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Quality Control Studies of CF-301 *versus* *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212

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Abstract

Background: CF-301, a novel, recombinantly-produced bacteriophage-derived lysin (cell wall hydrolase) is the first agent of this class to enter clinical development in the US for the treatment of bacteremia including endocarditis due to *S. aureus*. Previously, a modification of the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (BMD) was approved for susceptibility testing with CF-301 that uses CAMHB supplemented with 25% horse serum and 0.5 mM DL-Dithiothreitol solution (DTT)to eliminate several phenomenon that resulted in inaccurate and non-reproducible results using the standard method. The studies reported here were performed in order to determine BMD quality control (QC) ranges for CF-301 vs. *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 using the new modified broth method. Vancomycin was tested as a control for both studies.

Materials and Methods: CLSI M23-A4[1] (2016) Tier 1 and 2 QC studies were performed using the standard reference method BMD minimum inhibitory concentration (MIC) test (M7-A10[2], 2015) with the following exceptions: CAMHB was supplemented with 25% horse serum and 0.5 mM DTT. For Tier 1 studies 2 labs tested 5 or 8 replicates a day for 5 days in one lot of supplemented CAMHB. For Tier 2 studies frozen BMD panels containing CF-301 diluted in 3 lots of supplemented CAMHB were supplied to 8 labs and each lab tested 1- 4 replicates per day over a minimum of 3 days.

Results: **Table 1. Tier 1 and 2 Quality Control Ranges for CF-301**

QC Organism	Tier 1 QC Ranges (µg/mL)	Tier 2 Proposed QC Ranges (µg/mL)
S. aureus ATCC 29213	0.25-1	0.25-2
E. faecalis ATCC 29212	16-64	8-64

Conclusions: The Tier 1 study produced 3 dilution ranges for both organisms. Tier 2 studies using the modified BMD MIC test for CF-301 vs. *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 produced reproducible results from the 8 laboratories. These results suggested ranges of 0.25-2 µg/mL for *S. aureus* ATCC 29213 and 8-64 µg/mL for *E. faecalis* ATCC 29212. Vancomycin QC ranges were in control for all tests in both studies. Tier 2 ranges will be submitted to the CLSI for inclusion in the M100[3] document.

Introduction

CF-301, a novel, recombinantly-produced bacteriophage-derived lysin (cell wall hydrolase) is the first agent of this class to enter clinical development in the US for the treatment of bacteremia including endocarditis due to *S. aureus*.

Key Features of CF-301 lysin:

- **Rapid killing of bacteria by cleaving the structure of the bacterial peptidoglycan cell wall**
- **Synergy with existing antibiotics**
- **Rapid clearance of biofilms**
- **Low propensity to develop resistance**
- **Fully active against resistant bacteria (while sparing good bacteria)**

Acknowledgement

This study was sponsored by a grant from ContraFect Corporation

Materials and Methods

Tier 1 Studies:

- CMI and ContraFect each prepared CLSI frozen broth microdilution MIC test panels containing CF-301 and Vancomycin dilutions
- CF-301 dilutions were prepared in CAMHB supplemented with 25% horse serum and 0.5 mM DL-Dithiothreitol (DTT) solution (CAMHB lysin broth).
- Vancomycin dilutions were prepared in CAMHB.
- CMI tested 25 replicates of each QC strains over 5 days at 5 replicates per day.
- Contrafect tested 40 replicates of each QC over 5 days at 8 replicates per day.

Tier 2 Studies:

- CMI prepared CLSI frozen BMD MIC panels containing CF-301 diluted in 3 different manufacturer's lots of CAMHB lysin broth and vancomyin was included as a control agent in one lot of CAMHB
- CMI shipped MIC panels to 7 outside laboratories for a total of 8 labs doing the testing.
- Each laboratory tested 10 replicates of each QC strain over a 3 to 10 days period (minimum 4 replicates per day and no less than 3 days of testing).
- Labs were instructed to perform repeat tests if any vancomycin results were out of control on day of testing. Vancomycin QC ranges were listed per CLSI M100-S26 document.[3]
- Results were compiled an analyzed at CMI using CLSI M23-A4 guidelines followed by a second analysis using the RangeFinder Method[4]

Results

Figure 1. *S. aureus* ATCC 29213
CF-301 Broth Microdilution Tier 1 MIC Quality Control
Proposed Range 0.25 - 1 µg/mL 100% Included

MIC (ug/ml)	LOT 1	LOT 2	LAB 1	LAB 2	All Labs
0.12					
0.25					
0.5	24	25	24	25	49
1	1		1		1
2					
4					
8					
16					
32					
64					
128					

N	25	25	25	25	50
GKOMEAN	0.51	0.50	0.51	0.50	0.51
MODE	0.5	0.5	0.5	0.5	0.5
MIN	0.5	0.5	0.5	0.5	0.5
MAX	1	0.5	1	0.5	1
RANGE	2	1	2	1	2

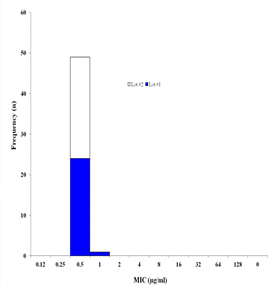


Figure 2. *E. faecalis* ATCC 29212
CF-301 Broth Microdilution Tier 1 MIC Quality Control
Proposed Range 16-64 µg/mL 100% Included

MIC (ug/ml)	LOT 1	LOT 2	LAB 1	LAB 2	All Labs
0.12					
0.25					
0.5					
1					
2					
4					
8					
16	7		7		7
32	18	25	18	25	43
64					
128					
0					

N	25	25	25	25	50
GKOMEAN	26.4	32.0	26.4	32.0	29.0
MODE	32	32	32.0	32.0	32.0
MIN	16	32	16.0	32.0	16.0
MAX	32	32	32	32	32
RANGE	2	1	2	1	2

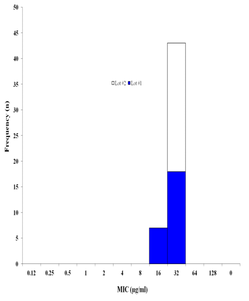
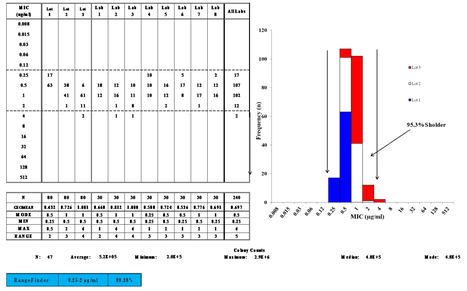
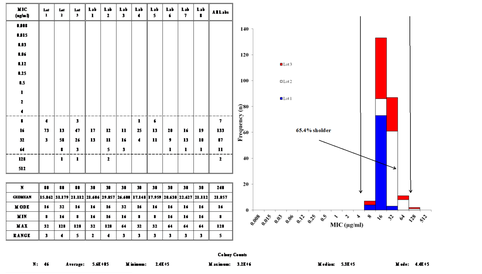


Figure 1. *S. aureus* ATCC 29213 Tier 2
CF-301 in CAMHB+25% Horse Serum and 0.5 mM DTT
Proposed Range: 0.25-2 µg/ml = 99.2% Included



RangeFinder 0.25-2 µg/ml 99.28%

Figure 3. *E. faecalis* ATCC 29212 Tier 2
CF-301 in CAMHB+25% Horse Serum and 0.5 mM DTT
Proposed Range: 8 - 64 µg/ml = 99.2% Included



RangeFinder 8-64 µg/ml 99.28%

Table 1. Tier 1 and Tier 2 Quality Control Ranges for CF-301

QC Organism	Tier 1 QC Ranges (µg/mL)	Tier 2 Proposed QC Ranges (µg/mL)
S. aureus ATCC 29213	0.25-1	0.25-2 µg/ml (99.2%)
E. faecalis ATCC 29212	16-64	8-64 µg/ml (99.2%)

Conclusions

- CF-301 Tier 1 and Tier 2 studies MIC quality control ranges were presented to the CLSI Antimicrobial Susceptibility Testing subcommittee in January 2017.
- 4 dilution ranges for both SA and EF were approved as listed in Table 1 Tier 2.
- The use of 8 labs in Tier 2 *vs.* 2 labs in Tier 1 slightly increased the variability in MIC ranges as expected.
- Tier 2 study results were within the maximum allowed 4 dilutions acceptable for CLSI MIC QC ranges.
- Vancomycin MIC results were in control for all tests in both studies.

References

1. Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters, 4th Edition, January 2016 Clinical Laboratory Standards Institute, Wayne PA.
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4. Turnidge, J., and G. Bordash. 2007. Statistical methods for establishing quality control ranges for antibacterial agents in Clinical and Laboratory Standards Institute susceptibility testing. Antimic.b. Agents Chemo.r. 51: 2483–2488.