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A Multi-Site Study Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI/ISO Broth Microdilution Method for Gepotidacin using Non-Fastidious Gram-Negative Organisms

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ABSTRACT

Background: Gepotidacin (GEP) (Glaxo SmithKline, Collegeville, PA), a novel bacterial type II topoisomerase inhibitor, demonstrates activity against gram-positive and gram-negative pathogens associated with conventional and biothreat infections. A study was conducted at 4 different sites to determine the accuracy and reproducibility of GEP susceptibility testing against non-fastidious gram-negative organisms using the Thermo Scientific™ Sensititre™ dried MIC susceptibility system compared with the CLSI (M07, M100)/ ISO 20776-1, ISO 20776-2 (CLSI/ISO) reference broth microdilution method (BMD). **Materials and Methods:** GEP, in a dilution range of 0.015-64µg/mL, was tested on the Sensititre system against 558 recent clinical and challenge isolates. 15 isolates were also included to assess MIC reproducibility. Microorganisms tested included 109 *Escherichia coli*, 143 *Klebsiella* spp., 107 *Enterobacter* spp., 61 *Proteus* spp., 26 *Serratia marcescens,* 101 *Pseudomonas aeruginosa,* and 1 *Acinetobacter baumannii,* and 25 *Moraxella catarrhalis.* The Sensititre dried MIC susceptibility system was inoculated per manufacturers' instructions. BMD was performed per CLSI/ISO guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within published ranges. **Results:**GEP MIC results for the non-fastidious gram-negative organisms tested on the Sensititre system compared with CLSI/ISO BMD for automated and manual reads resulted in 95.5% and 97% essential agreement (EA; +/- 1 $\log_2$ dilution), respectively. Overall agreement for GEP MIC reproducibility (+/- 1 $\log_2$ dilution of the modal MIC) using automated and manual reads was 98.7% and 99.4%, respectively. **Conclusion:** The Sensititre susceptibility system demonstrates an equivalent level of performance compared to the CLSI/ISO BMD method when testing GEP against non-fastidious gram-negative organisms. The high level of agreement obtained by the Sensititre susceptibility system and the CLSI/ISO BMD method suggests that this is an acceptable method for susceptibility testing of GEP.

INTRODUCTION

Gepotidacin belongs to the triazaacenapathylene class of antibiotics. This agent binds to DNA gyrase and topoisomerase IV in ways that distinguish it from other quinolone antibiotics. Gepotidacin has demonstrated *in vitro* activity and *in vivo* efficacy against gram-positive and gram-negative pathogens for skin and bio-threat infections including isolates resistant to existing classes of antimicrobials.
This *in vitro* comparison study was done to validate the performance of gepotidacin on the commercially manufactured Thermo Scientific Sensititre 18 – 24 hour Dried Susceptibility Plate with the standard reference broth microdilution method recommended by the Clinical and Laboratory Standards Institute and International Organization for Standardization (CLSI/ISO). To establish equivalency for both auto and manual read methodologies, a series of studies were conducted at 4 trial sites including testing of clinical, challenge isolates, reproducibility and quality control isolates.

MATERIALS AND METHODS

- The Sensititre 18-24 hour MIC or breakpoint susceptibility system (Thermo Fisher Scientific, Oakwood Village, OH) is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms.

Gepotidacin was tested against: (Table 1.)

- 461 recent clinical isolates across the four sites
- 97 challenge isolates at a single testing site
- 15 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 1 Quality Control Strain (ATCC)

MATERIALS AND METHODS Cont.

- Colony Counts and purity plates were performed on the inoculums of the clinical, challenge, reproducibility and QC strains on each day of testing.

- Essential agreement was calculated within plus or minus, one two-fold dilution of the Sensititre dried plates with the reference broth microdilution method.

- Each isolate was tested using a:

  - Dried Sensititre 18–24 susceptibility plate containing Gepotidacin (0.015-64µg/ml). The dried plates were set up and tested by both automated and manual reading methodologies according to the manufacturer's instructions.

  - Reference broth microdilution plates were prepared and tested on each isolate according to the current CLSI/ISO standard method.

Table 1. Organisms Tested	Number Tested
Clinical Isolates (4 sites)	461
CDC Challenge Isolates (one site)	97
Reproducibility Isolates (4 sites) (3 x day for 3 days)	15 (540)
ATCC Quality Control Strains (20 replicates of each strain at 4 sites)	1 (80)
TOTAL	**1178**

Quality Control

- Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.

- Colony counts were performed and fell within expected ranges
Reference 2-8X10^5 cfu/ml, Sensititre 5X10^4-5X10^5 cfu/ml

Table 2. Quality Control Strain	Gepotidacin CLSI QC Range (µg/ml)
Escherichia coli ATCC 25922	1-4

Results

Essential agreement for gepotidacin on the Sensititre susceptibility plate compared to the reference microdilution plate was calculated for each read method (Auto and Manual) using the +/- one $\log_2$ dilution standard. Essential agreement rates are shown for non-fastidious gram-negative isolates in **Tables 3 and 4**.

Clinical Isolates and Challenge Organisms

The overall essential agreement for gepotidacin within ±1 $\log_2$ dilution was **97.0%** for the manual method and **95.5%** for the auto read method.

Inter-laboratory Reproducibility

Reproducibility testing results for gepotidacin within ±1 $\log_2$ dilution from the modal MIC was **98.7%** for the auto read method and **99.4%** for the manual read method.

Table 3. Summary Data and % Essential Agreement of Non-Fastidious Gram-Negative Clinical and Challenge Isolates Using the Manual Read Method

The overall essential agreement for gepotidacin within +/- one $\log_2$ dilution, was 97.0% for the manual read method

Combined Total Isolates

Gepotidacin	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Escherichia coli	104	104	102	101	98.1%	97.1%
Klebsiella pneumoniae	94	93	89	88	94.7%	94.6%
Klebsiella oxytoca	43	43	41	41	95.3%	95.3%
Enterobacter cloacae	67	66	66	65	98.5%	98.5%
Klebsiella (Enterobacter) aerogenes	39	39	39	39	100.0%	100.0%
Serratia marcescens	26	26	26	26	100.0%	100.0%
Proteus vulgaris	24	24	24	24	100.0%	100.0%
Proteus mirabilis	36	36	36	36	100.0%	100.0%
Moraxella catarrhalis	25	24	23	22	92.0%	91.7%
Pseudomonas aeruginosa	100	99	95	94	95.0%	94.9%
Total	**558**	**554**	**541**	**536**	**97.0%**	**96.8%**

Table 4. Summary Data and % Essential Agreement of Non-Fastidious Gram-Negative Clinical and Challenge Isolates Using the Auto Read Method

The overall essential agreement for gepotidacin within +/- one $\log_2$ dilution, was 95.5% for the auto read method

Combined Total Isolates

Gepotidacin	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Escherichia coli	104	104	101	100	97.1%	96.2%
Klebsiella pneumoniae	94	93	88	87	93.6%	93.5%
Klebsiella oxytoca	43	43	41	41	95.3%	95.3%
Enterobacter cloacae	67	66	66	65	98.5%	98.5%
Klebsiella (Enterobacter) aerogenes	39	39	37	37	94.9%	94.9%
Serratia marcescens	26	26	24	24	92.3%	92.3%
Proteus vulgaris	24	24	22	22	91.7%	91.7%
Proteus mirabilis	36	36	36	36	100.0%	100.0%
Moraxella catarrhalis	25	23	23	22	92.0%	95.7%
Pseudomonas aeruginosa	100	99	95	94	95.0%	94.9%
Total	**558**	**553**	**533**	**528**	**95.5%**	**95.5%**

RESULTS Cont.

Table 5. Inter-laboratory Reproducibility, % Essential Agreement and ±1 $\log_2$ dilution from the Modal Value

Gepotidacin	Auto Read	Manual Read
Between-site total isolates tested (N)	540	540
Between-site isolates within +/- 1 well from mode (n)	533	537
Between-site reproducibility %	**98.7%**	**99.4%**
Total essential agreement (n)	533	537
Essential agreement %	**98.7%**	**99.4%**

CONCLUSIONS

This study validates that the Sensititre 18–24 hour susceptibility system (both auto read and manual read) demonstrated an equivalent level of performance compared to the (CLSI/ISO) reference broth microdilution plate when testing gepotidacin against non-fastidious gram-negative clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of gepotidacin.

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