GSK2140944 MIC Quality Control Ranges Using a Multi-laboratory Study Design

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ABSTRACT

Objectives: To conduct a study to establish MIC quality control (QC) ranges for GSK2140944, a type II topoisomerase inhibitor, using the reference CLSI broth microdilution (BMD) method. This antimicrobial is being developed for the treatment of conventional and biothreat pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA).

Methods: An eight laboratory study design was compliant with CLSI M23-A3 guidelines. Four QC strains were tested (*S. aureus* ATCC 29213 [SA], *E. coli* ATCC 25922 [EC], *H. influenzae* ATCC 49247 [HI], and *S. pneumoniae* ATCC 49619 [SP]) using three media lots (three manufacturers) of cation-adjusted Mueller-Hinton broth (MHB), Haemophilus Test Medium (HTM) and MHB with 5% lysed horse blood. Ten replicate tests were performed for each QC organism generating 320 BMD values/QC strain (1,280 total). Levofloxacin, linezolid and azithromycin were used as control agents.

Results: The table lists the proposed MIC QC ranges for GSK2140944. A four $\log_2$ dilution range was only required for SA with GSK2140944 due to a dominant "shoulder" MIC at 0.25 mg/L, which had 61.7% of the MIC values compared to the modal occurrences at 0.5 mg/L. A range was established for GSK2140944 with *E. coli* of 1 – 4 mg/L which included all reported results and a solid mode (255 of 320 results) at 2 mg/L. Only a three doubling dilution range was necessary for both HI and SP to also include all reported results. No significant differences were noted among media lots for GSK2140944. Only one value of 1,120 generated control results was outside of the CLSI published QC ranges. All GSK2140944 values were within the proposed QC ranges for all processed organisms. The CLSI Subcommittee on Antimicrobial Susceptibility Testing approved these QC ranges in January 2012 for publication after the selection of the compound's chemical name.

Conclusions: Proposed MIC QC ranges for GSK2140944 should accurately guide clinical or reference laboratories participating in the testing of clinical trial isolates, and facilitate the regulatory review process for this investigational antimicrobial combination exhibiting a unique mode of action.

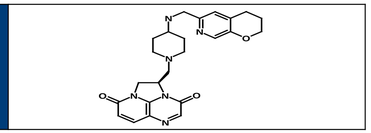
Abstract Table

QC organism (ATCC no.)	Proposed QC ranges for BMD tests (MIC in mg/L; % in proposed range) GSK2140944
S. aureus ATCC 29213	0.12 – 1 (100.0)
E. coli ATCC 25922	1 – 4 (100.0)
H. influenzae ATCC 49247	0.25 – 1 (100.0)
S. pneumoniae ATCC 49619	0.06 – 0.25 (100.0)

INTRODUCTION

GSK2140944 (**Figure 1**) is a bacterial type II topoisomerase inhibitor that has demonstrated *in vitro* activity against the key causative pathogens of community-acquired bacterial pneumonia, acute bacterial skin and skin structure infections (including methicillin-resistant *Staphylococcus aureus* [MRSA]) and biothreat pathogens, including against isolates resistant to existing classes of antimicrobials. GSK2140944 selectively inhibits bacterial DNA replication by interacting with the GyrA subunit of bacterial DNA gyrase and the ParC subunit of bacterial topoisomerase IV in a manner that is distinct from fluoroquinolones. This highly specific interaction to bacterial topoisomerases is evidenced by weak inhibition of human topoisomerase II, supporting the selective activity of GSK2140944 against the bacterial targets. A Clinical Laboratory and Standards Institute (CLSI) M23 style quality control (QC) study was performed to establish disk diffusion and broth microdilution QC ranges for five bacterial strains to assist clinical laboratories in monitoring the activity of this compound during ongoing clinical trials.

Figure 1. Chemical structure of GSK2140944.



MATERIALS AND METHODS

Eight laboratories were used in these two separate studies to establish a QC range. These laboratories were experienced microbiology facilities and each followed the CLSI procedures for disk diffusion and broth microdilution methods.

For MIC tests, reference frozen-form broth microdilution panels were prepared by ThermoFisher Scientific (Cleveland, Ohio, USA) according to Good Manufacturing Practice (GMP) guidelines and shipped frozen to all sites. Panels contained four lots of cation-adjusted Mueller-Hinton broth (Oxoid, Hampshire UK; BBL, Sparks, Maryland, USA; and Difco [two lots], Detroit, Michigan, USA). Also, panels containing four lots of Haemophilus Test Medium and four lots of Mueller-Hinton broth supplemented with 2.5-5% lysed horse blood were provided by the same vendors. Azithromycin, levofloxacin, and linezolid were utilized as control agents. For broth microdilution testing, each laboratory tested 10 replicates of *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Haemophilus influenzae* ATCC 49247 and *Streptococcus pneumoniae* ATCC 49619. Colony counts of the inoculum were performed on drug-free agar media and resulted in the following average counts by QC organism: *S. aureus* ATCC 29213 (3.2 x 10^5 CFU/ml), *E. coli* ATCC 25922 (3.6 x10^5 CFU/ml), *H. influenzae* ATCC 49247 (5.0 x10^5 CFU/ml) and *S. pneumoniae* ATCC 49619 (2.7 x 10^5 CFU/ml).

For disk diffusion tests, two lots of 10-µg GSK2140944 disks were manufactured by two companies: MAST Group, Merseyside, UK (lot #309972) and BD, Franklin Lakes, New Jersey, USA (lot #2340189). Single lots of comparator disks from BD were used: azithromycin 15-µg (lot #2304320), levofloxacin 5-µg (lot #2272187) and linezolid 30-µg (lot #2291112). Three manufacturers (Remel, Lenexa, Kansas; Hardy Diagnostics, Santa Maria, California; and BBL) were used to produce lots of Mueller-Hinton agar (lot #302846, #13042, #2356035) and Mueller-Hinton agar with 5% sheep blood (lot #304123, #H12361, #3024248). For disk diffusion tests, each laboratory tested 10 replicates of *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *S. pneumoniae* ATCC 49619.

RESULTS

- The GSK2140944 broth microdilution MIC and disk diffusion QC results are summarized as CLSI approved ranges in **Table 1**. All *S. aureus* ATCC 29213 MIC results were included in the approved 0.12 – 1 mg/L range (**Figure 2A**). The number of MIC values at 0.25 mg/L represented 61.7% of the number of modal MIC at 0.5 mg/L, therefore, a four dilution range was required per CLSI M23-A3 document. All *E. coli* ATCC 25922 results were within the approved range of 1 – 4 mg/L with a clear mode at 2 mg/L (**Table 1**, **Figure 2B**). For *H. influenzae* ATCC 49247 and *S. pneumoniae* ATCC 49619, all GSK2140944 MIC results were within the approved three doubling dilution ranges (**Table 1**; **Figures 2C** and **2D**).
- All broth media lots and laboratories failed to exhibit any skewing of MIC results and all modal values were within one MIC dilution step, regardless of the QC strain tested. Only one MIC value among 1,120 control drug results was outside of the CLSI published range.
- The zone diameters reported by eight laboratories for *E. coli* ATCC 25922 are shown in **Figure 2A**; and proposed ranges are found in **Table 1**. Using M23-A3 criteria, a seven mm range of 19 – 25 mm included only 91.3% of reported zone diameters. By adding 1 mm to the range (19 – 26 mm), 95.6% of data was included and was considered acceptable. The CLSI antimicrobial susceptibility committee (January 2014) approved a QC range of 18 – 26 mm (98.7% of results). The zone diameters reported for *S. aureus* ATCC 25923 produced a QC range of 23 – 29 mm (7 mm range) which included 96.4% of these results (**Figure 3B**). The Range Finder programme recommended a range of 24 – 29 mm, but identified Laboratory G as an outlier laboratory. Deleting that laboratory and recalculating the range resulted in the same range, but included 99.3% of results for this QC strain. A range of 22 – 28 mm was proposed for *S. pneumoniae* ATCC 49619 and included 99.4% of all zone diameters. However, the Range Finder programme determined an outlier site and could be deleted from analysis (**Figure 3C**), thus providing a 22-28 mm range that included all reported zone diameters.
- *H. influenzae* ATCC 49247 was tested by disk diffusion but medium variations prevented a QC range from being established. The control disks (azithromycin, levofloxacin, and linezolid) provided valid internal quality assurance for this study with 99.5% of reported participant zones found within the CLSI published QC ranges.

Table 1. CLSI approved quality control (QC) ranges of GSK2140944 broth microdilution and disk diffusion susceptibility tests.

QC organism	Broth microdilution: Approved MIC range (mg/L)	Broth microdilution: % results in approved range	Disk diffusion zone diameters: Approved zone range (mm)	Disk diffusion zone diameters: % results in approved range
S. aureus ATCC 29213	0.12 – 1	100.0	NA[a]	NA[a]
S. aureus ATCC 25923	NA[a]	NA[a]	23 – 29 (24 – 29)[b]	96.4 (96.4)[b]
E. coli ATCC 25922	1 – 4	100.0	18 – 26 (17 – 27)[b]	98.7 (99.6)[b]
H. influenzae ATCC 49247	0.25 – 1	100.0	NA[a]	NA[a]
S. pneumoniae ATCC 49619	0.06 – 0.25	100.0	22 – 28	99.4

a. NA = not applicable
b. Range Finder results in parenthesis, if different

Figure 2. CLSI approved MIC quality control ranges for GSK2140944.

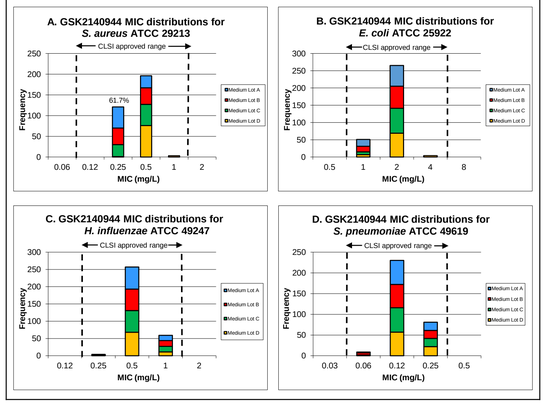
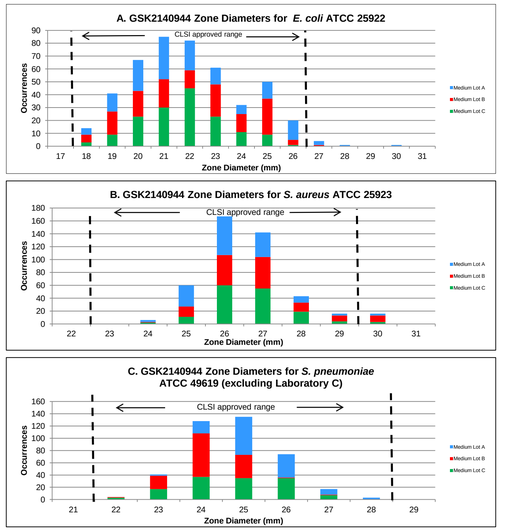


Figure 3. CLSI approved disk diffusion quality control ranges for GSK2140944.



CONCLUSIONS

- These results (**Table 1**) from two multi-laboratory investigations provide initial GSK2140944 QC ranges for routine susceptibility testing using disk diffusion and broth microdilution methods, as this new bacterial type II topoisomerase inhibitor is developed for oral and intravenous treatment of bacterial infections.
- The CLSI Subcommittee on Antimicrobial Susceptibility Testing approved these MIC QC ranges in January 2012 and disk diffusion zone QC ranges in January 2014 for publication after the selection of the compound's chemical name.

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