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Initial MIC Quality Control Ranges for GSK1322322 Using the CLSI Multi-Laboratory M23-A3 Study Design

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Abstract

Background: This study followed the CLSI M23-A3 (2008) guideline for establishing MIC quality control (QC) ranges for CLSI reference and standardized broth microdilution (BMD) tests when using GSK1322322, an investigational compound. GSK1322322 is an antibacterial agent that inhibits peptide deformylase function, a clinically unexploited target. GSK1322322 demonstrates antibacterial activity against multi-drug resistant community-acquired respiratory and cutaneous infection pathogens, including MRSA, and represents a new antimicrobial class with a novel mode of action.

Methods: CLSI BMD methods were utilized in an eight laboratory study design compliant with M23-A3 specifications. Three QC strains were tested (*S. aureus* ATCC 29213 [SA], *H. influenzae* ATCC 49247 [HI], and *S. pneumoniae* ATCC 49619 [SPN]) using four media lots (three manufacturers) of cation-adjusted Mueller-Hinton (MH) broth (with 2-5% lysed horse blood for testing SPN) and Haemophilus Test Medium. Ten replicate tests were performed for each QC organism generating 320 BMD values per strain (960 total). Colony counts were performed each day of testing. Levofloxacin, linezolid and azithromycin were used as antimicrobial test controls. Nearly all control agent MIC results (954/960; 99.4%) were within CLSI published ranges.

Results: The table lists the recommended MIC QC ranges for GSK1322322. Modal MIC values (% of total) observed for each strain were: SA at 2 µg/ml (75.0), HI at 1 µg/ml (63.9), and SPN at 0.25 µg/ml (76.6). A four $\log_2$ dilution range was proposed for HI due to a "shoulder" at 2 µg/ml, which has 70.9% of MIC values compared to the modal occurrences at 1 µg/ml. No significant differences were noted among media lots or testing site performance for GSK1322322 results (See Table 2).

Conclusions: Proposed MIC QC ranges for GSK1322322 will guide clinical or reference laboratories involved in the testing of clinical trial isolates and facilitate the regulatory review process for this novel, investigational agent.

Introduction

An investigational antimicrobial, GSK1322322, has a novel mode of action which inhibits peptide deformylase function. This novel drug demonstrates activity against multidrug-resistant (MDR) respiratory and skin infection pathogens, including MRSA. As the prevalence of resistances in staphylococcal strains increases, GSK1322322 could become an important option for treatment.

This broth microdilution quality control (QC) study of GSK1322322 was performed following the Clinical Laboratory Standards Institute (CLSI) M23-A3 (2008) guideline document using eight laboratories, different manufacturers of media and three antimicrobial control agents. The results are presented as proposed QC ranges in µg/ml concentrations for three American Type Culture Collection (ATCC) strains: *Staphylococcus aureus* ATCC 29213, *Haemophilus influenzae* ATCC 49247 and *Streptococcus pneumoniae* ATCC 49619.

Methods

A total of eight laboratories were recruited to provide data for this QC investigation. Broth microdilution panels included four cation-adjusted Mueller-Hinton (MH) broth media lots produced by Difco Laboratories (Detroit, MI), Becton Dickinson (BD; Sparks, MD), and Oxoid (Hampshire, United Kingdom [UK]). Four cation-adjusted MH broth lots supplemented with 2-5% lysed horse blood and four lots of Haemophilus Test Medium were also supplied by Difco, BD and Oxoid. GSK1322322 was provided by GlaxoSmithKline (GSK; Collegeville, PA); levofloxacin, linezolid, and azithromycin were acquired from Sigma-Aldrich (St. Louis, MO). Panels were prepared by a certified GMP source (TREK Diagnostics Cleveland, OH). Appropriate inoculum concentrations were established by performing colony counts from the broth microdilution trays which were subcultured onto drug-free agar plates.

Ten replicates of each of three ATCC strains produced 960 MIC values for GSK1322322. *S. aureus* ATCC 29213 was incubated at 16-20 hours at 35°C in ambient air according to CLSI document M07-A8 (2009). All sites were provided verbal and written instructions including photographs of panels for reading GSK1322322 MIC endpoints. Sites were informed that this drug may have a 1 to 3 well trailing endpoint and a 100% complete inhibition of growth should be recorded as the MIC endpoint. Some antimicrobials require 24 hour incubation for a more defined MIC endpoint. In this study, laboratories were instructed to place the panels back in the incubator after the initial 16-20 hour reading and incubate the panels to a total of 24 hours. Panels were read again at 24 hours.

Results

- Colony counts were performed on the panels with the average colony counts for the participating centers ranging from 1.7 X 10^5 to 8.1 X 10^5 CFU/ml for *S. aureus* ATCC 29213; 1.4 X 10^5 to 10.4 X 10^5 CFU/ml for *H. influenzae* ATCC 49247; and 0.4 X 10^5 to 3.8 X 10^5 CFU/ml for *S. pneumoniae* ATCC 49619.
- The results of the *S. aureus* ATCC 29213 testing from eight laboratories are shown in Table 1 and Figure 1 (16 to 20 hours incubation). Using M23 criteria to establish MIC ranges, 95.6% of all results were within the proposed limits of 1 – 4 µg/ml.
- Reading the *S. aureus* ATCC 29213 at 24 hours of incubation resulted in 99.6% of all reported results within the proposed limits of 1 – 4 µg/ml (data not shown). Although 24 hours of incubation resulted in more defined endpoints, the reference time of 16 to 20 hours provided acceptable ranges.
- *H. influenzae* ATCC 449247 MIC results are shown in Figure 2 with 100.0% of results within the proposed limits of 0.5 – 4 µg/ml. A four dilution range of is proposed due to the "shoulder" at 2 µg/ml, which has 70.9% of MIC values compared to the number of occurrences at the mode of 1 µg/ml.
- Figure 3 shows *S. pneumoniae* ATCC 49619 MIC results with a proposed range of 0.12 – 0.5 µg/ml. This range included 99.4% of reported results for GSK1322322.

Results-Continued

- All internal control results for levofloxacin and azithromycin were within the CLSI published range. One laboratory reported six linezolid control values outside of the published range for *S. aureus* ATCC 29213. Acceptable levofloxacin values were utilized as a valid control agent for *S. aureus.*
- There was no significant difference in performance among lots of Mueller-Hinton, HTM, or Mueller-Hinton with blood when testing GSK1322322 (see Table 1, as an example). The modal GSK1322322 MIC was the same for all media lots, regardless of QC strain tested (2 µg/ml for *S. aureus*, 1 µg/ml for *H. influenzae,* and 0.25 µg/ml for *S. pneumoniae*).

Table 1. Media lot comparisons and inter- and intra-laboratory comparisons of GSK1322322 MIC results versus *S. aureus* ATCC 29213 with 16-20 hour incubation.

MIC (µg/ml)	Occurrences by lot:				Laboratory code (occurrences):								Total
	A	B	C	D	A	B	C	D	E	F	G	H	
0.25													
0.5			14		2					10	2		14
1	15	15	18	15	22	1	8			30	2		63[a]
2	64	65	48	63	16	39	32	40	40		33	40	240[a]
4	1			2							3		3[a]
8													
Total	80	80	80	80	40	40	40	40	40	40	40	40	320
Mode	2	2	2	2	1	2	2	2	2	1	2	2	2
Geomean	1.8	1.8	1.3	1.8	1.3	2.0	1.7	2.0	2.0	0.8	1.9	2.0	1.7
Range	3	2	3	3	3	2	2	1	1	2	4	1	4

a. 95.6% of qualified results in proposed QC range (1 – 4 µg/ml)

Table 2. Proposed GSK1322322 broth microdilution MIC quality control ranges.

QC organism (ATCC no.)	GSK1322322 proposed range for BMD (MIC in µg/ml; % in proposed range)
S. aureus ATCC 29213	1 – 4 (95.6)
H. influenzae ATCC 49247	0.5 – 4 (100.0)
S. pneumoniae ATCC 49619	0.12 – 0.5 (99.4)

Figures 1 – 3.

Figure 1. GSK1322322 MIC distributions for *S. aureus* ATCC 29213 at 16-20 hours incubation

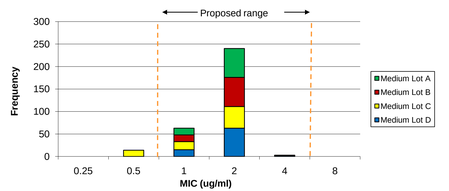


Figure 2. GSK1322322 MIC distributions for *H. influenzae* ATCC 49247

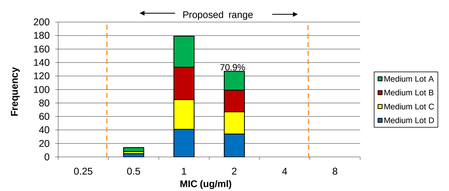
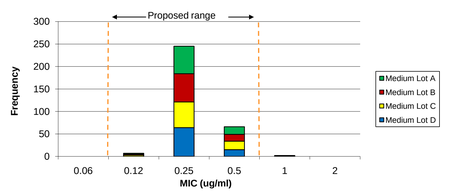


Figure 3. GSK1322322 MIC distributions for *S. pneumoniae* ATCC 49619



Conclusions

- The proposed QC ranges for broth microdilution methods showed that GSK1322322 has generally good inter- and intra-laboratory reproducibility for the commonly utilized control strains: *S. aureus* ATCC 29213, *H. influenzae* ATCC 49247 and *S. pneumoniae* ATCC 49619 (see Table 2).
- These proposed ranges were presented to the CLSI quality control working group in June 2010 and all proposed ranges were approved by the AST subcommittee as stated here.
- This study established QC ranges that can be utilized to support accurate testing for susceptibility of GSK1322322 during clinical trials and continued product development.
- GSK1322322 appears to be a potentially useful treatment option for infections caused by MDR staphylococci, including methicillin-resistant *S. aureus* (MRSA) and *S. pneumoniae*.

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