For Educational Purposes Only.
Conclusion on page 3 shows that Argentyn 23 kills 99.99% of MRSA in 3 minutes

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Antimicrobial Efficacy Testing
Modified USP <51> M-015

Sponsor:	Natural-Immunogenics Corporation	STS Study No.:	N/A
	3265 West McNab Road	STS Test No.:	M07-4059A Amend. 2
	Pompano Beach, FL 33069	PO Number	111507MM3
		Start Date:	12-6-07

Attention: Mary Ellen Mahon, Laboratory Director and Kay Mitchen, RA/QA Director

Test Article/Lot No./STS Sample #: Argentyn 23, 23 ppm/MV65/2

Control Materials: H_2O was used to evaluate the initial inoculum concentrations.

Purpose: To challenge a preserved product with a standard inoculum of a representative range of microorganisms and establish the extent of viability loss at predetermined time intervals.

Test System Reference: Testing was completed according to the procedures and specifications as described in General Chapter <51> of the current edition of the USP.

Materials:

Challenge Organisms

Methicillin Resistant *Staphylococcus aureus* (MRSA), ATCC No. 33591

Sterile Media/Lot No.

Trypticase Soy Agar (TSA)
Sterile Diluent: H_2O

Validated Neutralizing Media/Lot No.

Dey-Engley Neutralizing Broth (DEB) or equivalent

Additional Equipment/Lot No.(if applicable)

Spectrophotometer
Colony Counter
Centrifuge

Incubators:
30-35°C
45-50°C (agar medium equilibration)
Pipette aid
Vortex

Procedure:

Preparation of Challenge Organisms

Cultures were maintained in the manner recommended by the curator of the culture collection. Cultures were no greater than 5 passes removed from the depository stock (ATCC).

Bacteria

Challenge organism, MRSA, was aseptically transferred onto fresh TSA slants and incubated for 18-24 hours at 30-35°C.

Subsequent to incubation, the bacteria was harvested using sterile diluent and a sterile cotton swab to dislodge the organisms from the agar surface. The resulting suspension was washed using centrifugation at 20-25°C for no longer than 10 minutes at 4000 x g. After washing, the supernatant was decanted, the pellet resuspended in fresh sterile diluent and the suspension washed a second time. Following the second wash the pellet was resuspended in fresh sterile diluent. Note: centrifuge calibrated for time and RPM.

Preparation of Standardized Inoculum

All challenge organisms were spectrophotometrically adjusted in sterile diluent to a concentration of $1.0x10^7$-$1.0x10^8$ colony forming units (cfu)/ml. A 1/3 post-dilution was required.

Preparation of Test Samples

For each challenge organism one, 20 ml sample of the test formulation and one, 20 ml sample of diluent (inoculum control) were aseptically dispensed into appropriate sample containers.

Recovery Medium Control

For each challenge organism, the test formulation was diluted 1:10 using DEB. A control sample was prepared using 10 mL of TSB. Both tubes were inoculated with sufficient inoculum to result in 10-100 cfu of challenge organisms per plate (post diluted organism suspensions serially diluted with 9.0 mL water blanks). One (1) mL from each tube was plated in triplicate with TSA. Plates were incubated in the same manner as the test and control samples, counts recorded, and organism recovery in the neutralizer broth compared to organism recovery in the control tube was calculated.

Determination of Preservative Effectiveness

All test and inoculum control samples were inoculated with 0.1 ml of an appropriate inoculum suspension to achieve a final concentration of $1.0x10^5$-$1.0x10^6$ cfu/ml and thoroughly mixed.

Immediately after inoculation, a 1.0 ml aliquot from the inoculum control, for each challenge organism, was aseptically transferred to a 9.0 ml DEB blank. Subsequent to neutralization, all inoculum control samples were serially diluted using additional DEB blanks and appropriate dilutions plated in triplicate with TSA.

At 45 seconds, 1.5 and 3 minutes after inoculation, a 1.0 ml aliquot from the test sample, for each challenge organism, was aseptically transferred to 9.0 mL of DEB. One (1) mL was plated in triplicate for the 10^{-1} dilution and 1.0 mL was serial diluted with additional 9.0 mL DEB blanks and the 10^{-2} through the 10^{-4} dilutions were plated in triplicate with TSA.

All bacterial plates were incubated at 30-35°C for 3-5 days

Results: The plate count results for the recovery medium control and determination of preservative efficacy were entered into a validated Microsoft Excel spreadsheet, and the recovery efficiency and log reduction values, respectively, were calculated. The data tables were printed out and are appended to the report. For the purpose of calculations, all averages less than 10 were equated to zero (0).

Conclusions:

Bacteria

At three (3) minutes, both Sovereign Silver and Argentyn 23 killed 99.99% of the challenge organism, Methicillin Resistant *Staphylococcus aureus.*

Inoculum Controls

All initial inoculum concentrations were within specifications.

☒ Criteria Met ☐ Criteria Not Met

Recovery Medium Control

For test article, the recovery in the neutralizer broth as compared to diluent was >50% for all of the challenge organisms evaluated per STS Test Number M05-0297.

☒ Criteria Met ☐ Criteria Not Met

Media Release (Per current edition of USP)

☒ Criteria Met ☐ Criteria Not Met

References:

1. Current Edition of the United States Pharmacopoeia
2. M-059 "Maintenance and Use of Stock Cultures"
3. M-015 "Preservative Efficacy of Multi-Dose Preserved Contact Lens Care Products"
4. M-060 "Challenge Microorganism Preparation, Harvesting and Spectrophotometric Determination"

Test Performed By/Date: 9-22-11

Test Reviewed By/Date: 22, Sept 2011

Table I

Formulation: Argentyn 23

Challenge Organism: *MRSA*, ATCC 33591

Sample Description	Exposure Time	Dilution	Colony Forming Units Recovered				Log Reduction (Compared to initial or rechallenge inoculum)	
			Plate #1	Plate #2	Plate #3	Mean		
Initial inoculum	0	1.00E+04	71	73	75	7.3E+05		
Argentyn 23	45 sec.	1.00E+02	107	124	117	1.2E+04	1.8	
	1.5 min.	1.00E+02	60	62	85	6.9E+03	2.0	
	3 min.	1.00E+01	0	0	0	0.0E+00	5.9	

Shaded cells are unlocked for data entry

Note that all calculations performed by Microsoft Excel are done to at least 6 significant figures. Entries in the table above are shown using the convention of rounding values equal to or greater than 0.5 to the next higher integral value; rounding is performed as the final step in the calculation.

Table II

Summary of Log Reduction Data

Formulation: Argentyn 23

Challenge Organism	Exposure Time	Log Reductions (from Tables I-V)
	Bacteria	
MRSA ATCC 33591	45 seconds	1.8
	1.5 minutes	2.0
	3 minutes	5.9

Table III

Recovery Medium Control

Formulation: Argentyn 23

Challenge Organism	Colony Forming Units in DEB Medium					Colony Forming Units in TSB Medium					Mean cfu in DEB/ Mean cfu in TSB (= % Recovery)
	Dilution	Plate 1	Plate 2	Plate 3	Mean	Dilution	Plate 1	Plate 2	Plate 3	Mean	
MRSA ATCC 33591	1.00E+00	14	10	13	1.2E+01	1.00E+00	16	11	15	1.4E+01	88

Shaded cells are unlocked for data entry

Note that all calculations performed by Microsoft Excel are done to at least 6 significant figures. Entries in the table above are shown using the convention of rounding values equal to or greater than 0.5 to the next higher integral value; rounding is performed as the final step in the calculation.