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A Multi-Site Study Comparing an 18-24h Sensititre® Susceptibility System to the CLSI Broth Microdilution Method for Iclaprim Using Fastidious and Non-Fastidious Gram-Positive Organisms

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Abstract

Background: Iclaprim (ICL) (Arpida Ltd., Reinach, Switzerland) is a new selective dihydrofolate inhibitor for the treatment of severe hospital infections caused by gram positive pathogens including, methicillin-resistant *Staphylococcus aureus* (MRSA). An evaluation was performed to determine the accuracy and reproducibility of ICL susceptibility testing using the Sensititre® 18 – 24 hour dried susceptibility system (TREK Diagnostic Systems, Cleveland, OH) compared with the CLSI M07 reference broth microdilution method (BMD). Both automated and manual reading methodologies were performed.

Materials and Methods: ICL (0.002-32µg/mL) was tested against 860 fresh clinical isolates, 150 challenge isolates and 50 reproducibility isolates. These isolates consisted of: 117 coagulase-negative *Staphylococcus* spp., 172 *Staphylococcus aureus*, 120 *Enterococcus* spp., 329 *Streptococcus* spp. (Beta-hemolytic group), 187 *Streptococcus pneumoniae,* and 135 *Streptococcus* spp. (Viridans group). Dried plates were inoculated per manufacturers' instructions. BMD was performed per CLSI M07 guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the CLSI published QC ranges.

Results: Comparisons of MIC results on the Sensititre® system to the CLSI M07 BMD for both automated and manual reads resulted in 98.0% and 99.2% essential agreement for ICL (+/- one $\log_2$ dilution), respectively. Overall agreement for the reproducibility (+/- one $\log_2$ dilution of the modal MIC) using automated and manual reads were 98.5% and 98.5%, respectively.

Conclusions: The results for ICL indicates that the Sensititre® 18 – 24 hour susceptibility system for all clinical and challenge isolates gave reliable results using either the automated or manual read methods when compared to the CLSI reference BMD.

Introduction

Iclaprim is a novel diaminopyrimidine derivative which has shown extended spectrum in-vitro activity against gram positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-intermediate *Staphylococcus aureus* (VISA). Activity has also been demonstrated against penicillin-resistant *Streptococcus pneumoniae.* Here we are reporting results from a multi-site study, performing a series of evaluations to determine the accuracy and reproducibility of the Sensititre® 18 – 24 hour susceptibility system with **Iclaprim** compared to the CLSI reference Broth Microdilution Method (CLSI M07).

Materials & Methods

• Indications for use: The Sensititre® 18 – 24 hour MIC or breakpoint susceptibility system is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms.

• Each isolate was tested using a Sensititre® 18 – 24 hour susceptibility plate containing **Iclaprim** (0.002-32µg/mL) (Arpida Ltd., Reinach, Switzerland). The dried plates were set-up and tested according to the manufacturers' instructions.

• The CLSI reference broth microdilution plate was prepared and tested on each isolate according to the Clinical Laboratory Standards Institute (CLSI M07).

• Testing consisted of 860 fresh clinical isolates (combined 3 sites); approximately 309 gram positive isolates, and 551 fastidious isolates from each site. 150 Centers for Disease Control and Prevention (CDC) challenge isolates consisted of: 75 gram positive and 75 fastidious supplied to a single testing site (Tables 1 and 2).

• Reproducibility testing consisted of 25 gram positive and 25 *Streptococcus* spp. isolates tested at all 3 sites on the Sensititre® 18 – 24 hour susceptibility plate (Table 1). The test plate results were compared with those of the CLSI reference broth microdilution plate.

• Quality control (QC) was assured by testing 20 replicates of each ATCC strain including *S. aureus* 29213, *E. faecalis* 29212, and *S. pneumoniae* 49619, at each site (Tables 1 and 3).

• Colony counts were performed on the inoculum of the QC strains on each day of testing.

Table 1. Organisms Tested

Organisms Tested	Number Tested
Clinical Isolates (combined 3 sites)	
(309 gram-positive, 551 *Streptococcus* spp.)	860
CDC Challenge Isolates (1 site)	
(75 gram-positive, 75 *Streptococcus* spp.)	150
Reproducibility Isolates (combined 3 sites)	
(25 gram-positive, 25 *Streptococcus* spp.)	50
CLSI Quality Control Strains	
(20 replicates of each strain at 3 sites)	3 x 20

Table 2. Clinical and Challenge Isolates Tested

Gram-positive Organisms	Number Tested
Coagulase Negative *Staphylococcus*	112
Staphylococcus aureus	163
Enterococcus spp.	114
Beta Streptococcus (MHB)	87
Total	476
***Streptococcus* spp.**	Number Tested
Streptococcus pneumoniae	177
Viridans Group *Streptococcus*	130
Beta Streptococcus (MHB with LHB)	227
Total	534

Table 3. Quality Control Strains

Quality Control Strains	CLSI MIC Ranges (µg/ml)
Staphylococcus aureus ATCC 29213	0.06-0.25
Enterococcus faecalis ATCC 29212	0.004-0.03
Streptococcus pneumoniae ATCC 49619	0.03-0.12

Results

Table 4. Summary Data and % Essential Agreement of Gram-positive Clinical and Challenge Isolates Using the Manual Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	93	78	93	78	100.0%	100.0%
Staphylococcus aureus	136	132	136	132	100.0%	100.0%
Enterococcus spp.	97	56	97	56	100.0%	100.0%
Beta Streptococcus (MHB)	75	68	73	67	97.3%	98.5%
Total	**401**	**334**	**399**	**333**	**99.5%**	**99.7%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	19	18	19	18	100.0%	100.0%
Staphylococcus aureus	27	25	27	25	100.0%	100.0%
Enterococcus spp.	17	11	17	11	100.0%	100.0%
Beta Streptococcus (MHB)	12	12	10	10	83.3%	83.3%
Total	**75**	**66**	**73**	**64**	**97.3%**	**97.0%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	112	96	112	96	100.0%	100.0%
Staphylococcus aureus	163	157	163	157	100.0%	100.0%
Enterococcus spp.	114	67	114	67	100.0%	100.0%
Beta Streptococcus (MHB)	87	80	83	77	90.3%	90.9%
Total	**476**	**400**	**472**	**397**	**99.2%**	**99.3%**

Table 5. Summary Data and % Essential Agreement of Gram-positive Clinical and Challenge Isolates Using the Auto Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	92	78	90	77	97.8%	98.7%
Staphylococcus aureus	136	132	135	131	99.3%	99.2%
Enterococcus spp.	91	56	91	56	100.0%	100.0%
Beta Streptococcus (MHB)	75	70	72	68	96.0%	97.1%
Total	**394**	**336**	**388**	**332**	**98.5%**	**98.8%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	19	18	19	18	100.0%	100.0%
Staphylococcus aureus	27	25	27	25	100.0%	100.0%
Enterococcus spp.	17	12	17	12	100.0%	100.0%
Beta Streptococcus (MHB)	12	12	11	11	91.7%	91.7%
Total	**75**	**67**	**74**	**66**	**98.7%**	**98.5%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Coagulase Negative *Staphylococcus*	111	96	109	95	98.9%	99.4%
Staphylococcus aureus	163	157	162	156	99.7%	99.6%
Enterococcus spp.	108	68	108	68	100.0%	100.0%
Beta Streptococcus (MHB)	87	82	83	79	93.9%	94.4%
Total	**469**	**403**	**462**	**398**	**98.5%**	**98.8%**

Table 6. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Manual Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	152	135	149	133	98.0%	98.5%
Viridans Group *Streptococcus*	105	86	105	86	100.0%	100.0%
Beta Streptococcus (MHB with LHB)	202	152	200	151	99.0%	99.3%
Total	**459**	**373**	**454**	**370**	**98.9%**	**99.2%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	25	25	25	25	100.0%	100.0%
Viridans Group *Streptococcus*	25	19	25	19	100.0%	100.0%
Beta Streptococcus (MHB with LHB)	25	18	25	18	100.0%	100.0%
Total	**75**	**62**	**75**	**62**	**100.0%**	**100.0%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	177	160	174	158	99.0%	99.3%
Viridans Group *Streptococcus*	130	105	130	105	100.0%	100.0%
Beta Streptococcus (MHB with LHB)	227	170	225	169	99.5%	99.7%
Total	**534**	**435**	**529**	**432**	**99.1%**	**99.3%**

Table 7. Summary Data and % Essential Agreement of *Streptococcus* spp. Clinical and Challenge Isolates Using the Auto Read Method

Clinical Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	150	129	144	126	96.0%	97.7%
Viridans Group *Streptococcus*	105	86	104	86	99.0%	100.0%
Beta Streptococcus (MHB with LHB)	202	152	198	149	98.0%	98.0%
Total	**457**	**367**	**446**	**361**	**97.6%**	**98.4%**

Challenge Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	25	25	25	25	100.0%	100.0%
Viridans Group *Streptococcus*	25	19	25	19	100.0%	100.0%
Beta Streptococcus (MHB with LHB)	25	18	23	18	92.0%	100.0%
Total	**75**	**62**	**73**	**62**	**97.3%**	**100.0%**

Total Isolates

	Number of Isolates		Essential Agreement		% Essential Agreement	
Organism Group	All	Evaluable	Total	Evaluable	Total	Evaluable
Streptococcus pneumoniae	175	154	169	151	98.0%	98.9%
Viridans Group *Streptococcus*	130	105	129	105	99.5%	100.0%
Beta Streptococcus (MHB with LHB)	227	170	221	167	95.0%	99.0%
Total	**532**	**429**	**519**	**423**	**97.6%**	**98.6%**

Essential agreement for **Iclaprim** on the Sensititre® susceptibility plate compared to the CLSI reference microdilution plate was calculated for each method (autoread and manual read) using the +/- one $\log_2$ dilution standard. Essential agreement rates are shown for gram positive in tables 4 and 5, and for *Streptococcus* spp. isolates, in tables 6 and 7.

Clinical Isolates and CDC Challenge Organisms

• **Gram positive Isolates:** The overall essential agreement for **Iclaprim,** within +/- one $\log_2$ dilution, was 99.2% for the manual method and 98.5% for the autoread method (Tables 4 and 5).

• ***Streptococcus* spp. Isolates:** The overall essential agreement for **Iclaprim,** within +/- one $\log_2$ dilution, was 99.1% for the manual method and 97.6% for the autoread method (Tables 6 and 7).

Interlaboratory Reproducibility

• **Gram positive Isolates:** Reproducibility testing results for **Iclaprim**, within +/- one $\log_2$ dilution from the modal MIC was, 97.3% for the autoread method and 98.6% for the manual read method (Table 8).

• ***Streptococcus* spp. Isolates:** Reproducibility testing results for **Iclaprim**, within +/- one $\log_2$ dilution from the modal MIC was, 100% for the autoread method and 100% for the manual read method (Table 8).

Table 8. Interlaboratory Reproducibility % Essential Agreements +/- one $\log_2$ Dilution of the Modal MIC for Iclaprim

	Auto gram-positive	Manual gram-positive	Auto *Streptococcus* spp.	Manual *Streptococcus* spp.
Between-site total isolates tested	75	75	75	75
Between-site isolates within +/- 1 well from mode	73	73	75	75
Between-site reproducibility ratio	73/75	73/75	75/75	75/75
Between-site reproducibility %	97%	97%	100%	100%
Total essential agreement	73	74	75	75
Essential agreement %	97%	99%	100%	100%

Conclusions

This study validates that the Sensititre® 18 – 24 hour susceptibility system (both autoread and manual read) demonstrated an equivalent level of performance compared to the CLSI M07 reference broth microdilution plate when testing **Iclaprim** against gram positive and *Streptococcus* spp. clinical and challenge isolates. The high level of essential agreement obtained by the Sensititre® 18 – 24 hour susceptibility method and the CLSI reference method suggests that this is an acceptable method for susceptibility testing of **Iclaprim**.

References

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