



CLSI Methodology WG

Early Interaction for S-649266

- Background information
 - When developing an AST methodology for S-649266, Chelex-treated IsoSensitest Broth (ISB) produced the most consistent MIC results for the bacteria tested. Shionogi has already completed a M23 QC study for Chelex-treated ISB and CAMHB. These were presented at the Jan 2015 meeting but no decision was made regarding the methodology issues.
 - CLSI did approved the QC range using CAMHB in Jan 2015.
- Discussion points
 - 1) Iron-depleted MHB
 - Does CLSI endorse the QC ranges of S-649266 in Chelex (or other cation-binding resins)-treated MHB if appropriate results are obtained in QC range studies?
 - 2) Alternative MIC method for *Acinetobacter* spp.
 - Does CLSI agree the agar dilution method as alternative MIC methodology for *Acinetobacter baumannii*?

Discussion point (1)

Iron-depleted MHB



- Shionogi investigated whether other chelating agents (e.g., amberlite or CR-11) can be used instead of Chelex. Chelex, CR-11 and Amberlite treated MHB produced similar and reproducible MICs for QC strains (see slide 10).
- Future plan is as follows:
 - Broth microdilution MIC quality control study for S-649266 in Chelex-treated MHB (ongoing)
 - Broth microdilution MIC reproducibility for clinical isolates (planned for 2015)
- We would like to request that the broth microdilution quality control study be added to the agenda for the Quality Control Working Group for CLSI meeting at next January 2016.

Discussion point (1)

Iron-depleted MHB



- Does CLSI endorse the MIC methodology of S-649266 in iron-depleted MHB (cation-binding resin treated MHB) if appropriate results are obtained in QC range studies?
 - Test strains:
 - *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853
 - Test medium:
 - Chelex-treated MHB

Discussion point (2)

Alternative MIC method for *Acinetobacter* spp.



- Regarding *A.baumannii*, trailing phenomenon observed in Chelex-treated CAMHB.
- MIC reproducibility of S-649266 against *A. baumannii* was poor due to trailing phenomenon, but it seems to be improved when OD90% endpoint is used.
- The appropriate endpoint was investigated MIC correlation between agar dilution method using MHA and BMD using CT-ISB was compared.
 - Agar MICs were similar and highly reproducible when compared with MICs in Chelex-treated ISB (see slide 10).

Species	Number of strains	Frequency of trailing phenomenon (%)			
		CAMHB	Chelex-treated CAMHB	Chelex-treated ISB	MHA
<i>A. baumannii</i>	133 or 48	17% (23/133)	29% (39/133)	7% (9/133)	0% (0/48)

- Future plan is as follows:
 - The additional studies to determine MIC using Chelex-treated MHB and MHA are ongoing. The appropriate method will be determined from the reproducibility and PK/PD relationship with in vivo efficacy.
 - If MHA were selected as an appropriate method, the following studies will be conducted.
 - Agar dilution MIC Quality Control Study for S-649266 in MHA
 - Agar dilution MIC reproducibility using clinical isolates.
 - Does CLSI agree the MHA dilution method as alternative MIC methodology for *Acinetobacter baumannii* if appropriate results are obtained?



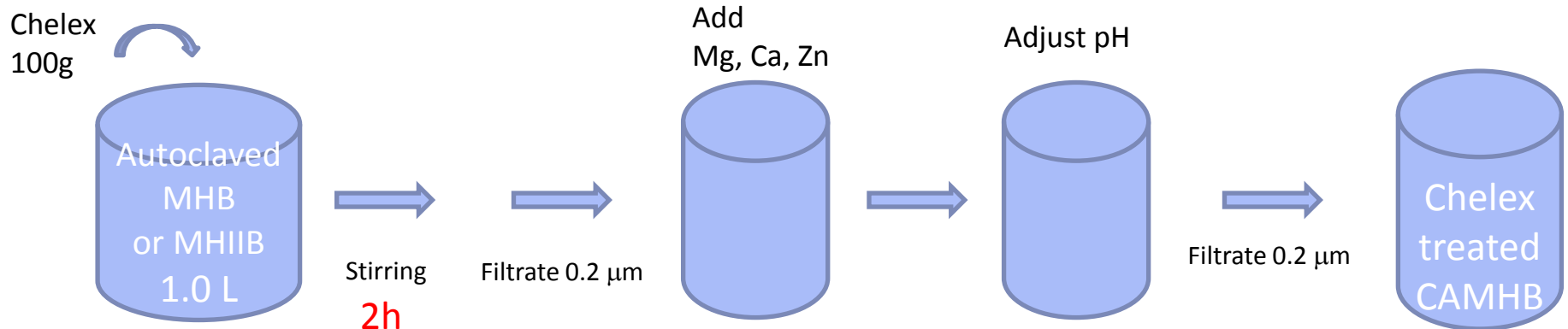
Appendix

Revised Chelex treatment method for CAMHB

Outline of detailed method:

1. Autoclaved MHB or MHIIB return to room temperature
2. Add 100 g Chelex (Bio-Rad) to 1 L of autoclaved MHB or MHIIB
3. Stir at room temperature for approximately **2 hours**
4. Filter using 0.2 μm filter
5. Add Ca^{2+} (final: 20 - 25 mg/mL, CaCl_2), Mg^{2+} (final: 10 – 12.5 mg/mL, MgCl_2) and Zn^{2+} (final: 10 μM , ZnSO_4).
6. Adjust pH to 7.2-7.4 by using HCl
7. Filter using 0.2 μm filter

*: Step 6 and 7 can be changed. (Filtration can be conducted after adjustment of pH if the solutions of Ca, Mg, and Zn are pre-sterilized and aseptically added.)



		Chelex			CR11			Amberlite			Normal broth		
		Occurrences by lot			Occurrences by lot			Occurrences by lot			Occurrences by lot		
	MIC	A	B	C	A	B	C	A	B	C	A	B	C
E. coli ATCC25922	0.016	0	0	0	0	0	0	0	0	0	0	0	0
	0.031	0	0	0	0	1	2	3	2	3	1	1	1
	0.063	0	3	3	2	2	1	0	1	0	2	1	1
	0.125	3	0	0	1	0	0	0	0	0	0	0	1
	0.25	0	0	0	0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	1	0
Total		3	3	3	3	3	3	3	3	3	3	3	3
Range		1	1	1	2	2	2	1	2	1	2	6	3

		Chelex			CR11			Amberlite			Normal broth		
		Occurrences by lot			Occurrences by lot			Occurrences by lot			Occurrences by lot		
	MIC	A	B	C	A	B	C	A	B	C	A	B	C
P. aeruginosa ATCC27853	0.031	0	0	0	0	0	0	0	0	0	0	0	0
	0.063	1	0	0	0	0	2	2	0	2	0	0	0
	0.125	0	0	1	0	0	1	0	2	0	0	0	0
	0.25	1	2	1	1	1	0	1	1	0	2	0	0
	0.5	1	1	1	2	2	0	0	0	1	1	0	0
	1	0	0	0	0	0	0	0	0	0	0	2	2
	2	0	0	0	0	0	0	0	0	0	0	1	1
4		0	0	0	0	0	0	0	0	0	0	0	0
Total		3	3	3	3	3	3	3	3	3	3	3	3
Range		4	2	3	2	2	2	3	2	4	2	2	2

- Reproducible MICs were observed among cation binding resins.

S-649266 MIC correlation between BMD and Agar dilution

BMD in CT ISB vs BMD in CT-MHIB

CT-MHIB MIC		>32				1		1	1				
		32			1		2		1	1	1		
		16					1	3		1		1	
		8			2	2	2		1				
		4				6	1	1					
		2				1			1				
		1				1							
		0.5				2							
		0.25				1							
		0.125			3								
		0.063		1	1	2							
		≤0.031	1	1									
	MIC (μg/mL)	≤0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	>32
		CT-ISB											

BMD in CT ISB vs MHA

Agar MIC	>32								1	1			
	32												
	16								1				
	8					1							
	4							2	1				
	2			1		1	2	1					
	1				2	5	1	1					
	0.5			3	9						1		
	0.25		1	2	3		1						
	0.125		1		1								
	0.063	1		1									
	≤0.031												
MIC (μg/mL)	≤0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	>32	
	CT-ISB												

- Agar MICs was similar and highly reproducible compared with MICs in Chelex-treated ISB.
 - ✓ Trailing phenomenon was not observed on MHA

Plan 1 for next action: MIC Quality Control Study



- Broth Microdilution MIC Quality Control Study for S-649266 sodium Drug Product in Chelex-Treated Mueller Hinton Broth
 - QC ranges have not been tested yet with Chelex-treated CAMHB. QC MIC study will be conducted because reproducible MIC was obtained in Chelex-treated, CR-11 and Amberlite treated MHB
- Study design
 - Total eight laboratory include
 - Medium: CAMHB (three lots from at least two manufacturers).
 - Chelex 100 Resin used to remove cations; then Ca, Mg, Zn added
 - Frozen panel was manufactured at Thermo Fisher Scientific
 - QC organism: *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853
 - Control drug: cefepime

Plan 2 for next action:

Broth Microdilution MIC reproducibility



- Broth Microdilution MIC reproducibility
 - To determine the reproducibility of MIC values for clinical isolates when testing S-649266 using standard cation-adjusted Mueller Hinton broth (CAMHB) and iron-depleted CAMHB.
- Study design
 - Single laboratory testing (JMI Laboratories, North Liberty, Iowa)
 - CAMHB (three lots from at least two manufacturers).
 - The iron-depletion method that will be used is removal of iron by using cationic-exchange resins (resins from 3 manufacturers [Chelex, CR11, and Amberlite])
 - Test strains (N= 20 clinical isolates)
 - *E. coli* (4, including KPC, CTX-M, NDM strains), *P. aeruginosa* (4, including KPC, IMP or VIM, OprD- strains), *K. pneumoniae* (6, including KPC, NDM and SHV strains), *A. baumannii* (6, including OXA-23 and -24 strains)
 - Quality control strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853
- Control drug: meropenem

Plan 3 for next action:

Broth vs disc susceptibility test



- Broth vs Disk correlation for Susceptibility Testing of S-649266 sodium Drug Product
- Study design
 - Methods
 - Agar dilution MIC with Mueller Hinton agar
 - Disk diffusion with Mueller Hinton Agar
 - Broth microdilution MIC with two conditions: Cation-adjusted Mueller Hinton broth (CAMHB) and Chelex treated CAMHB
 - Test strains total number of strain is 250
 - 82 carbapenem resistant isolates from the CAPITAL 2012 study
 - 46 isolates from IHMA's collection carrying the PER beta-lactamase gene
 - 120 isolates from the previous IHMA susceptibility study
 - 2 quality control strains (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853)
 - Control drug: cefepime