

Independent Laboratory Study: ATS Labs Protocol # SJC01051614.EXVO

Ex-Vivo Antibacterial Evaluation of Topical Products Using a Vitro-Skin® Model- Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575) Matthew Sathe, B.S. Senior Microbiologist

Background

Enterococci are an emerging pathogen in hospitalized patients [1]. These pathogens ubiquitously occur in the hospital environment and show a high tenacity on inanimate surfaces [2,3,4]. As a result, enterococcal infections emerge with a rising frequency. Additionally, enterococci have the ability of acquiring resistances to multiple antimicrobial agents and the capacity to transfer resistances to other pathogens via mobile genetic elements [5,6,7]. For this reason the prevalence of vancomycin resistant enterococci (VRE) has increased intensively [1]. Vancomycin resistance is associated with enhanced mortality, e.g. among patients with enterococcal blood stream infections [8]. Within hospital settings prevention of VRE transmission is therefore a major objective.

Method

A film of the test organism dried onto a 1" x 1" demarcated area of 1.5" x 1.5" rehydrated Vitro-Skin® carriers was treated by wiping each carrier over and back twice with a saturated towelette for a total of 4 passes. Following treatment and exposure, each carrier was neutralized and assayed for survivors. Appropriate culture purity, neutralizer sterility, carrier sterility, population and neutralization confirmation controls were performed. Percent and Log₁₀ reductions were determined for the test based on the test population control results.

Results

Theraworx Technology Lot 141291, ready to use, demonstrated a >99.99% (>4.80 log₁₀) reduction of Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575) following a 15-minute exposure time when tested at ambient temperature (20.90°C).

TABLE 3: POPULATION CONTROL RESULTS

Test Organism	Carrier #	CFU/Carrier	Log ₁₀ of CFU/Carrier	Average Log ₁₀	Geometric Mean (CFU/Carrier)
Vancomycin Resistant <i>Enterococcus faecalis</i> - VRE (ATCC 51575)	1	1.3 x 10 ⁶	6.11	6.10	1.26 x 10 ⁶
	2	1.2 x 10 ⁶	6.08		

CFU = Colony Forming Unit

1. European Center for Disease Prevention and Control. Data from the ECDC Surveillance Atlas - Antimicrobial resistance. <https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc>. Accessed 05 Nov 2017.
2. Kramer A, Schebek I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis.* 2006;6:130.
3. Sample ML, Gravel D, Oxley C, Toye B, Garber G, Ramotar K. An outbreak of vancomycin-resistant enterococci in a hematology-oncology unit: control by patient cohorting and terminal cleaning of the environment. *Infect Control Hosp Epidemiol.* 2002;23:468-70.
4. McDermott H, Skally M, O'Rourke J, Humphreys H, Fitzgerald-Hughes D. Vancomycin-resistant enterococci (VRE) in the intensive care unit in a nonoutbreak setting: identification of potential reservoirs and epidemiological associations between patient and environmental VRE. *Infect Control Hosp Epidemiol.* 2018;39:40-5.
5. Pinholt M, Gumpert H, Bayliss S, Nielsen JB, Vorobieva V, Pedersen M, et al. Genomic analysis of 495 vancomycin-resistant enterococcus faecium reveals broad dissemination of a vanA plasmid in more than 19 clones from Copenhagen, Denmark. *J Antimicrob Chemother.* 2017;72:40-7.
6. Bender JK, Kalmbach A, Fleige C, Klare I, Fuchs S, Werner G. Population structure and acquisition of the vanB resistance determinant in German clinical isolates of *Enterococcus faecium* ST192. *Sci Rep.* 2016;6:21847.
7. Gawryszewska I, Zabička D, Hryniewicz W, Sadowy E. Linezolid-resistant enterococci in Polish hospitals: species, clonality and determinants of linezolid resistance. *Eur J Clin Microbiol Infect Dis.* 2017;36:1279-86.
8. DiazGranados CA, Zimmer SM, Klein M, Jernigan JA. Comparison of mortality associated with vancomycin-resistant and vancomycin-susceptible enterococcal bloodstream infections: a meta-analysis. *Clin Infect Dis.* 2005;41:327-33.

FINAL STUDY REPORT

STUDY TITLE

Ex-Vivo Antibacterial Evaluation of Topical Products Using a Vitro-Skin® Model

Test Organism:

Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)

PRODUCT IDENTITY

Theraworx Technology Lot 141291

AUTHOR

Matthew Sathe, B.S.
Senior Microbiologist

STUDY COMPLETION DATE

July 15, 2014

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Mercy R&D
524 N. Boonville Ave
Springfield, MO 65806

PROJECT NUMBER

A16931

Page 1 of 5

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Ex-Vivo Antibacterial Evaluation of Topical Products Using a Vitro-Skin® Model
Project Number: A16931
Protocol Number: SJC01051614.EXVO

TEST SUBSTANCE IDENTITY

Test Substance Name: Theraworx Technology
Lot/Batch(s): 141291

STUDY DATES

Date Sample Received: June 27, 2014
Study Initiation Date: July 2, 2014
Experimental Start Date: July 11, 2014
Experimental End Date: July 14, 2014
Study Completion Date: July 15, 2014

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Vancomycin Resistant <i>Enterococcus faecalis</i> - VRE	51575	Tryptic Soy Agar with 5% Sheep Blood (BAP)	35-37°C, aerobic

The test organism used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Test Substance Dilution: Ready to Use (RTU)
Test Exposure Time: 15 minutes
Exposure Temperature: Ambient (20.90°C)
Number of Carriers Tested/Lot: 2 test and 2 control carriers
Organic Soil Load Description: None
Neutralizer: Lethen Broth + 0.07% Lecithin + 0.5% Tween 80
Agar Plate Medium: BAP
Carrier Wiping: Over and back twice for a total of 4 passes

EXPERIMENTAL DESIGN

A film of the test organism dried onto a 1" x 1" demarcated area of 1.5" x 1.5" rehydrated Vitro-Skin® carriers was treated by wiping each carrier over and back twice with a saturated towelette for a total of 4 passes. Following treatment and exposure, each carrier was neutralized and assayed for survivors. Appropriate culture purity, neutralizer sterility, carrier sterility, population and neutralization confirmation controls were performed. Percent and Log₁₀ reductions were determined for the test based on the test population control results.

Per Sponsor's direction, the study was not required to be conducted under U.S. EPA 40 CFR Part 160 or U.S. FDA 21 CFR Part 58.

STUDY RESULTS

TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control		Results
Purity Control	Vancomycin Resistant <i>Enterococcus faecalis</i> - VRE (ATCC 51575)	Pure
Neutralizer Sterility Control		No Growth
Carrier Sterility Control		No Growth

TABLE 2: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Neutralization Confirmation (CFU)		Log ₁₀ Difference	Pass/Fail (±1 log ₁₀)
		Numbers Control	Results		
Theraworx Technology 141291	Vancomycin Resistant <i>Enterococcus faecalis</i> - VRE (ATCC 51575)	106,132	116,124	0.00	Pass

CFU = Colony Forming Units

TABLE 3: POPULATION CONTROL RESULTS

Test Organism	Carrier #	CFU/Carrier	Log ₁₀ of CFU/Carrier	Average Log ₁₀	Geometric Mean (CFU/Carrier)
Vancomycin Resistant <i>Enterococcus faecalis</i> - VRE (ATCC 51575)	1	1.3 x 10 ⁶	6.11	6.10	1.26 x 10 ⁶
	2	1.2 x 10 ⁶	6.08		

CFU = Colony Forming Unit

**TABLE 4: TEST RESULTS FOR Theraworx Technology 141291
 AGAINST Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)**

Dilution (Volume Plated)	Survivors	
	Replicate #1	Replicate #2
	15 Minute Exposure Time	
10 ⁰ (1.00 mL)	0,0	0,0
10 ⁰ (0.100 mL)	0,0	0,0
10 ⁻¹ (0.100 mL)	0,0	0,0
10 ⁻² (0.100 mL)	0,0	0,0
10 ⁻³ (0.100 mL)	0,0	0,0
CFU/Carrier	<2 x 10 ¹	<2 x 10 ¹
Log ₁₀ CFU/Carrier	<1.30	<1.30
Average Log ₁₀	<1.30	
Geometric Mean (CFU/Carrier)	<2.00 x 10 ¹	
Log ₁₀ Reduction =	>4.80	
Percent Reduction =	>99.99%	

CFU = Colony Forming Units

A value of <1 was used in place of zero for calculation purposes


CONTROL RESULTS

The control results for culture purity, neutralizer sterility, carrier sterility, population and neutralization confirmation were all acceptable.

ANALYSIS

Theraworx Technology Lot 141291, ready to use, demonstrated a >99.99% (>4.80 log₁₀) reduction of Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575) following a 15 minute exposure time when tested at ambient temperature (20.90°C).

PREPARED BY:



Matthew Sathe, B.S.
Senior Microbiologist

7-15-14
Date

The use of the ATS Labs name, logo or any other representation of ATS Labs without the written approval of ATS Labs is prohibited. In addition, ATS Labs may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the expressed written permission of ATS Labs.