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Analysis of MIC Testing Methods and Variables for GSK1322322 and Comparators Against *Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus pyogenes* and *Moraxella catarrhalis*

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Abstract

Background: GSK1322322 is an antibacterial agent with a novel mode of action (inhibition of peptide deformylase function) and activity against multi-drug resistant respiratory and skin pathogens. This study was conducted to determine the effect of various testing parameters on *in vitro* activity of GSK132232 against relevant pathogens. **Methods:** MICs were determined for 5 *M. catarrhalis*, 12 *H. influenzae*, 14 *S. pneumoniae*, 15 *S. pyogenes*, and QC strains 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 *M. catarrhalis*, 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 streptococci, 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, inoculum concentration and by BMD only: pH, calcium, magnesium, zinc, potassium, thymidine, polysorbate 80, albumin, serum, and lung surfactant. **Results:** There was good correlation of MICs by all MIC methods. Variables that most affected BMD MICs are shown in the table. Lung surfactant and 0.002% P80 had no effect on GSK1322322 activity. GSK1322322 AD MICs were affected by atmosphere, incubation time and inoculum concentration for *M. catarrhalis*, and high inoculum concentration for streptococci.

Bacteria	Testing Variable	GSK1322322 BMD MICs compared to reference BMD MICs (average dilution difference)	
		GSK1322322	Levofloxacin or Linezolid[1]
M. catarrhalis	Inoculum (10^6CFU/mL and 10^7CFU/mL)	↑ 1.2 and 2.2	No difference and ↑ 1.0
H. influenzae	Inoculum (10^7CFU/mL)	↑ 1.3	↑ 0.3
S. pyogenes, S. pneumoniae	Inoculum (10^7CFU/mL)	↑ >4	↑ >3
S. pneumoniae	Inoculum (10^6CFU/mL)	↓ 1.3	↓ 0.8
M. catarrhalis	Calcium (5.67 ug/mL) and Magnesium (3.1 ug/mL)	↓1.6	↓ 1.4
H. influenzae[2]	Zinc (+5 and 10 mmol/L)	↓1.1 and 0.8	No difference and ↓0.9
H. influenzae, M.catarrhalis	pH 5.5	No growth	No growth
H. influenzae, S. pyogenes, S. pneumoniae[2]	40°C	↓ 1.8	↓ 0.5
M. catarrhalis	Thymidine (+5 mcg/mL)	↑1.41	↑ 0.8
M. catarrhalis	Serum (25% and 50%)	↑1.61 and 2.21	No difference and ↑1.6
H. influenzae	Serum (25% and 50%) and Albumin (4 mg/dL)	↑ 2	↑ 0.5

[1]Linezolid for *S. pneumoniae* and *S. pyogenes*; Levofloxacin for *H. influenzae* and *M. catarrhalis*
[2]No growth for *M. catarrhalis*

Conclusions: When performing susceptibility testing with GSK1322322 it is important to control the inoculum concentration. An increased incubation temperature, thymidine, cation concentration and addition of serum or albumin may also affect MICs.

Introduction

- The effect of various testing parameters on the *in vitro* activity of the antimicrobial agent, GSK1322322, and a comparative agent, levofloxacin (against MC and HF) or linezolid (against SP and PY), were tested against 5 *M. catarrhalis*, 12 *H. influenzae*, 14 *S. pneumoniae* and 15 *S. pyogenes*.
- The effect of lung surfactant on the *in vitro* activity of GSK1322322 was studied with 15 methicillin susceptible *S. aureus* and 15 methicillin resistant *S. aureus*
- Quality control strains *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 449766 and *H. influenzae* NCTC 8648 were tested.

Methods

Reference Methods Summary

Organism	Method	Final Inoculum Preparation (from 0.5 McFarland)	Media Used	Incubation Time (hours)	QC Organism (ATCC No.)	GSK1322322 Concentration range (mcg/mL)	Levofloxacin / Linezolid Conc. range (mcg/mL)
M. catarrhalis	Broth microdilution	0.75 mL to 11 mL	CAMHB	24	29213	0.03 – 32	0.004 – 4
	Agar dilution[1]	1 mL to 9 mL	MHA			0.06 – 4	0.008 – 0.5
	Macro dilution	0.4 mL to 40 mL	CAMHB			0.03 – 8	0.008 – 1
S. pneumoniae	Broth microdilution	2 mLs to 11 mLs	CAMHB + 5% LHB	24	49619	0.03 – 32	0.004 – 4
	Agar dilution[1]	1 mL to 9 mLs	MHA + 5% SB			0.015 – 4	0.25 – 4
	Macro dilution	0.4 mL to 40 mLs	CAMHB + 5% LHB			0.12 – 16	0.25 – 4
S. pyogenes	Broth microdilution	0.5 mLs to 11 mLs	CAMHB + 5% LHB	24	49619	0.015 – 16	0.015 – 16
	Agar dilution[1]	1 mL to 9 mLs	MHA + 5% SB			0.03 – 8	0.25 – 4
	Macro dilution	0.4 mL to 40 mLs	CAMHB + 5% LHB			0.03 – 8	0.25 – 4
H. influenzae	Broth microdilution	0.5 mL to 11 mL	HTM broth	24	49247, 49766, 8648	0.03 – 32	0.004 – 4
	Macro dilution	0.4 mL to 40 mLs				0.06 – 16	0.004 – 0.25

[1]Agar dilution plates were incubated in 5% CO_2 according to CLSI (there are no CLSI guidelines available for *M. catarrhalis* agar dilution, however, 5% CO_2 is recommended for disk diffusion).

Variables Studied

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

Results

GSK1322322 Geometric Mean MICs of Reference Method

Figure 1. Geometric Mean MICs (μg/mL) of GSK1322322 for 5 *Moraxella catarrhalis* by CLSI Broth Microdilution Performed Over Study Testing Period

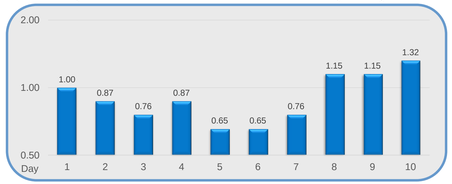


Figure 2. Geometric Mean MICs (μg/mL) of GSK1322322 for 12 *Haemophilus influenzae* by CLSI Broth Microdilution Performed Over Study Testing Period

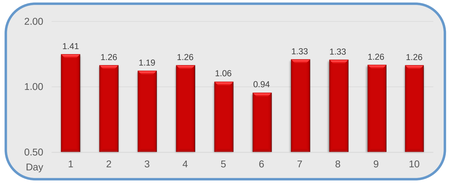


Figure 3. Geometric Mean MICs (μg/mL) of GSK1322322 for 14 *Streptococcus pneumoniae* by CLSI Broth Microdilution Performed Over Study Testing Period

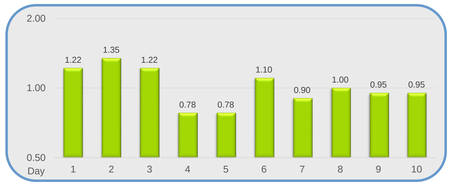
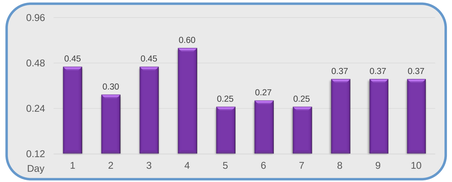


Figure 4. Geometric Mean MICs (μg/mL) of GSK1322322 for 15 *Streptococcus pyogenes* by CLSI Broth Microdilution Performed Over Study Testing Period



GSK1322322 MIC methods - Broth Microdilution, Broth Macrodilution and Agar Dilution (Tables 1-3)

- **BMD MICs did not vary by more than one doubling dilution during the course of the study (Tables 1-3)**
- **Macrodilution MICs differed slightly compared to broth microdilution (BMD) MICs (Tables 1 and 3)**
- **Agar dilution (MH-AD) MICs were slightly higher compared to BMD MICs (Tables 1-3)**

Table 1. Mean Dilution Difference MICs of GSK1322322 for 5 *Moraxella catarrhalis* 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	-0.27
CAMHB BMD	MHA AD	15	-0.67
MHA AD	ISA AD	15	-1.01
CAMHB BMD	ISB BMD	15	-0.81

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

Table 2. Mean Dilution Difference MICs of GSK1322322 for 12 *Haemophilus 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	36	0.09
HTM BMD	BSB BMD	36	-0.08
HTM BMD	EUB BMD	36	-0.03

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 3. Mean Dilution Difference MICs of GSK1322322 for 14 *Streptococcus pneumoniae* and 15 *Streptococcus pyogenes* as determined by broth microdilution, macrodilution and agar dilution methodologies

		S. pneumoniae		*S. pyogenes*	
CLSI Reference Method	Comparative Method	n	Mean Dilution Difference (Comparative - CLSI reference)	n	Mean Dilution Difference (Comparative - CLSI reference)
LHB BMD	LHB MD	42	-0.26	45	-0.16
LHB BMD	SBA AD	42	0.12	45	0.09
SBA AD	BSA AD	42	-0.64	45	0.18
SBA AD	EUA AD	42	-0.21	45	0.02
LHB BMD	BSB BMD	42	0.95	45	-0.42

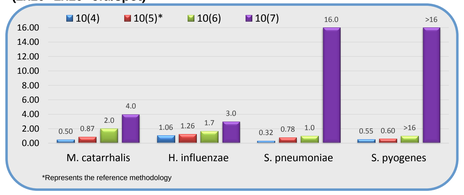
*CAMHB (MC) or HTM (HF) represents the reference methodology
**NG = No Growth

LHB – Cation adjusted Mueller Hinton broth + 5% lysed horse blood
SBA – Cation adjusted Mueller Hinton agar + 5% sheep blood
BSA – IsoSensitest Agar + 5% defibrinated horse blood
EUA – Cation adjusted 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)

- **Of all agar dilution variables tested, inoculum concentration was the only variable to show a significant impact on the MIC of GSK1322322**

Figure 5. Geometric Mean MICs (μg/mL) of GSK1322322 by agar dilution against all study strains utilizing varying inoculum concentration levels ($2x10^3$-$2x10^6$ cfu/spot)

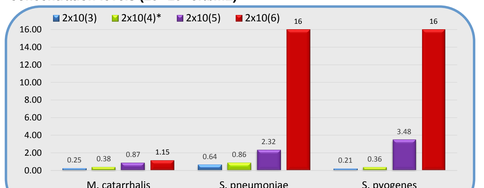


*Represents the reference methodology

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

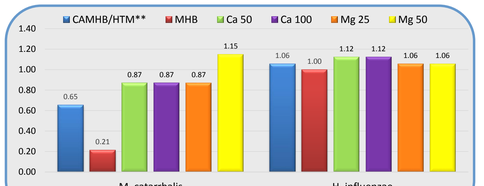
- **The majority of all MICs were within one doubling dilution compared to the reference BMD MICs. The variables that impacted the MICs (as shown below) were inoculum concentration, calcium, magnesium and zinc concentrations, incubation temperature and the addition of human serum and albumin.**

Figure 6. Geometric Mean MICs (μg/mL) of GSK1322322 by broth microdilution against all study strains utilizing varying inoculum concentration levels (10^4-10^7 cfu/mL)



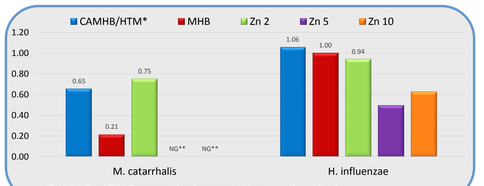
*Represents the reference methodology

Figure 7. Geometric Mean MICs (μg/mL) of GSK1322322 by broth microdilution against 5 *Moraxella catarrhalis* and 12 *Haemophilus influenzae* utilizing varying concentrations of calcium (Ca) (μg/mL) and magnesium (Mg) (μg/mL)



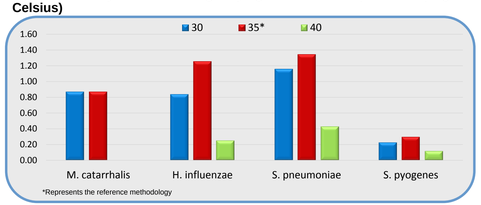
**CAMHB (MC) or HTM (HF) represents the reference methodology

Figure 8. Geometric Mean MICs (μg/mL) of GSK1322322 by broth microdilution against 5 *Moraxella catarrhalis* and 12 *Haemophilus influenzae* utilizing varying concentrations of zinc (2, 5 and 10 μg/mL)



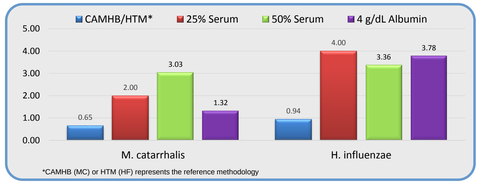
*CAMHB (MC) or HTM (HF) represents the reference methodology **NG = No Growth

Figure 9. Geometric Mean MICs (μg/mL) of GSK1322322 by broth microdilution against all study strains utilizing varying incubation temperatures (degrees Celsius)



*Represents the reference methodology

Figure 10. Geometric Mean MICs (μg/mL) of GSK1322322 by broth microdilution against 5 *Moraxella catarrhalis* and 12 *Haemophilus influenzae* utilizing varying concentrations of human serum and addition of albumin



*CAMHB (MC) or HTM (HF) represents the reference methodology

Quality Control

All GSK1322322 MICs were within the CLSI recommended QC range, with the exception of the following variables:

- **Higher MICs with BMD using inoculum concentration of 10^6 and 10^7 cfu/mL and agar dilution using inoculum concentration of 10^7 and 10^8 cfu/mL**
- **Lower MICs with BMD incubated at 40°C**
- **Lower MICs with a pH concentration of 5.5 and 8.5**
- **Higher MICs with BMD using the addition of thymidine, human serum and albumin**

Table 4. Quality Control Result Summary (number of results at each MIC)

	GSK1322322 MIC (μg/mL)						Levofloxacin (SA & HF) / Linezolid (SP) MIC (μg/mL)								
Quality Control Strain	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
SA29213					12						1	11			
HF49247				7	3			9	1						
HF49766				2	8			10							
HF8648					5	5		9	1						
SP49619	2	3	5	4									2	8	5

CLSI Expected Range

Conclusions

- **Lung surfactant did not affect the GSK1322322 MIC results**
- **Most variables had no or only a minor effect on GSK1322322 MICs.**
- **Variables shown to impact GSK1322322 MIC results were incubation temperature of 40°C, low calcium and magnesium concentrations, inoculum concentration, elevated zinc, addition of albumin and addition of serum.**
- **When performing susceptibility testing with GSK1322322, therefore, it is important to be aware of these differences and control these particular variables if possible according to standardized methods.**

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