

An Equivalency Study of the Sensititre® Dried MIC System Compared with the CLSI Broth Microdilution Reference Method for TR-700 and Comparator Antimicrobials

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ABSTRACT

Background: TR-700 is a novel antibacterial oxazolidinone. It demonstrates potent activity against numerous gram positive species. An evaluation was undertaken to determine the accuracy and reproducibility of the Sensititre 18 - 24 hour dried susceptibility system (manufactured by TREK Diagnostic Systems, Cleveland, OH) with TR-700 compared to the CLSI (M07) reference broth microdilution method (BMD). TR-700 was tested over the range of 0.015 – 32 µg/mL.

Materials and Methods: Two hundred isolates (30 *Streptococcus pneumoniae*, 20 *Streptococcus pyogenes*, 20 *Streptococcus agalactiae*, 20 *Streptococcus* species Viridans group, 40 *Staphylococcus aureus*, 30 *Staphylococcus* species, 40 *Enterococcus* species) were tested with TR-700 and 8 comparators using the Sensititre 18 - 24 hour dried susceptibility plate and by CLSI (M07) BMD for comparison. For reproducibility testing, 10 isolates were tested 3 times daily over a period of three days. Recommended CLSI quality control organisms were tested daily and were within the CLSI expected quality control ranges for all antimicrobials.

Results: Comparison of the Sensititre plate to the CLSI (M07) BMD for TR-700 resulted in 98.3% agreement (+/- one log₂ dilution) before repeat testing and 100% agreement (+/- one log₂ dilution) after repeat testing. Comparison of the Sensititre plate to the CLSI (M07) BMD for comparators resulted in 99.6% agreement (+/- one log₂ dilution) before repeat testing and 100% agreement (+/- one log₂ dilution) after repeat testing. Reproducibility was calculated as the percentage of results within +/- one log₂ dilution of the modal value. Overall agreement for the reproducibility of TR-700 and for comparators was 100%.

Conclusions: This evaluation indicates that the Sensititre 18 - 24 hour dried susceptibility plate with TR-700 yields highly reproducible results equivalent to the CLSI (M07) BMD. Thus the Sensititre plate is an acceptable method for susceptibility testing of TR-700.

INTRODUCTION

TR-700 is an oral/intravenous antimicrobial drug that is a member of the oxazolidinone class. TR-700 demonstrates an 8 fold greater activity than Linezolid against gram positive organisms including MRSA and VRE's.

PURPOSE OF THE STUDY

In this report, we performed a series of studies to determine the accuracy and reproducibility of the Sensititre® 18 – 24 hour susceptibility system with TR-700 compared to the CLSI reference broth microdilution method (M07).

MATERIALS & METHODS

Organisms Tested

- 200 Clinical and Challenge isolates consisting of:

| Organism | Number of Isolates Tested |
|---|---------------------------|
| <i>Streptococcus pneumoniae</i> | 30 |
| <i>Streptococcus pyogenes</i> | 20 |
| <i>Streptococcus agalactiae</i> | 20 |
| <i>Streptococcus</i> species Viridans group | 20 |
| <i>Staphylococcus aureus</i> | 40 |
| <i>Staphylococcus</i> species | 30 |
| <i>Enterococcus</i> species | 40 |

- 10 Reproducibility isolates (with on-scale endpoints)

| Organisms Tested | |
|--|--|
| Coagulase-negative <i>Staphylococcus</i> species (CNS) | |
| Methicillin-susceptible <i>Staphylococcus aureus</i> (MSSA) | |
| Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) | |
| <i>Enterococcus</i> species (<i>Enter</i> spp.) | |
| <i>Enterococcus faecium</i> (<i>E. faec.</i>) | |
| <i>Streptococcus pneumoniae</i> (<i>S. pneumo</i>) | |
| <i>Streptococcus pyogenes</i> (<i>S. pyog.</i>) | |
| <i>Streptococcus agalactiae</i> (<i>S. agal.</i>) | |
| <i>Streptococcus</i> species Viridans Group x2 (Viridans grp.) | |

- CLSI quality control strains (20 replicates of each tested)

| Organism | ATCC Number |
|---------------------------------|-------------|
| <i>Staphylococcus aureus</i> | ATCC 29213 |
| <i>Enterococcus faecalis</i> | ATCC 29212 |
| <i>Streptococcus pneumoniae</i> | ATCC 49619 |

- Quality Control isolates were tested daily on both the reference and dried plates and were within the CLSI Quality Control ranges.
- Colony counts were performed daily and fell within expected ranges.

MATERIALS & METHODS cont.

Antimicrobials Tested

| Antimicrobials Tested | Range Tested µg/ml | Supplied By |
|-----------------------|--------------------|-------------------------------------|
| TR-700 | 0.015-32 | Trius Therapeutics San Diego, CA |
| Linezolid | 0.06-64 | Pfizer Inc. Groton, CT |
| Clindamycin | 0.015-32 | Sigma Aldrich St. Louis, MO |
| Vancomycin | 0.06-64 | Amresco Inc. Solon, OH |
| Daptomycin | 0.03-8 | Cubist Lexington, MA |
| Levofloxacin | 0.03-8 | Johnson & Johnson Raritan, NJ |
| Penicillin | 0.06-16 | Amresco Inc. Solon, OH |
| Tigecycline | 0.008-1 | Wyeth Pearl River, NY |
| Oxacillin | 0.06-4 | Sigma Aldrich St. Louis, MO |

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a custom dried plate (Sensititre 18 – 24h susceptibility system) containing TR-700 and was set up according to manufacturers' instructions.
- The reference broth microdilution plates were prepared and tested according to The Clinical Laboratory Standards Institute (CLSI- M07).
- Repeat testing was performed for any organism/antimicrobial combination that resulted in a greater than +/- one log₂ dilution error. These were repeated in triplicate on both the dried test plate and the reference plate.
- Reproducibility: 10 Clinical/ challenge strains that had on-scale endpoints were tested in triplicate on 3 separate days. Any results on reproducibility testing with a greater than +/- one log₂ dilution difference were retested as per the criteria for repeat testing.

RESULTS

Essential agreements for the 200 isolates were calculated using the +/- one log₂ dilution standard for comparison studies. The agreement rates for the 200 isolates were as follows:

| Antimicrobials | # Isolates Tested | % Essential Agreement (B/A)* |
|----------------|-------------------|------------------------------|
| TR-700 | 200 | 98.3/100 |
| Linezolid | 200 | 100 |
| Clindamycin | 200 | 100 |
| Vancomycin | 200 | 99.6/100 |
| Daptomycin | 200 | 100 |
| Levofloxacin | 200 | 100 |
| Penicillin | 200 | 98.7/100 |
| Oxacillin | 200 | 100 |
| Tigecycline | 200 | 98.6/100 |

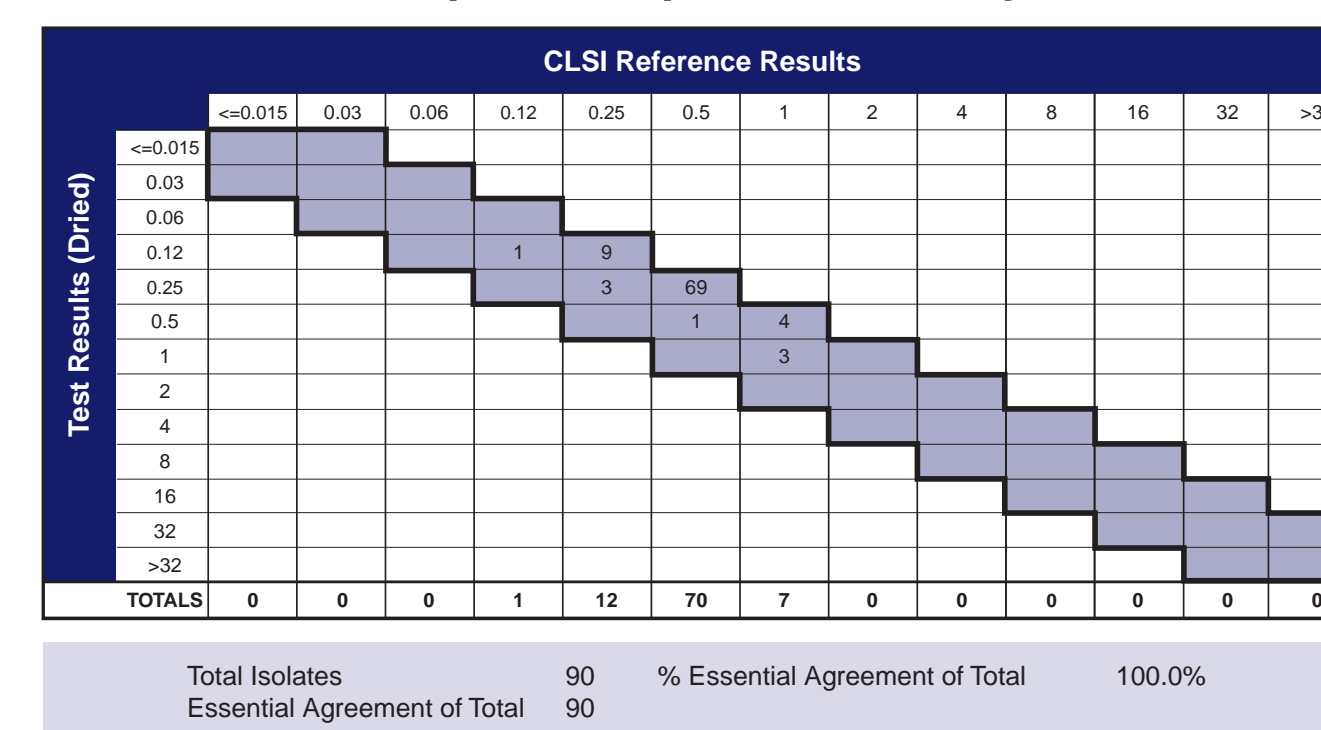
* (B/A) before/after retesting

Reproducibility results for TR-700 and the comparator antimicrobials within +/- one log₂ dilution of the CLSI reference broth microdilution plate

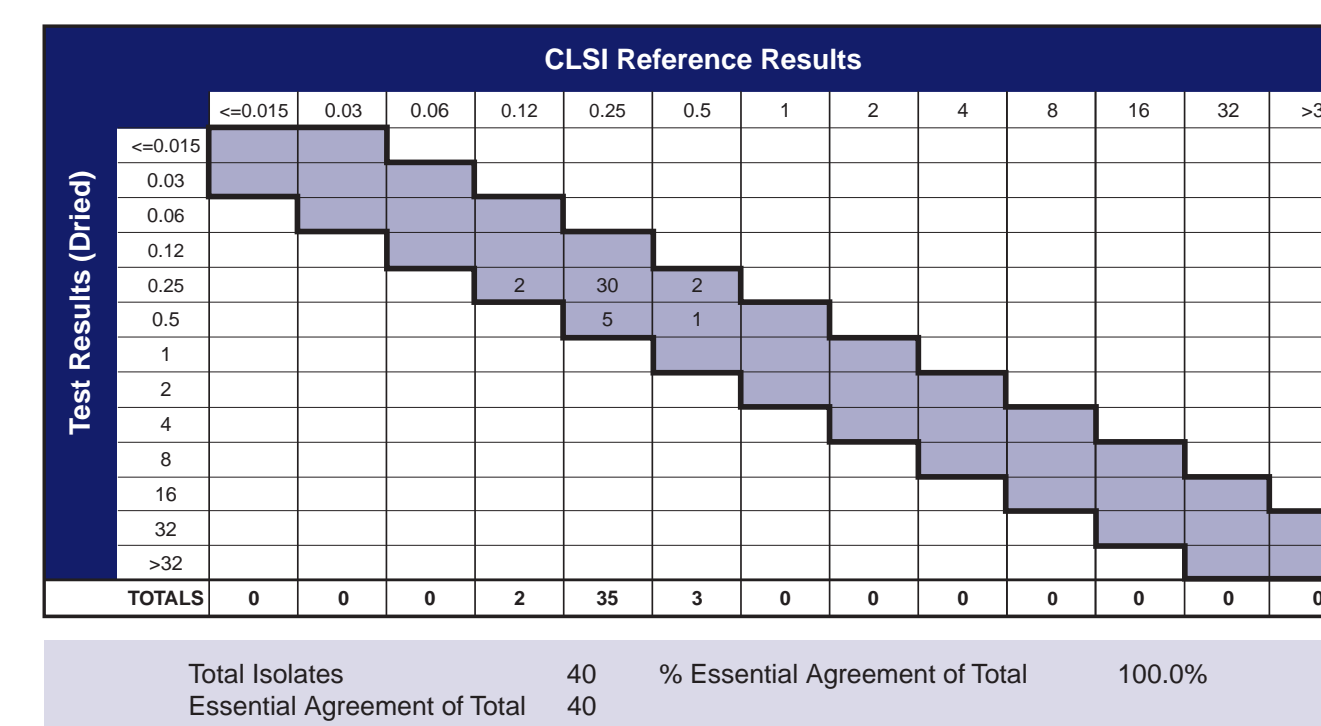
| Antimicrobials | # Isolates Tested | % Essential Agreement |
|----------------|-------------------|-----------------------|
| TR-700 | 10 | 100 |
| Linezolid | 10 | 100 |
| Clindamycin | 10 | 100 |
| Vancomycin | 10 | 100 |
| Daptomycin | 10 | 100 |
| Levofloxacin | 10 | 100 |
| Penicillin | 10 | 100 |
| Oxacillin | 10 | 100 |
| Tigecycline | 10 | 100 |

RESULTS cont.

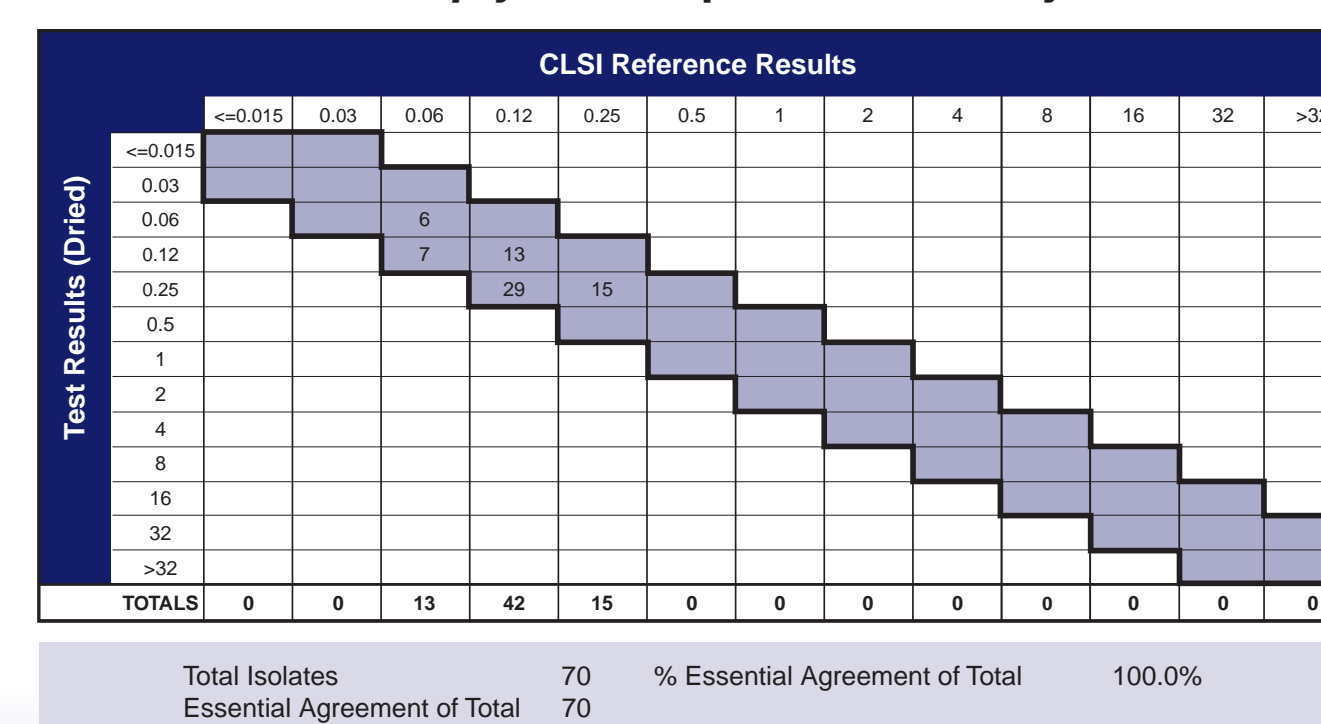
Streptococcus species TR-700 Study



Enterococcus species TR-700 Study



Staphylococcus species TR-700 Study



REFERENCES

- Clinical and Laboratory Standards Institute. 2009. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-eighth edition*. Approved document M07-A8. Wayne, PA:CLSI.
- Clinical and Laboratory Standards Institute. 2009. *Performance standards for antimicrobial susceptibility testing. 19th informational supplement M100-S19*. Wayne, PA:CLSI.



RESULTS cont.

Reproducibility Results for TR-700

| Isolate | Day 1 | | | Day 2 | | | Day 3 | | | MODE |
|-------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------|------|
| | REF. TR-700 | TEST TR-700 | TEST TR-700 | TEST TR-700 | TEST TR-700 | TEST TR-700 | TEST TR-700 | TEST TR-700 | | |
| CNS | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.25 | |
| MSSA | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.5 | |
| MRSA | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.25 | |
| <i>Enter</i> spp. | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.5 | |
| <i>E. faec.</i> | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.25 | |
| <i>S. pneumo.</i> | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.25 | |
| <i>S. pyog.</i> | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 1 | |
| <i>S. agal.</i> | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.5 | |
| Viridans Grp. | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.25 | |
| Viridans Grp. | A | A B C | A B C | A B C | A B C | A B C | A B C | A B C | 0.12 | |

CONCLUSION

The Sensititre 18-24 hour dried susceptibility system demonstrated an equivalent level of performance to the CLSI, M07 reference broth microdilution plate for susceptibility testing of TR-700.

Isolates Tested:

- The overall essential agreement for TR-700, within a +/- one log₂ dilution range, was 100%, after repeat testing, using the manual method.
- The overall essential agreement for comparators, within a +/- one log₂ dilution range, was 100%, after repeat testing, using the manual method.

Reproducibility:

- Interlaboratory reproducibility was 100% for TR-700 and all comparator drugs.

This study validates that the Sensititre 18 – 24 hour Dried Susceptibility system performs equivalent to the CLSI M07 reference microdilution method and is an acceptable method for susceptibility testing of TR-700.