



TECHNICAL SUMMARY

INHIBITION OF BACTERIA, FUNGI, AND VIRUSES IN VETERICYN PLUS®

Vetericyn Plus® contains hypochlorous acid, as a known antimicrobial, that inhibits the growth of microorganisms in the product. A series of studies were conducted to characterize the growth inhibition of bacteria, yeast, mold, and virus within the product based on USP and ASTM methods.

1. MODIFIED USP <51> PRESERVATIVE EFFECTIVENESS TEST

A 10 mL aliquot of Vetericyn Plus® was transferred into 10 separate sterile test tubes. Each tube was inoculated with 0.1 mL of respective organism to yield a final organism concentration of 10^7 CFU per mL of the test article. Inoculum volume was 0.5 – 1.0% of the product volume. Theoretical organism concentration per mL of the test article was calculated by the verification of the inoculum suspension. The inoculum concentration was verified by standard plate count method, using SCDA for bacteria and SDA for fungi.

At each sampling time point (15, 30, and 60 seconds) each tube was mixed thoroughly and then duplicate. 1.0 mL aliquot of mixture was removed from each tube and filtered separately. Each filter for bacteria was transferred to SCDA plate and incubated at 30 - 35°C for 3-5 days. Each filter for yeast and mold was transferred to SDA plate and incubated at 20-25°C for 3-5 days (yeast) and 3-7 days (mold), respectively. After the incubation, colonies were enumerated and log reduction for each organism was calculated. Log reduction results are summarized in Table 1.

TABLE 1. Log Reduction of Bacteria, Yeast, and Mold In Presence of Vetericyn Plus®

15 SECONDS			
MICROORGANISMS	INOCULUM CONCENTRATION (CFU/ML)	POST-EXPOSURE SURVIVORS (CFU/ML)	Log ₁₀ REDUCTION
<i>K.pneumoniae</i>	1.32×10^7	$< 1.00 \times 10^0$	> 7.12
<i>E.faecalis</i>	4.10×10^7	$< 1.00 \times 10^0$	> 7.61
<i>S.aureus</i> MRSA	6.55×10^7	$< 1.00 \times 10^0$	> 7.82
<i>S.epidermidis</i>	5.80×10^7	$< 1.00 \times 10^0$	> 7.76
<i>A. baumannii</i>	8.50×10^7	$< 1.00 \times 10^0$	> 7.93
<i>S. aureus</i>	8.75×10^7	$< 1.00 \times 10^0$	> 7.94
<i>P.aeruginosa</i>	5.20×10^7	$< 1.00 \times 10^0$	> 7.72
<i>E.coli</i>	8.90×10^7	$< 1.00 \times 10^0$	> 7.95
<i>C.albicans</i>	2.30×10^7	$< 1.00 \times 10^0$	> 7.36

**TABLE 1. Log Reduction of Bacteria, Yeast, and Mold In Presence of Vetericyn Plus®
(Continued)**

30 SECONDS / 60 SECONDS			
MICROORGANISMS	INOCULUM CONCENTRATION (CFU/ML)	POST-EXPOSURE SURVIVORS (CFU/ML)	Log ₁₀ REDUCTION
<i>K.pneumoniae</i>	1.32×10^7	$< 1.00 \times 10^0$	> 7.12
<i>E.faecalis</i>	4.10×10^7	$< 1.00 \times 10^0$	> 7.61
<i>S.aureus</i> MRSA	6.55×10^7	$< 1.00 \times 10^0$	> 7.82
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<i>S. aureus</i>	8.75×10^7	$< 1.00 \times 10^0$	> 7.94
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<i>C.albicans</i>	2.30×10^7	$< 1.00 \times 10^0$	> 7.36

2. BACTERIAL TIME KILL STUDIES PER ASTM METHODS

Microbial inhibition characteristics of Vetericyn Plus® were evaluated *in vitro* according to ASTM E2315-03 Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill, except for *Clostridium difficile* which was evaluated in accordance with E2839-11 Standard Test Method for Production of *Clostridium difficile* Spores for Use in Efficacy Evaluation of Antimicrobial Agents.

A 0.1 mL aliquot of the standard inoculum was added to 1.9 mL Vetericyn Plus® representing the start of the test exposure. The inoculated product was immediately vortex mixed and held for the remainder of the specified exposure time ranging from 15 – 90 seconds at room temperature (21 °C). The mixture was neutralized at the end of each exposure time to cease the antimicrobial effect of Vetericyn Plus® on any remaining bacterial colonies.

Using standard microbiological spread plate procedure, serial dilutions of 1 mL aliquots (10^0 – 10^{-3}) were plated in duplicate on appropriate recovery medium for the test organism. All subculture plates were incubated for 3 days at 35-37 °C under aerobic or anaerobic conditions suitable for the test organism. Following incubation, the agar plates were visually examined for the presence of growth and enumerated. Log₁₀ reductions were determined for each exposure time. Log reduction results are summarized in Table 2.

3. VIRAL TIME KILL STUDIES PER ASTM METHODS

Antiviral characteristics of Vetericyn Plus® were evaluated *in vitro* according to a modification of the Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension (ASTM E1052). The stock virus was obtained from the American Type Culture Collection, Manassas, VA (ATCC). Cultures of various indicator cells were seeded and maintained at 36-38 °C in a humidified atmosphere of 5-7% CO₂ until use. On the day of testing, the cells were verified for proper integrity and confluency before subjected to the infectivity assay.

TABLE 2. Microbial Log Reduction In Vetericyn Plus®

15 SECONDS			
MICROORGANISMS	INOCULUM CONCENTRATION (CFU/ML)	POST-EXPOSURE SURVIVORS (CFU/ML)	Log ₁₀ REDUCTION
<i>C difficile, spore form</i>	1.13 x 10 ⁶	5.2 x 10 ³	2.33
<i>B fragilis</i>	3.9 x 10 ⁶	< 5	>5.89
<i>H influenzae</i>	3.9 x 10 ⁶	< 5	> 4.44
<i>S pyogenes</i>	3.9 x 10 ⁶	< 5	>5.79
<i>S epidermidis</i>	6.0 x 10 ⁵	<5	>5.08
<i>S haemolyticus</i>	5.1 x 10 ⁵	<5	>5.01
<i>S hominis</i>	1.04 x 10 ⁶	<5	>5.32
<i>S saprophyticus</i>	7.0 x 10 ⁵	<5	>5.15
<i>E aerogenes</i>	3.8 x 10 ⁶	<5	>5.88
<i>E coli</i>	2.06 x 10 ⁶	<5	>5.61
<i>K pneumoniae</i>	1.31 x 10 ⁶	<5	>5.42
<i>M luteus</i>	1.46 x 10 ⁵	<5	>4.46
<i>A baumannii</i>	1.41 x 10 ⁶	<5	>5.45
<i>E faecium</i>	1.00 x 10 ⁶	<5	>5.30
<i>S aureus (MRSA)</i>	1.14 x 10 ⁶	<5	>5.36
<i>E faecalis (VRE)</i>	1.82 x 10 ⁶	<5	>5.56
<i>P mirabilis</i>	4.2 x 10 ⁶	<5	>5.92
<i>S marcescens</i>	1.34 x 10 ⁶	<5	>5.65
<i>P aeruginosa</i>	2.24 x 10 ⁶	<5	>5.43
<i>C albicans</i>	1.02 x 10 ⁶	<5	>5.31
<i>M bovis</i> - BCG	1.64 x 10 ⁶	3.6 x 10 ³	2.65

For the exposure time assayed, a 1.8 mL aliquot of Vetericyn Plus® was dispensed into a sterile tube and mixed with a 200 µL aliquot of stock virus suspension. The mixtures were vortex mixed for 10 seconds and held for the remainder of the specified exposure time ranging from 30-60 seconds at room temperature (21 °C). Following the exposure time, a 200 µL aliquot was removed from the tube and the mixture was immediately titered by 10-fold serial dilutions and assayed for the presence of virus.

For infectivity assay, cells contained in cell culture labware were inoculated in quadruplicate with 250 µL of the dilutions prepared from virus control and test substance. The inoculum was allowed to adsorb overnight at 36-38 °C in a humidified atmosphere of 5-7% CO₂. Following adsorption period, a 1 mL aliquot of the test medium was added to each culture well. The cultures were incubated at 36-38 °C in a humidified atmosphere of 5-7% CO₂ for approximately ten days. The test medium was aspirated from each culture well and replaced with fresh medium as needed through the incubation period. The cultures were scored for cytopathic effect, cytotoxicity, and for viability.

Viral titers were expressed as log10 of the 50% titration endpoint for infectivity (TCID₅₀) as calculated by the method of Spearman Karber. Log reduction was calculated from the difference between TCID₅₀ of the virus control and TCID₅₀ of Vetericyn Plus® solution sample. Log reduction results are summarized in Table 3.

**TABLE 2. Microbial Log Reduction In Vetericyn Plus®
(Continued)**

30 SECONDS / 60 SECONDS / 90 SECONDS			
MICROORGANISMS	INOCULUM CONCENTRATION (CFU/ML)	POST-EXPOSURE SURVIVORS (CFU/ML)	Log ₁₀ REDUCTION
<i>C difficile, spore form</i>	1.13 x 10 ⁶	< 5	>5.35
<i>B fragilis</i>	3.9 x 10 ⁶	< 5	>5.89
<i>H influenzae</i>	3.9 x 10 ⁶	< 5	> 4.44
<i>S pyogenes</i>	3.9 x 10 ⁶	< 5	>5.79
<i>S epidermidis</i>	6.0 x 10 ⁵	<5	>5.08
<i>S haemolyticus</i>	5.1 x 10 ⁵	<5	>5.01
<i>S hominis</i>	1.04 x 10 ⁶	<5	>5.32
<i>S saprophyticus</i>	7.0 x 10 ⁵	<5	>5.15
<i>E aerogenes</i>	3.8 x 10 ⁶	<5	>5.88
<i>E coli</i>	2.06 x 10 ⁶	<5	>5.61
<i>K pneumoniae</i>	1.31 x 10 ⁶	<5	>5.42
<i>M luteus</i>	1.46 x 10 ⁵	<5	>4.46
<i>A baumannii</i>	1.41 x 10 ⁶	<5	>5.45
<i>E faecium</i>	1.00 x 10 ⁶	<5	>5.30
<i>S aureus (MRSA)</i>	1.14 x 10 ⁶	<5	>5.36
<i>E faecalis (VRE)</i>	1.82 x 10 ⁶	<5	>5.56
<i>P mirabilis</i>	4.2 x 10 ⁶	<5	>5.92
<i>S marcescens</i>	1.34 x 10 ⁶	<5	>5.65
<i>P aeruginosa</i>	2.24 x 10 ⁶	<5	>5.43
<i>C albicans*</i>	1.02 x 10 ⁶	<5	>5.31
<i>M bovis</i> - BCG	1.64 x 10 ⁶	6 x 10 ¹	4.43

*Log reduction of 4.43 at 30 seconds

TABLE 3. Viral Log Reduction In Vetericyn Plus®

30 SECONDS / 60 SECONDS / 90 SECONDS			
MICROORGANISMS	Log ₁₀ REDUCTION 30 Sec	Log ₁₀ REDUCTION 60 Sec	Log ₁₀ REDUCTION 90 Sec
Hepatitis B virus	≥5.25	*	*
Rhinovirus type 37	>3.50	>3.75	>3.75
Influenza A (H1N1)	>5.75	>5.75	*

*Not evaluated.