thermoscientific

A multi-site study comparing a commercially prepared dried MIC susceptibility system to the CLSI broth microdilution method for Debio 1452 (FAB) using non-fastidious Gram-positive organisms

***S.M. Andrus[1], C. C. Knapp[1], N. M. Holliday[1], S. B. Killian[1], J.M. Lindley[2], J. M. Streit[2], R. K. Flamm[2], B.J. Olson[3], T.R. Fritsche[3], K. Becker[4], E.A. Idelevich[4], E. Scopes[5], A.M. Leonte[5]**

[1]Thermo Fisher Scientific, Cleveland, OH; [2]JMI Laboratories, North Liberty, IA; [3]Division of Laboratory Medicine, Marshfield Clinic, Marshfield WI; [4]University Hospital Münster, Münster Germany; [5]Thermo Fisher Scientific, Basingstoke, UK

ABSTRACT

Background: Debio 1452 (FAB) is a novel selective antimicrobial active against *Staphylococcus* species by inhibiting FabI, an enzyme critical in fatty acid biosynthesis. A 4-site study was performed to determine the accuracy and reproducibility of Debio 1452 susceptibility testing using the Thermo Scientific™ Sensititre™ dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI (M07, 2016) reference broth microdilution method (BMD; ISO 20776-1). Both automated (Thermo Scientific™ Sensititre™ OptiRead™) and manual (Thermo Scientific™ Sensititre™ Vizion™) reading methodologies were employed.
Methods: Debio 1452 (0.0005-2µg/mL) was tested against 484 recent clinical isolates, 114 challenge isolates, and 48 reproducibility isolates. These isolates consisted of 540 *Staphylococcus aureus* (249 methicillin resistant *S. aureus* (MRSA) & 251 methicillin susceptible *S. aureus* (MSSA)), 26 *Staphylococcus haemolyticus,* 22 *Staphylococcus lugdunensis,* 27 *Staphylococcus epidermidis,* 10 *Staphylococcus hominis,* 10 *Staphylococcus simulans,* and 10 *Staphylococcus saprophyticus.* The Sensititre dried MIC susceptibility system was inoculated per manufacturers' instructions and the BMD method was performed per CLSI (M07) and ISO 20776-1 guidelines. Recommended CLSI quality control (QC) organisms were tested daily and were within the published QC ranges.
Results: Comparison of *Staphylococcus* spp. MIC results on the Sensititre system to the CLSI/ISO 20776-1 BMD method for automated and manual reads resulted in 97.3% and 98.3% essential agreement (EA, +/- 1 $\log_2$ dilution) for Debio 1452, respectively. Overall the essential agreements for reproducibility (+/- 1 $\log_2$ dilution of the modal MIC) using automated and manual reads were 100% and 99.3%, respectively.*
Conclusion: This study validates that the Sensititre susceptibility system (both automated and manual read) demonstrated an equivalent level of performance compared to the CLSI/ISO 20776-1 BMD method when testing Debio 1452 against *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. The high level of agreement obtained by the Sensititre susceptibility system and the CLSI/ISO 20776-1 BMD method suggests that this is an acceptable method for susceptibility testing of Debio 1452. Debio 1450, the prodrug of Debio 1452, is currently in late stage clinical development for treatment of staphylococcal infections.
**This abstract has been amended from its original version after further data analysis.*

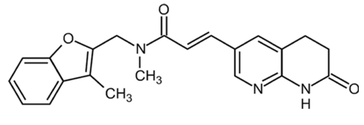
INTRODUCTION AND OBJECTIVES

Debio 1452 (Fab) (Figure 1) is a FabI inhibitor developed by Debiopharm Group, with potent activity against *Staphylococcus aureus* (including methicillin-resistant, MRSA, and methicillin-susceptible, MSSA, isolates) and Coagulase Negative *Staphylococcus* species . Debio 1452's mechanism targets *Staphylococcus* species through inhibition of the staphylococcal FabI enoyl-Acyl carrier protein (ACP) reductase that catalyzes the last step in the elongation process of the fatty acid chain in these bacteria (5).

This *in vitro* multi-site comparison study was done to evaluate the performance of Debio 1452 on the commercially manufactured Sensititre® 18-24 hour susceptibility system, for both automated and manual read, compared against the Clinical Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method (M07, ISO 20776-1).

To establish equivalency between the test and reference methods, a series of studies were conducted and the MIC results obtained using the Sensititre dried plate technology were compared to the MIC results obtained from the CLSI frozen reference plate.

Figure 1: Chemical Structure of Debio 1452



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.
- Debio 1452 was tested against (Table 1):
  - 484 recent clinical isolates (4 sites)
  - 114 challenge isolates (1 site)
  - 48 reproducibility isolates (4 sites)
    S. aureus (MRSA and MSSA), *S. haemolyticus, S. epidermidis*

Table 1. Number of isolates tested

Organisms	Number tested
Clinical Isolates (4 sites)	484
CDC Challenge Isolates (1 site)	114
Reproducibility Isolates (4 sites) (3 x day for 3 days)	12 (432)
CLSI Quality Control Strains (20 replicates of each strain at 4 sites)	1 (80)
TOTAL	**1110**

- Colony Counts were performed on the inoculums of the Clinical, Challenge, Reproducibility, and QC strains on each day of testing
  - Reference Colony Count Range: 2-8X10^5
  - Sensititre Colony Count Range: 5X10^4-5X10^5
- Each isolate was tested using:
  - Dried Sensititre 18–24 susceptibility plate containing Debio 1452 (0.0005-2µg/mL). The dried plates were set up and tested by both automated and manual reading methodologies according to the manufacturer's instructions.
  - CLSI reference broth microdilution plate was prepared and tested on each isolate according to the current Clinical Laboratory Standards Institute standard method (CLSI M07/M100 and ISO 20776-1).
- Reproducibility testing consisted of 12 isolates (*S. aureus* (MRSA and MSSA), *S. haemolyticus, S. epidermidis*) tested at 4 sites on the Sensititre 18-24 hour susceptibility plate in triplicate on three consecutive days.
- Quality control (QC) was assured by testing 20 replicates of each ATCC strain including *Staphylococcus aureus* ATCC 29213 (recommended by CLSI) across four sites (Table 2).

Table 2. Quality control

QC Organism	Range (µg/ml)
Staphylococcus aureus ATCC 29213	0.002-0.015

RESULTS

Essential agreement for Debio 1452 on the Sensititre susceptibility plate compared to the CLSI reference microdilution plate was calculated for both automated and manual read methods using the +/- one $\log_2$ dilution standard.

Clinical and Challenge Isolate Results for Gram Positive Non-Fastidious Organisms
- The overall essential agreement for Debio 1452 within +/- one log dilution was 97.3% for the automated read method (Table 3) and 98.3% for the manual read method (Table 4).

Interlaboratory Reproducibility Isolate Results for Gram Positive Non-Fastidious Organisms
- Reproducibility testing results for Debio 1452 within +/- one log dilution from the mode MIC was 100.0% for the automated read method and 99.3% for the manual read method (Table 5).

Table 3. Data summary and percent essential agreement of Gram positive non fastidious clinical and challenge isolates for the Automated Read Method

Combined total isolates (4 sites)

	Number of isolates		Essential agreement		% Essential agreement	
Organism Group	**All**	**Evaluable**	**Total**	**Evaluable**	**Total**	**Evaluable**
Staphylococcus aureus (MSSA)	251	251	245	245	97.6%	97.6%
Staphylococcus aureus (MRSA)	249	249	247	247	99.2%	99.2%
Coagulase-Negative *Staphylococcus* spp.	96*	96	88	88	91.7%	91.7%
Total	**596**	**596**	**580**	**580**	**97.3%**	**97.3%**

The overall essential agreement for Debio 1452 within +/- one $\log_2$ dilution, was 97.3% for the Automated Read Method.

Table 4. Data summary and percent essential agreement of Gram positive non-fastidious clinical and challenge isolates for the Manual Read Method

Combined total isolates (4 sites)

	Number of isolates		Essential agreement		% Essential agreement	
Organism Group	**All**	**Evaluable**	**Total**	**Evaluable**	**Total**	**Evaluable**
Staphylococcus aureus (MSSA)	251	251	249	249	99.2%	99.2%
Staphylococcus aureus (MRSA)	249	249	248	248	99.6%	99.6%
Coagulase-Negative *Staphylococcus* spp.	98	98	91	91	92.9%	92.9%
Total	**598**	**598**	**588**	**588**	**98.3%**	**98.3%**

The overall essential agreement for Debio 1452 within +/- one $\log_2$ dilution, was 98.3% for the Manual Read Method.

Table 5. Interlaboratory (4 sites) reproducibility results for Automated and Manual Read of Gram positive non-fastidious organisms

Debio 1452	Auto Read	Manual Read
Total isolates tested	432	432
Isolates within +/- 1 well from mode	432	429
Reproducibility ratio	432/432	429/432
Reproducibility percent	**100.0%**	**99.3%**
Total essential agreement	432/432	429/432
Essential agreement percent	**100.0%**	**99.3%**

CONCLUSIONS

This study validates that the Sensititre 18–24 hour susceptibility system (both automated read and manual read methods) demonstrated an equivalent level of performance compared to the CLSI M07/M100 and ISO 20776-1 reference broth microdilution plate when testing Debio 1452 against non-fastidious Gram positive clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of Debio 1452.

REFERENCES

1. Clinical and Laboratory Standards Institute. 2015. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-tenth edition.* Approved document M07-A10. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute. 2017. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-seventh Informational Supplement* M100-S27. Wayne, PA: CLSI.
3. FDA *Guidance for Industry and FDA Class II Special Controls Guidance Document:* Antimicrobial Susceptibility Test (AST) Systems, August 28, 2009.
4. Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases (ISO 20776-1 :2006).
5. Kaplan, *et al.* 2012. *Antimicrob Agents Chemotherapy*. 56 (11) 5864-74. Pubmed 22948878.

ThermoFisher SCIENTIFIC

© 2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

*Two isolates, Clinical isolate TC105 *S. haemolyticus* and Challenge isolate GPCH0113 *S. saprophyticus*, did not generate a signal for the automated read method.