F-280 Analysis of MIC Testing Methods and Variables for GSK2140944 and Levofloxacin Against Gram-negative and Gram-positive Bacterial Isolates

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Revised Abstract

Background: GSK2140944 is an antimicrobial that inhibits bacterial DNA gyrase and topoisomerase IV by a novel mechanism and is being developed as an oral and IV treatment of Gram-positive (including MRSA) and biothreat pathogens. This study was conducted to determine the effect of various testing parameters on in vitro activity of GSK2140944 against relevant pathogens. **Methods:** 10 *Staphylococcus aureus*, 10 *Streptococcus pneumoniae*, 10 *Haemophilus influenzae*, 5 *Escherichia coli*, and QC strains were tested by 3 methods: CLSI broth microdilution (BMD), macrodilution and agar dilution (AD). The effect of media was determined using BMD and AD methods with cation adjusted Mueller Hinton broth (CAMHB) and agar (MHA) and IsoSensitest Broth (ISB) and agar (ISA) (for *S.aureus* and *E. coli*), CAMHB+5% lysed horse blood (LHB), ISB + 5% LHB, MHA+5% Sheep Blood, MHA+5% defibrinated horse blood (DHB)+20 mg/L NAD and ISA+5% DHB (for *S. pneumoniae*), Haemophilus Test Medium (HTM), CAMHB+5% LHB +20 mg/L NAD and ISB+5% LHB +20 mg/L NAD (for *H. influenzae*). The following variables were studied by BMD and AD: temperature, incubation time, atmosphere and inoculum concentration. The following variables were studied by BMD only: pH, calcium, magnesium, zinc, potassium, thymidine, polysorbate 80 (P80), albumin, serum and lung surfactant. **Results:** There was good correlation of broth methods. AD MICs were similar with exception of GSK2140944 for *S. aureus* (AD MICs 1.5 dilutions higher compared to BMD MICs). Variables that affected BMD MICs are shown in the table. Lung surfactant and 0.002% P80 did not affect GSK2140944 MICs. AD GSK2140944 MICs were increased with high inoculum concentration. *S. aureus* AD GSK2140944 and levofloxacin MICs were increased with CO^2 incubation.

Bacteria	Testing Variable	BMD MICs compared to reference BMD MICs (average dilution difference)	
		GSK2140944	Levofloxacin
S. aureus	Inoculum 10^7CFU/mL)	↑ 6	↑ 0.7
E. coli, S. pneumoniae, H. influenzae	Inoculum (10^5CFU/mL)	↑ >4	↑ >5
S. aureus, E. coli[2]	pH 5.5	↑ 1.4	↑ 0.3, ↑ 0.6
S. pneumoniae	pH 6.5	↑ 1.8	↑ 0.4
E. coli, S. pneumoniae, H. influenzae	pH 8.5	↓ 1.2, ↓1.6, ↓2.6	0, ↓ 0.2, ↓0.1
S. pneumoniae	40ºC	↓ 1.04	↓ 0.2
H. influenzae	40ºC	↓ 2.4	↑ 0.1
S. pneumoniae	CO^2 (10%)	↑ 1.01	↑ 0.5
S. pneumoniae, H. influenzae	Serum (50%)	↓ 1.6, ↓ 1.1	↑ 0.7, ↓0.1

[2]No growth for *S. pneumoniae* and *H. influenzae*

Conclusions: When performing susceptibility testing with GSK2140944 it is important to control the inoculum concentration and pH. An increased incubation temperature and addition of serum will also affect *S. pneumoniae* and *H. influenzae* MICs.

Introduction

- GSK2140944 is an antimicrobial that inhibits bacterial DNA gyrase and topoisomerase IV by a novel mechanism and is being developed as an oral and IV treatment of Gram-positive (including MRSA) and biothreat pathogens.
- The effect of various testing parameters on the *in vitro* activity of the antimicrobial agent, GSK2140944, and a comparative agent, levofloxacin, were tested against 5 *E. coli*, 10 *H. influenzae*, 10 *S. aureus* and 10 *S. pneumoniae* (Tables 1 and 2).

Methods

Table 1. Reference Methods Summary

Organism (n)	Method	Final Inoculum Preparation (from 0.5 McFarland)	Media Used	Incubation Time (hours)	QC Organism (ATCC No.)	GSK2140944 Concentration range (µg/mL)
E. coli (5)	Broth microdilution	0.5 mL to 11 mL	CAMHB	20	25922	0.03 – 32
	Agar dilution	1 mL to 9 mL's	MHA			0.06 – 8
	Macro dilution	0.2 mL to 20 mL's	CAMHB			0.03 – 16
S. aureus (10)	Broth microdilution	0.5 mL to 11 mL	CAMHB		29213	0.03 – 32
	Agar dilution	1 mL to 9 mL's	MHA			0.06 – 8
	Macro dilution	0.2 mL to 20 mL's	CAMHB			0.03 – 16
S. pneumoniae (10)	Broth microdilution	2 mL's to 11 mL's	CAMHB + 5% LHB	24	49619	0.03 – 32
	Agar dilution	1 mL to 9 mL's	MHA + 5% SB			0.12 – 4
	Macro dilution	0.725 mL to 20 mL's	CAMHB + 5% LHB			0.06 – 2
H. influenzae (10)	Broth microdilution	0.5 mL to 11 mL	HTM broth	24	49247	0.06 – 64
	Macro dilution	0.2 mL to 20 mL's				0.12 – 4

CAMHB – Cation adjusted Mueller Hinton broth
MHA –Mueller Hinton agar
HTM – Haemophilus Test Medium
LHB – Lysed Horse Blood

Table 2. Variables Studied

Variable Description	Method Tested	Specific Variables Tested
Temperature	Broth microdilution and agar dilution	30, 35, and 40ºC
Incubation time	Broth microdilution and agar dilution	16, 20, 24 and 48 hours
Atmospheric conditions	Broth microdilution	Ambient, 5% and 10% CO_2
Broth Comparison	Broth microdilution	CAMHB, ISB, LHB, BSB, HTM, EUB, BSB-NAD
Agar Comparison	Agar dilution	SBA, EUA, BSA, MHA and ISA
Inoculum	Broth microdilution and agar dilution	10^3, 10^4, 10^5, 10^6, 10^7 CFU/mL or CFU/spot
Calcium	Broth microdilution	5.7, 22.1, 48.5 and 102.7 mg/L
Magnesium	Broth microdilution	3.1, 10.4, 27.9 and 56.5 mg/L
pH	Broth microdilution	5.56, 6.46, 7.24 and 8.57
Serum	Broth microdilution	25% and 50%
Albumin	Broth microdilution	4 mcg/dL
Polysorbate 80	Broth microdilution	0.002%
Thymidine	Broth microdilution	1 and 5 mg/L
Zinc	Broth microdilution	2, 5 and 10 mmol/L
Potassium	Broth microdilution	12.5, 25 and 50 mmol/L
Lung Surfactant	Broth microdilution	1% and 5%

(See Tables 3-6 for abbreviation definitions)

Results

GSK2140944 Geometric Mean MICs of Reference Method

Figure 1. Geometric Mean MICs (µg/mL) of GSK2140944 against 5 *E. coli* by CLSI Broth Microdilution Performed Over Study Testing Period

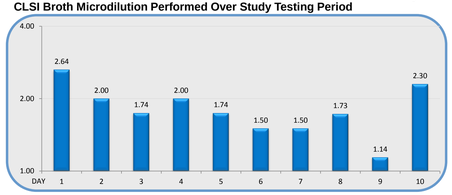


Figure 2. Geometric Mean MICs (µg/mL) of GSK2140944 against 10 *S. aureus* by CLSI Broth Microdilution Performed Over Study Testing Period

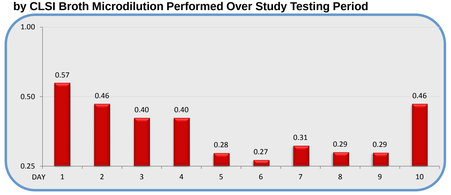


Figure 3. Geometric Mean MICs (µg/mL) of GSK2140944 against 10 *H. influenzae* by CLSI Broth Microdilution Performed Over Study Testing Period

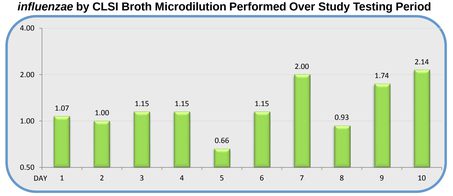
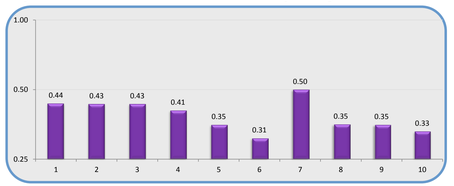


Figure 4. Geometric Mean MICs (µg/mL) of GSK2140944 for 10 *S. pneumoniae* by CLSI Broth Microdilution Performed Over Study Testing Period



MIC methods - Broth Microdilution (BMD), Broth Macrodilution (MD) and Agar Dilution (AD) (Tables 3-6)

- **BMD MICs did not vary by more than one doubling dilution (Figures 1-4)**
- **MD MICs were not significantly impacted compared to BMD MICs (Tables 3-6)**
- **AD MICs were 1.23 dilutions higher compared to BMD MICs against *S. aureus* (Table 4); all other AD MICs were not significantly impacted.**
- **Levofloxacin results were comparable to GSK2140944**

Table 3. Mean Dilution Difference MICs of GSK2140944 against 5 *E. coli* as determined by broth microdilution, macrodilution and agar dilution methodologies

CLSI Reference Method	Comparative Method	n	Mean Dilution Difference (Comparative - CLSI Reference)
CAMHB BMD	CAMHB MD	15	0.13
CAMHB BMD	MHA AD	15	-0.13
MHA AD	ISA AD	15	-0.20
CAMHB BMD	ISB BMD	15	0.47

CAMHB – Cation adjusted Mueller Hinton broth
MHA –Mueller Hinton agar
ISA – IsoSensitest agar
ISB – IsoSensitest broth

Table 4. Mean Dilution Difference MICs of GSK2140944 against 10 *S. aureus* as determined by broth microdilution, macrodilution and agar dilution methodologies

CLSI Reference Method	Comparative Method	n	Mean Dilution Difference (Comparative - CLSI Reference)
CAMHB BMD	CAMHB MD	30	0.51
CAMHB BMD	MHA AD	30	1.23
MHA AD	ISA AD	30	0.67
CAMHB BMD	ISB BMD	30	0.37

CAMHB – Cation adjusted Mueller Hinton broth
MHA –Mueller Hinton agar
ISA – IsoSensitest agar
ISB – IsoSensitest broth

Table 5. Mean Dilution Difference MICs of GSK2140944 against 10 *H. influenzae* as determined by broth microdilution, macrodilution and agar dilution methodologies

CLSI Reference Method	Comparative Method	n	Mean Dilution Difference (Comparative - CLSI Reference)
HTM BMD	HTM MD	30	0.17
HTM BMD	BSB BMD	30	-0.17
HTM BMD	EUB BMD	30	0.00

HTM – Haemophilus Test Medium
BSB – IsoSensitest broth + 5% lysed horse blood + 20 mg/L NAD
EUB – Cation adjusted Mueller Hinton broth + 5% lysed horse blood + 20 mg/L NAD

Table 6. Mean Dilution Difference MICs of GSK2140944 for 10 *S. pneumoniae* as determined by broth microdilution, macrodilution and agar dilution methodologies

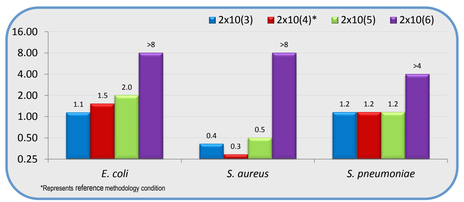
CLSI Reference Method	Comparative Method	n	Mean Dilution Difference (Comparative - CLSI Reference)
LHB BMD	LHB MD	30	-0.37
LHB BMD	SBA AD	30	0.54
SBA AD	BSA AD	30	0.33
SBA AD	EUA AD	30	0.23
LHB BMD	BSB BMD	30	-0.07

LHB – Cation adjusted Mueller Hinton broth + 5% lysed horse blood
SBA – Mueller Hinton agar + 5% sheep blood
BSA – IsoSensitest Agar + 5% defibrinated horse blood
EUA – Mueller Hinton agar + 5% defibrinated horse blood + 20 mg/L NAD
BSB – IsoSensitest broth + 5% lysed horse blood

Agar Dilution – Effect of Testing Variables (Figure 5, 10)

- **Of all agar dilution variables tested, inoculum concentration (Figure 5) and CO_2 incubation (Figure 10) were the only variables to show a significant impact on the MIC of GSK2140944**

Figure 5. Geometric Mean MICs (µg/mL) of GSK2140944 by agar dilution against all study strains utilizing varying inoculum concentration levels (2x10^3-2x10^6 cfu/spot)



Broth Microdilution – Effect of Testing Variables (Figures 6-10)

- **The majority of MICs were within one doubling dilution compared to the reference BMD MICs. The variables that impacted the MICs (as shown in Figures 6-10) were inoculum concentration, pH, incubation temperature, atmospheric condition and the addition of human serum**
- **The levofloxacin MICs were similarly impacted by the same variables impacting GSK2140944**

Figure 6. Geometric Mean MICs (µg/mL) of GSK2140944 by broth microdilution against all study strains utilizing varying inoculum concentration levels (10^4-10^7 cfu/mL)

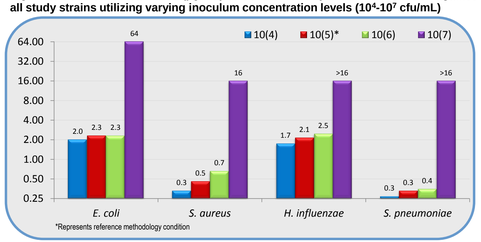


Figure 7. Geometric Mean MICs (µg/mL) of GSK2140944 by broth microdilution against all study strains utilizing varying levels of pH

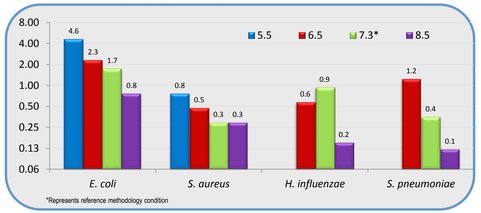


Figure 8. Geometric Mean MICs (µg/mL) of GSK2140944 by broth microdilution against 10 *H. influenzae* and 10 *S. pneumoniae* utilizing varying incubation temperatures (degrees Celsius)

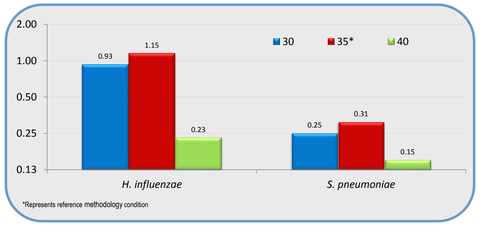


Figure 9. Geometric Mean MICs (µg/mL) of GSK2140944 by broth microdilution against 10 *H. influenzae* and 10 *S. pneumoniae* utilizing varying levels of human serum

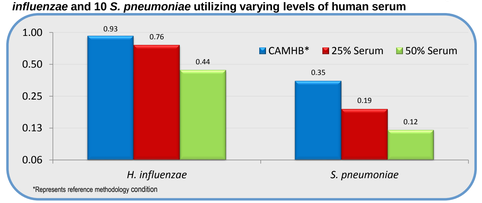
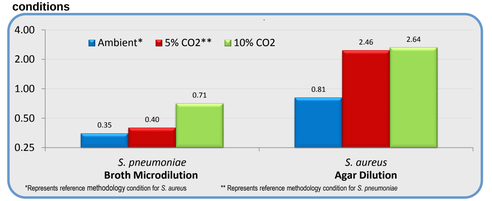


Figure 10. Geometric Mean MICs (µg/mL) of GSK2140944 against 10 *S. pneumoniae* (by broth microdilution) and 10 *S. aureus* (by agar dilution) using different atmospheric incubation conditions



Quality Control (QC)

- **Established CLSI QC ranges for GSK2140944 were available after the completion of this study.**
- **All but 2 GSK2140944 MIC results for the QC strains were within the recently established CLSI QC ranges.**
- **All MIC results for levofloxacin were within the established CLSI QC ranges**
- **The variables that impacted the MIC results of the QC strains were similar to those that affected the study isolate MIC results**

Table 7. CLSI Reference Method Quality Control Result Summary (number of results at each MIC)

Quality Control Strain	GSK2140944 MIC (µg/mL)							Levofloxacin MIC (µg/mL)							
	0.06	0.12	0.25	0.5	1	2	4	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2
E. coli ATCC 25922					4	4		3	2	3						
S. aureus ATCC 29213			6	3							2	3	4			
H. influenzae ATCC 49247				1	8	1			8	1						
S. pneumoniae ATCC 49619		1	7	1										5	4	

CLSI Expected Range

Conclusions

- **Lung surfactant and 0.002% P80 did not affect GSK2140944 MICs.**
- **GSK2140944 reference BMD geometric mean MICs, over a ten day period, varied as much as 1.5 µg/mL for *E. coli* and *H. influenzae*.**
- **Most variables had no or only a minor effect on GSK2140944 MICs.**
- **Variables shown to impact GSK2140944 MIC results by broth microdilution were high inoculum concentration, atmospheric condition, addition of human serum, incubation temperature and pH levels.**
- **Agar dilution GSK2140944 MICs were increased with high inoculum concentration and CO2 incubation.**
- **When performing susceptibility testing with GSK2140944, therefore, it is important to be aware of these differences and control these particular variables if possible according to standardized methods.**

Acknowledgement

This project was supported by GlaxoSmithKline Pharmaceuticals and has been funded in whole or in part with Federal funds awarded by the Defense Threat Reduction Agency under Agreement No. HDTRA1-07-9-0002.