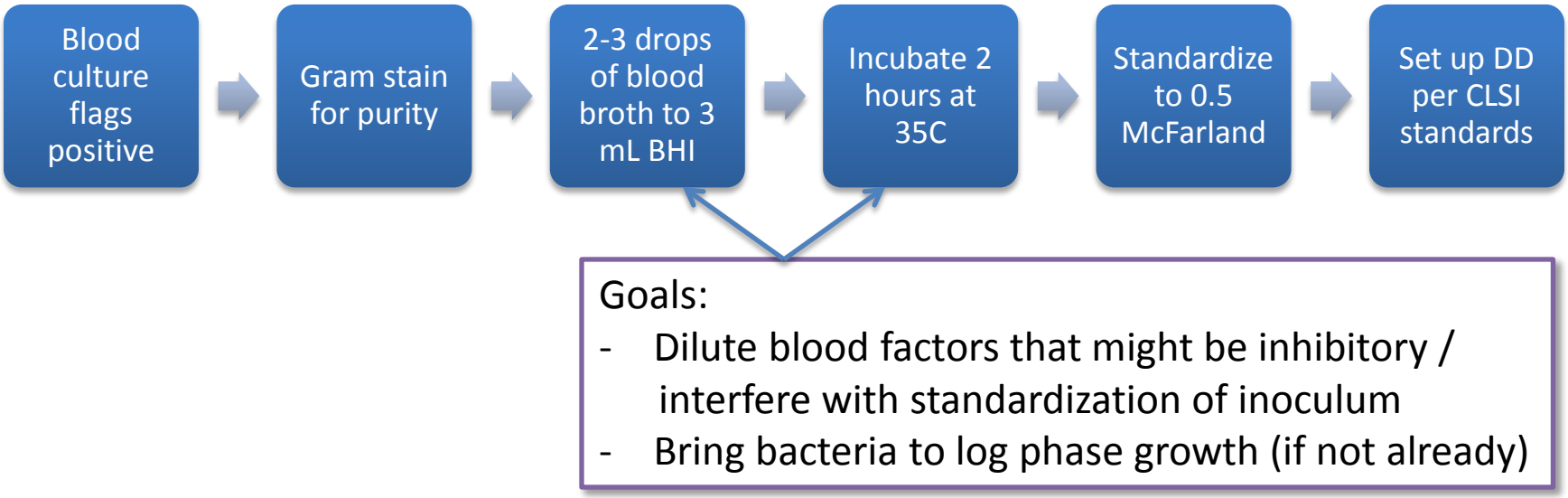
- Much discussion!
- Desire is to develop a standard method for labs
  - Will still need to be verified by each performing lab (per CLIA)
  - Is an Laboratory Developed Test (LDT)
- Desire is to be able to report “final” result from direct AST

Progress Since January Meeting

- 2 teleconferences
- Preliminary studies performed on inoculum, BMD direct AST

- June meeting:
  - Reviewed progress to date regarding effect of inoculum method on CFU/mL
  - Reviewed and refined proposed validation studies, with input from group

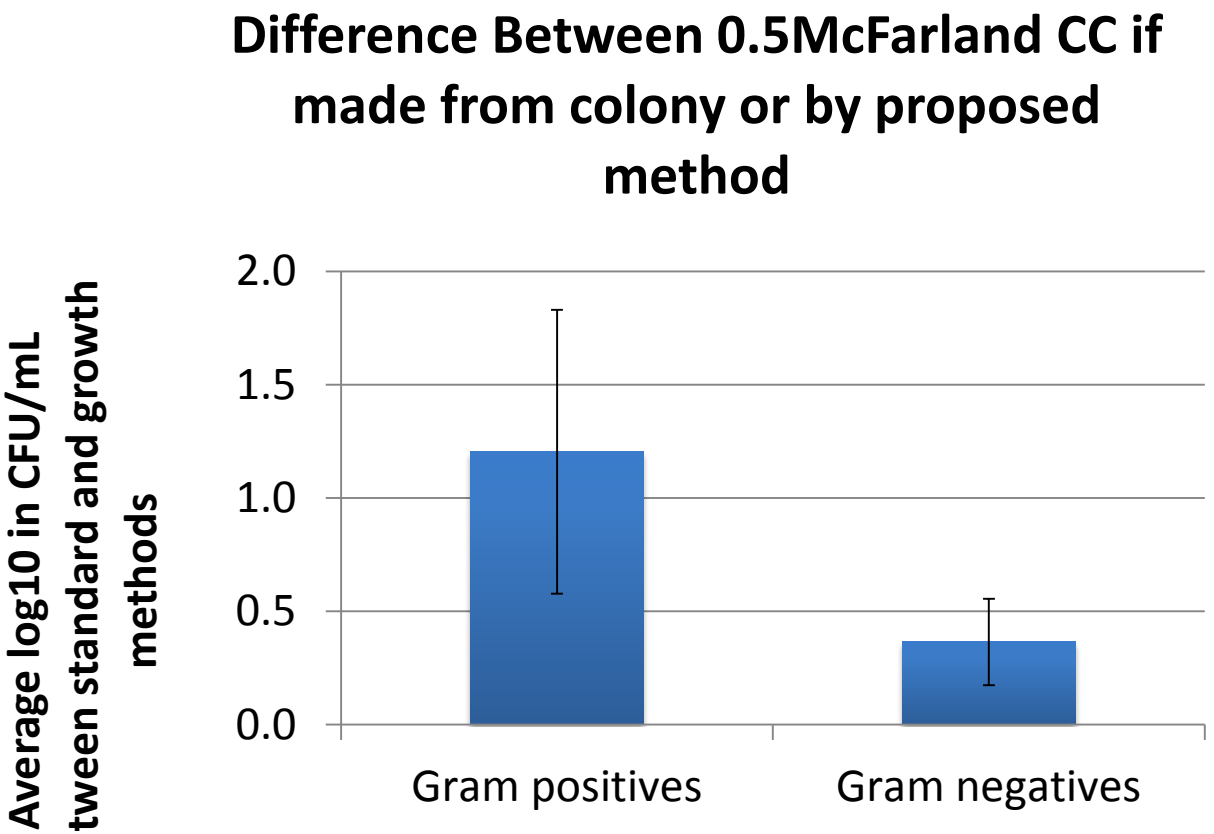
Method Overview



Results to Date

- Extensive literature review
  - Studies almost all in 1980 -1990s
  - Methods varied
  - Very good performance across the board
- Review of UCLA data (BMD)
  - Excellent performance with a few exceptions
  - Data in agenda book for reference

Effect of modified method to standardized inoculum?



Note: observed variability in CFU/mL of 0.5 McFarland by standard method is

± 0.68 $\log_{10}$ CFU/mL for Gram positives

± 0.11 $\log_{10}$ CFU/mL for Gram negatives

1-log difference in Inoculum density does not appear to affect DD result

Organism	Isolate #	Clindamycin			Erythromycin			Cefoxitin			Gentamicin			Penicillin			SXT			Vancomycin		
		UD	1:10	difference	UD	1:10	difference	UD	1:10	difference	UD	1:10	difference	UD	1:10	difference	UD	1:10	difference	UD	1:10	difference
S. aureus	ATCC213	26	28	2	26	29	3	25	26	1				19	21	2	28	31	3	17	19	2
S. aureus	ATCC923	25	29	4	24	28	4	24	27	3	22	26	4	28	34	6	26	30	4	17	19	2
S. aureus	clinical isolate	28	27	-1	26	27	1	26	26	0	23	24	1	41	41	0	28	29	1	17	18	1
S. aureus	clinical isolate	27	28	1	8	8	0	15	15	0	23	24	1	28	29	1	28	31	3	19	20	1
S. aureus	clinical isolate	23	26	3	25	28	3	25	25	0	19	21	2	39	38	-1	27	28	1	16	18	2
S. aureus	clinical isolate	25	28	3	24	22	-2	25	25	0	21	23	2	15	18	3	26	29	3	17	19	2
S. aureus	clinical isolate	26	29	3	6	6	0	11	13	2	20	24	4	7	9	2	27	29	2	17	18	1
S. aureus	clinical isolate	24	27	3	6	6	0	6	6	0	20	24	4	7	7	0	24	27	3	17	20	3
S. aureus	clinical isolate	25	28	3	6	6	0	12	12	0	20	23	3	7	7	0	25	27	2	18	20	2
S. aureus	clinical isolate	25	27	2	6	6	0	25	25	0	22	25	3	12	16	4	26	25	-1	17	19	2
S. aureus	clinical isolate	24	28	4	25	27	2	24	26	2	25	28	3	21	26	5	25	28	3	18	21	3
S. aureus	clinical isolate	25	29	4	26	30	4	25	26	1	24	27	3	22	25	3	27	31	4	18	20	2

Green – susceptible; yellow intermediate; red resistant; blue no data

UD, undiluted 0.5 McFarland; 1:10 is a 1:10 dilution of the 0.5 McFarland

Difference = difference in zone size between undiluted and diluted

Results from WG discussion

- Can blood culture broth, directly from bottle, be used to inoculate MHA for disk test?

Data from BD: Colony Count of bacteria in positive blood cultures

Organism	Coll.	Strain	TTD (hrs)	n	Mean PBC BACTEC Bottle Plate Count at Time of Positivity (cfu/mL)
Gram-Negative					
E. coli	ATCC	25922	10.3	4	3.9×10^{8}
P. aeruginosa	ATCC	27853	15.3	4	6.3×10^{8}
Gram-Positive					
E. faecalis	ATCC	29212	11.1	5	4.8×10^{8}
S. aureus	ATCC	29213	12.4	5	1.1×10^{7}
S. aureus MRSA	ATCC	43300	12.5	3	1.9×10^{7}
S. aureus MRSA	ATCC	33591	19.0	3	2.2×10^{6}
S. epidermidis	POS	3568	17.0	6	8.1×10^{7}
S. pneumoniae	ATCC	49619	11.6	4	9.8×10^{8}

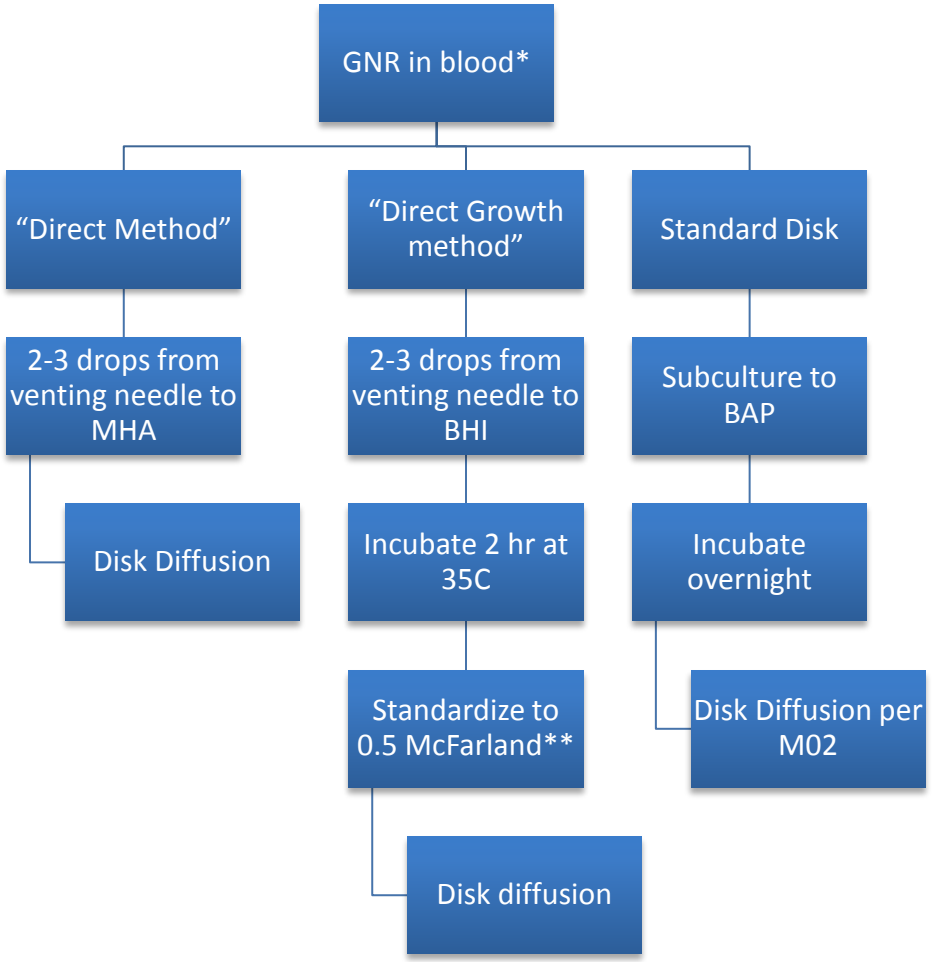
***** BACTEC Aerobic Plus (with Resin)***

TTD, time to detection

Phase I Study

- I-A Gram Negative Study
  - One media manufacturer, n=3 sites
  - Challenge set:
    - CRE, MRAb, MDR P.aeruginosa, QC organisms
    - spiked to blood per standard protocol (from Accelerate Dx)
  - Clinical isolates (n=25 per site)
  - Evaluate data
    - Require >90% EA, >90% CA, <2% VME, ME
    - Require >95% QC results to be in control
    - Will answer question if non-standardized inoculum method might work

Proposed Workflow



*Notes:

- Will define enrollment criteria to ensure patient mix
- Only enroll if one GS organism morphology
- if not Enterobacteriaceae, P. aeruginosa, A. baumannii, or mixed next day, do not read on day 2

**If not yet 0.5 McFarland (Psa), continue to incubate; if >0.5 McFarland, dilute in BHI

Gram negative Antimicrobials

Panel 1	Panel 2 (?)
1. Ampicillin 2. Ampicillin-sulbactam 3. Cefazolin 4. Ceftriaxone 5. Cefepime 6. Ertapenem 7. Imipenem 8. Meropenem 9. Piperacillin-tazobactam 10. Ciprofloxacin 11. Trimethoprim-sulfa	1. Aztreonam 2. Ceftazidime 3. Levofloxacin 4. Gentamicin 5. Tobramycin 6. Amikacin 7. Minocycline 8. Tigecycline 9. Cefoxitin

Phase II: Growth Medium Comparison (GN)

- Use protocol determined by direct inoculation vs. growth method
- Enroll sites to cover different manufacturers
- Have each site test a challenge set of organisms seeded in (n=10)
- Have each site test 50 patient isolates (will defined number to enroll), and compare to standard CLSI disk diffusion
- Expanded antimicrobials

Blood Media Types (US)

Manufacturer, Instrument	Bottle description	Total
BD, BACTEC	Aerobic - **Standard Aer/F**, **Plus Aer/F (resin)**, **Peds Plus/F (resin)** Anerobic - Standard Ana/F, Plus Ana/F (resin), Lytic Ana/F	6
Biomerieux, BacT/Alert	Aerobic: **SA**, FA (charcoal)*, PF (charcoal)*, **FA**+ (resin), PF+ (resin) Anaerobic- SN, FN (charcoal)*, FN+ (resin)	5
Biomerieux, Virtuo	Same bottles as BacT/Alert	
Thermo Fisher, VersaTrek	Aerobic - **Redox1** (resin) Anaerobic - Redox2 (resin)	2

*Planned to be phased out 2016 (will not evaluate)

Next Steps

- Identify study sites (please let Romney know if you are interested!!)
  - Hope for blood culture manufacturers to participate with seeded isolates
  - Need clinical labs for patient specimen testing
  - Clear guidelines on inclusion criteria to ensure good patient and organism mix
- Draft detailed proposal

Ad hoc “Atypical” *S. aureus* WG

- Romney Humphries - chair
- April Bobenchik (Recording Secretary)
- Stella Antonara
- Lars Westblade
- Eileen Burd
- Robin Patel
- Sandra Richter

Discussions to date

- Need AST for atypical *Staphylococcus aureus* (SA, and CoNS)
- 2 teleconferences to date, 1 hour meeting on Sunday
- What are atypical SA?
  - Small colony variants?
  - Thymidine vs. menadione vs. hemin auxotrophs?
- Definition :
  - Strains that do not grow on unsupplemented MHA or CAMHB at the time of testing

Planned Studies

- Phase I: AST for oxacillin resistance
  - Are PBP2a tests reliable for these poorly growing SA?
  - Can cefoxitin disk test be performed on BMHA, other media?
  - Reference = *mecA*
- Phase II:
  - Other antimicrobials?
    - CAMHB-LHB
    - WGS?

Preliminary Studies

- Romney Humphries, Shelley Miller, Janet Hindler, UCLA
- Atypical *S. aureus*
- Design and results presented
- Path forward shared

Testing for Oxacillin Resistance in *Staphylococcus pseudointermedius*

– Romney Humphries

Surrogate Testing ad hoc WG – Informational Update

Jim Jorgensen – chair

Members:

Mel Weinstein

Janet Hindler

Jim Lewis

Barb Zimmer

- First conference call held last Tuesday (June 9)
- Janet Hindler had previously submitted an inclusive spreadsheet wherein references to surrogate testing, etc. were listed
- Surrogate antimicrobial definition proposals are being discussed and drafts distributed at yesterday's meeting
  - Input
- Plan to have project completed for January, 2016 meeting

Cefazolin - Urine

- Provide input to T&T WG on comments from M100 review that pertain to methods

New Issues for Consideration?

- Typically we report combo drugs with inhibitors that do not have intrinsic activity as a ratio. But what should we do in situations wherein they both have activity?