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SCHOOL OF MEDICINE

UNIVERSITY OF MIAMI

*Department of Dermatology and Cutaneous Surgery
Wound Healing Research Laboratory*

Prontosan Study Report

Determination of the effects of Prontosan irrigation solution on methicillin resistant *Staphylococcus aureus* biofilms in a partial thickness porcine wound model

May 7, 2007

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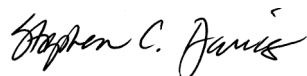
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INSTITUTIONAL POLICIES AND REGULATIONS

The following experiment was submitted for approval by University of Miami's Animal Use Committee. This study was conducted in compliance with the University of Miami's Department of Dermatology & Cutaneous Surgery's Standard Operating Procedures (SOPs) that comply with Good Laboratory Practices (GLP). Animals were monitored daily for any observable signs of pain or discomfort. In order to help minimize possible discomfort, an analgesic (Duragesic™ - fentanyl transdermal system: 25 µg/hr) was used during the entire experiment.

OBJECTIVE

The purpose of this study was to examine the activity of Prontosan irrigation solution on biofilms of methicillin resistant *Staphylococcus aureus* (MRSA) using a porcine partial thickness wound model.^{1,2,3,4}

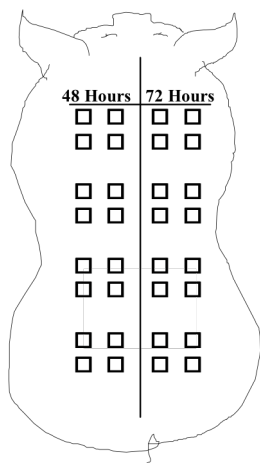
MATERIALS & METHODS

Experimental Animals & Design

Swine were used for our experimental research animal since their skin is morphologically similar to human skin. Three animals were used for this study. Young female specific pathogen free (SPF: Ken-O-Kaw Farms, Windsor, IL) pigs weighing 25-30 kg were kept in house for two weeks prior to initiating the experiment. These animals were fed a basal diet *ad libitum* and housed individually in our animal facilities (meeting USDA compliance) with controlled temperature (19-21°C) and lights (12h/12h LD). The animals were anesthetized with tiletamine HCl plus zolazepam (1.4 mg/kg) (Telazol; Laderle Parenterals Inc, Carolina, Puerto Rico), Xylazine (2 mg/kg), Atropine (0.05 mg/kg) I.M. and inhalation

of an isofluorane and oxygen combination during preparation, wounding, and treatment. The hair on the back of the pigs was clipped with standard animal clippers. Skin on both sides of the animals was prepared by washing with a non-antibiotic soap (Neutrogena®) and sterile water. The animals were blotted dry with sterile gauze. Thirty-two partial thickness wounds (10x7x0.3mm) were made on each animal by using a specialized electrokeratome. Wounds on the animals were inoculated with methicillin resistant *Staphylococcus aureus* (see wound inoculation section below). Wounds were covered with a polyurethane film for 24 hours after inoculation to allow biofilm development. Wounds were then randomly treated twice daily with 20ml of solution (2 x 10ml per treatment time – details of treatment regimen are described below) and recovered at 48 hours or 72 hours post initial treatment.

<u>Number of Animals</u>	<u>Treatment Groups</u>
3	1) Prontosan solution
	2) Ringer's solution
	3) Saline
	4) Untreated control



Treatment Groups

- ← Prontosan Solution (wound irrigation solution, B/Braun)
- ← Ringer Solution
- ← Saline
- ← Untreated

48 Hours- Wounds were treated twice per day and were recovered at 48 hours post treatment.

72 Hours- Wounds were treated twice per day and were recovered at 72 hours post treatment.

Note- all wounds were covered for 24 hours post inoculation and prior to treatment to allow biofilm development.

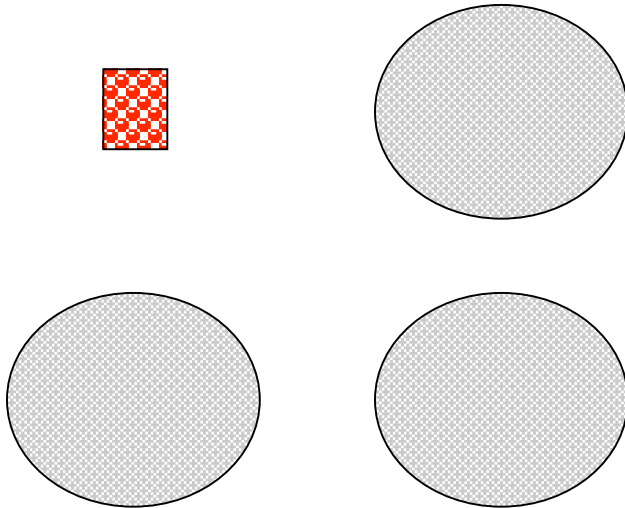
Wound Inoculation

A fresh culture of pathogenic isolate obtained directly from American Type Culture Collection (ATCC), Rockville, Maryland, was used in these studies. The inoculum was *Methicillin-Resistant Staphylococcus aureus* ATCC 33593 (MRSA). The frozen bacterium was recovered from glycerol stock (15% glycerol, -80°C). All inoculum suspensions were made by scraping the overnight growth from a culture plate into 5 ml of normal saline. This resulted in a suspension concentration of approximately 10^8 colony forming units/ml (CFU/ml). The 10^8 suspension was serially diluted to make an inoculum suspension with a concentration of 10^6 CFU/ml. A small amount of the inoculum suspension was plated onto culture media to quantitate the exact concentration of viable organisms. The inoculum suspension was used directly to inoculate each site. A 0.025 ml (25 μ l) aliquot of the suspension was deposited into the center of each wound. The suspension was lightly scrubbed into the test site for ten seconds using a sterile Teflon spatula and left for 3 minutes prior to covering the wounds with a polyurethane film dressing (each wound was dressed individually).

Treatment Regimen

Twenty-four hours post inoculation, the dressings were removed and a sterile 1 1/2" metal cap was placed over each wound and an ink marker was used to encircle each treatment area. During each treatment three of the four wounds in each group were covered with sterile 1 1/2" metal cap to prevent the rinse to flow onto the other wounds (see diagram below).

Metal Caps Covering Wounds During Treatments



Each wound was irrigated using 10ml syringes. During each irrigation (morning and late afternoon), each treatment was placed in a 10ml syringe and the syringe was held at a 45 degree angle over the wound and the entire wound area was irrigated twice (total 20ml). After irrigation, any excess fluid was dried with sterile gauze. The same individual performed all irrigations to provide a more reproducible condition.

Recovery Methods

Four wounds were cultured from each treatment group at 48 hours and 72 hours after initial treatment. Each site was cultured only once. The area was encompassed by a sterile surgical grade stainless steel cylinder (22mm internal diameter). One ml of all purpose neutralizer scrub solution (5% Tween 80 v/v, 2% Lecithin w/w, 0.05% Sodium oleate w/w, 0.05% Sodium thiosulfate w/w, 0.01% Proteose peptone w/w, and 0.01% Tryptone w/w) was pipetted into the glass cylinder and the site was scrubbed with a sterile Teflon spatula for 30 seconds. Serial dilutions were made of the recovered bacteria and were quantified using the Spiral Plater System, which deposits a small, defined amount (50 μ l) of suspension over the surface of a rotating agar plate. *MRSA* was selectively cultured on oxacillin

resistance selective media (ORSAB) overnight at 37°C. Colonies on the plates were counted and the colony forming units per ml (CFU/ml), Log CFU/ml, mean Log CFU/ml and standard deviation calculated.

RESULTS

Appendix 1 contains the raw data in tables for each pig [Table 1, (Pig#1); Table 2, (Pig#2); Table 3 (Pig#3)]. The data from all three animals was combined and tabulated (Table 4). Figures 1 and 2 represent the combined data for all treatments and times. Statistical analysis was performed on the data using an ANOVA analysis of the Log CFU/ml (Table 5).

As can be appreciated from comparison of the CFU/ml and the Log CFU/ml of the different treatments, Prontosan resulted in larger reductions of bacteria than irrigation with Ringer's solution or saline at both 48 and 72 hours (figure 1). Prontosan showed a significant reduction in the Log CFU/ml over all other groups by 48 hours (p-values; 0.033 versus Ringer's, 0.00125 versus saline, <0.001 versus untreated control) and at 72 hours (p-value < 0.001 versus all treatments) (Figure 2). Wounds treated saline solution resulted in slightly higher CFU/ml than did the Ringer's solution or the untreated control at 48 and 72 hour periods. However, these differences were not significant.

Additionally, treatment with Ringer's solution and saline resulted in a statistically insignificant increase in the Log CFU/ml from 48 to 72 hours. Treatment with Prontosan resulted in further reduction of Log CFU/ml 72 hours, however this decrease were not statistically significant.

Based on these results, it is possible that Prontosan irrigation may result in a greater reduction of wound bacteria after longer treatment periods.

Figure #1: Mean counts of MRSA present in wounds at 48 hours and 72 hours. Counts are expressed as colony forming units per mL (CFU/mL).

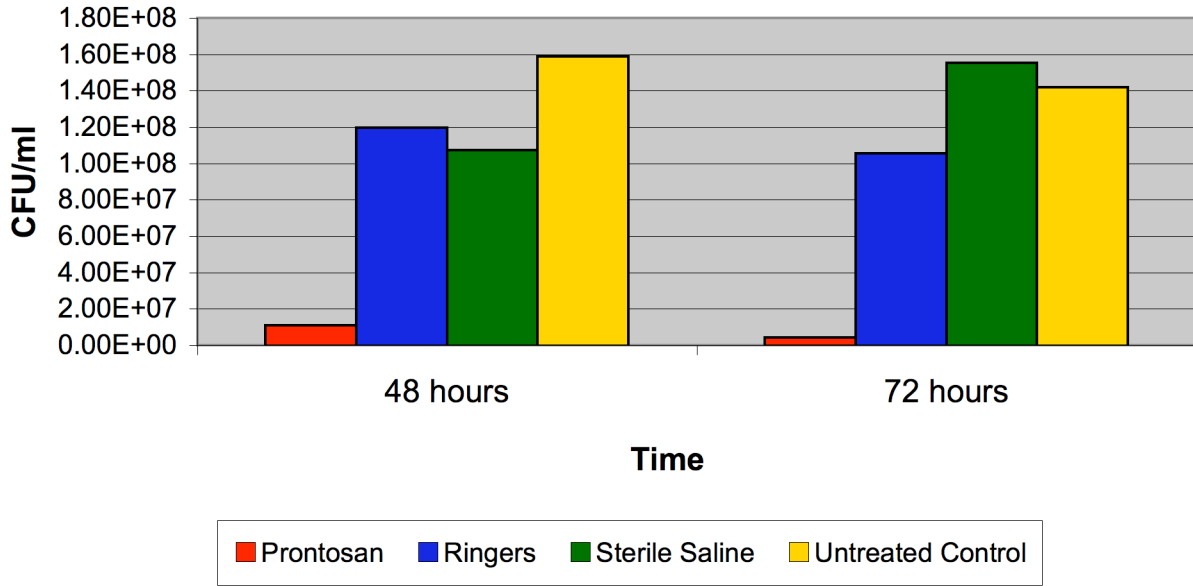
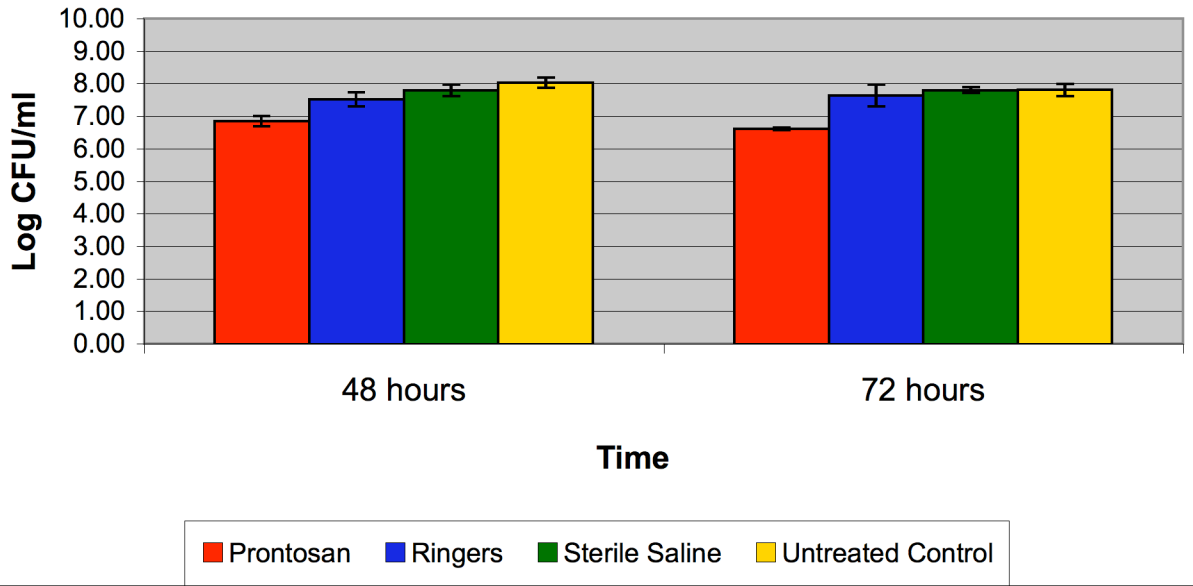


Figure #2: Mean counts of MRSA present in wounds at 48 hours and 72 hours. Counts are expressed as the Log colony forming units per mL (Log CFU/mL).



CONCLUSIONS

Irrigation of wounds with Prontosan solution resulted in a reduction of MRSA at 48 hours and 72 hours compared to all treatment groups. The reduction in the Prontosan treated group was statistically significant at 48 hours and 72 hours compared to all other treatment groups (p-value <0.05). Prontosan treatment resulted in the largest reduction of MRSA from 48 hours to 72 hours indicating that extended irrigation may provide additional benefits at reducing the bacterial load in the wound bed. It is also possible that if a higher irrigation pressure was used for these studies that larger reductions of MRSA counts may have been seen.

Appendix 1: Raw Data Tables

Table 1 Determination of the effects of Prontosan irrigation solution Pig #1

Bacteria	Dilution	Count	CFU/ml	Log CFU/ml
MRSA 33593	-5	52	1.04E+08	8.02

48 hours

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Prontosan	1	-3	168	3.36E+06	6.53	
	2	-3	414	8.28E+06	6.92	
	3	-4	152	3.04E+07	7.48	
	4	-3	450	8.99E+06	6.95	STDV
			Mean	1.28E+07	6.97	0.39

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Ringers	1	-4	62 / 1.214 μ l	5.11E+08	8.71	
	2	-4	123 / 5.5 μ l	2.24E+08	8.35	
	3	-3	91 / 1.214 μ l	7.50E+07	7.87	
	4	-2	536 / 1.214 μ l	4.42E+07	7.64	STDV
			Mean	2.14E+08	8.14	0.48

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Sterile Saline	1	-3	148 / 1.214 μ l	1.22E+08	8.09	
	2	-4	61 / 1.214 μ l	5.02E+08	8.70	
	3	-3	92 / 1.214 μ l	7.58E+07	7.88	
	4	-3	116 / 1.214 μ l	9.56E+07	7.98	STDV
			Mean	1.99E+08	8.16	0.37

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Untreated Control	1	-3	114 / 1.214 μ l	9.39E+07	7.97	
	2	-5	268	5.36E+08	8.73	
	3	-3	103 / 1.214 μ l	8.48E+07	7.93	
	4	-5	101	2.02E+08	8.31	STDV
			Mean	2.29E+08	8.24	0.37

72 hours

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Prontosan	1	-1	378 / 1.214 μ l	3.11E+06	6.49	
	2	-3	164	3.28E+06	6.52	
	3	-3	310	6.20E+06	6.79	
	4	-3	338	6.76E+06	6.83	STDV
			Mean	4.84E+06	6.66	0.18

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Ringers	1	-3	443 / 1.214 μ l	3.65E+08	8.56	
	2	-3	226 / 1.214 μ l	1.86E+08	8.27	
	3	-3	232 / 1.214 μ l	1.91E+08	8.28	
	4	-3	218 / 1.214 μ l	1.80E+08	8.25	STDV
			Mean	2.31E+08	8.34	0.15

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Sterile Saline	1	-4	178 / 5.5 μ l	3.24E+08	8.51	
	2	-3	334 / 1.214 μ l	2.75E+08	8.44	
	3	-2	1028 / 1.214 μ l	8.47E+07	7.93	
	4	-4	95 / 1.214 μ l	7.83E+08	8.89	STDV
			Mean	3.67E+08	8.44	0.39

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Untreated	1	-3	476 / 1.214 μ l	3.95E+08	8.60	
	2	-2	1262 / 1.214 μ l	1.04E+08	8.02	
Control	3	-3	243 / 1.214 μ l	2.00E+08	8.30	
	4	-4	92 / 1.214 μ l	7.58E+08	8.88	STDV
			Mean	3.64E+08	8.45	0.37

Table 2 Determination of the effects of Prontosan irrigation solution Pig #2

Bacteria	Dilution	Count	CFU/ml	Log CFU/ml
MRSA 33593	-3	58	1.16E+06	6.06

48 hours

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Prontosan	1	-4	82	1.64E+07	7.21	
	2	-4	61	1.22E+07	7.09	
	3	-2	75 / 1.214 μ l	6.18E+06	6.79	
	4	-4	159	3.18E+07	7.50	STDV
			Mean	1.66E+07	7.15	0.29

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Ringers	1	-4	28	5.60E+06	6.75	
	2	-3	58	1.16E+06	6.06	
	3	-3	107	2.14E+06	6.33	
	4	-4	51 / 1.214 μ l	4.20E+08	8.62	STDV
			Mean	1.07E+08	6.94	1.16

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Sterile Saline	1	-4	321	6.42E+07	7.81	
	2	-4	431	8.61E+07	7.94	
	3	-3	258 / 5.5 μ l	4.69E+07	7.67	
	4	-3	144 / 5.5 μ l	1.19E+08	8.07	STDV
			Mean	7.91E+07	7.87	0.17

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Untreated Control	1	-3	124 / 1.214 μ l	1.02E+08	8.01	
	2	-3	132 / 1.214 μ l	1.09E+08	8.04	
	3	-3	155 / 5.5 μ l	2.82E+07	7.45	
	4	-5	150	3.00E+08	8.48	STDV
			Mean	1.35E+08	8.00	0.42

72 hours

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Prontosan	1	-3	205	4.10E+06	6.61	
	2	-3	202	4.04E+06	6.61	
	3	-3	159	3.18E+06	6.50	
	4	-3	206	4.12E+06	6.61	STDV
			Mean	3.86E+06	6.58	0.06

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Ringers	1	-4	175	3.50E+07	7.54	
	2	-4	100	2.00E+07	7.30	
	3	-3	251	5.02E+06	6.70	
	4	-4	142	2.84E+07	7.45	STDV
			Mean	2.21E+07	7.25	0.38

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Sterile Saline	1	-4	281	5.02E+07	7.70	
	2	-5	22	4.40E+07	7.64	
	3	-4	66	1.32E+07	7.12	
	4	-4	50	9.99E+06	7.00	STDV
			Mean	2.93E+07	7.37	0.36

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Untreated Control	1	-4	74	1.48E+07	7.17	
	2	-4	86	1.72E+07	7.24	
	3	-4	124	2.48E+07	7.39	
	4	-5	34	6.80E+07	7.83	STDV
			Mean	3.12E+07	7.41	0.30

Table 3 Determination of the effects of Prontosan irrigation solution Pig #3

Bacteria	Dilution	Count	CFU/ml	Log CFU/ml
MRSA 33593	-3	58	1.16E+06	6.06

48 hours

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Prontosan	1	-2	90 / 2.968 µl	3.03E+06	6.48	
	2	-3	135	2.70E+06	6.43	
	3	-3	47 / 5.5 µl	8.55E+06	6.93	
	4	-2	324	6.48E+05	5.81	STDV
			Mean	3.73E+06	6.41	0.46

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Ringers	1	-4	88	1.76E+07	7.25	
	2	-4	381	7.62E+07	7.88	
	3	-4	222	4.44E+07	7.65	
	4	-2	159 / 1.214 µl	1.31E+07	7.12	STDV
			Mean	3.78E+07	7.48	0.35

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Sterile Saline	1	-3	242 / 5.5 µl	4.40E+07	7.64	
	2	-4	86	1.72E+07	7.24	
	3	-2	140 / 1.214 µl	1.15E+07	7.06	
	4	-4	150	3.00E+07	7.48	STDV
			Mean	2.57E+07	7.36	0.26

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Untreated Control	1	-4	51	1.02E+07	7.01	
	2	-4	257	5.14E+07	7.71	
	3	-5	64	1.28E+08	8.11	
	4	-5	22	4.40E+07	7.64	STDV
			Mean	5.84E+07	7.62	0.45

72 hours

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Prontosan	1	-3	203	4.06E+06	6.61	
	2	-3	333	6.66E+06	6.82	
	3	-3	168	3.36E+06	6.53	
	4	-3	106	2.12E+06	6.33	STDV
			Mean	4.05E+06	6.57	0.20

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Ringers	1	-4	21	4.20E+06	6.62	
	2	-5	97	1.94E+08	8.29	
	3	-2	115 / 2.968 µl	4.26E+06	6.63	
	4	-4	275	5.50E+07	7.74	STDV
			Mean	6.44E+07	7.32	0.83

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Sterile Saline	1	-4	62	1.24E+07	7.09	
	2	-4	96	1.92E+07	7.28	
	3	-5	52	1.16E+08	8.06	
	4	-4	343	6.86E+07	7.84	STDV
			Mean	5.41E+07	7.57	0.46

Treatment	Wounds	Dilution	Count	CFU/ml	Log CFU/ml	
Untreated Control	1	-4	41	8.20E+06	6.91	
	2	-4	102	2.04E+07	7.31	
	3	-4	202	4.04E+07	7.61	
	4	-4	179	3.58E+07	7.55	STDV
			Mean	2.62E+07	7.35	0.32

Table 4 Determination of the effects of Prontosan irrigation solution in the three pigs.

48 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Prontosan	1	7.60E+06	6.74	
	2	7.73E+06	6.81	
	3	1.50E+07	7.07	
	4	1.38E+07	6.75	STDV
Mean	1.10E+07	6.84	0.15	

72 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Prontosan	1	3.76E+06	6.57	
	2	4.66E+06	6.65	
	3	4.25E+06	6.61	
	4	4.33E+06	6.59	STDV
Mean	4.25E+06	6.60	0.03	

48 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Ringers	1	1.78E+08	7.57	
	2	1.00E+08	7.43	
	3	4.05E+07	7.28	
	4	1.59E+08	7.79	STDV
Mean	1.20E+08	7.52	0.22	

72 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Ringers	1	1.35E+08	7.57	
	2	1.33E+08	7.95	
	3	6.68E+07	7.20	
	4	8.78E+07	7.81	STDV
Mean	1.06E+08	7.64	0.33	

48 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Sterile Saline	1	2.02E+08	7.96	
	2	4.47E+07	7.54	
	3	8.15E+07	7.84	
	4	1.01E+08	7.80	STDV
Mean	1.07E+08	7.78	0.18	

72 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Sterile Saline	1	1.13E+08	7.79	
	2	7.13E+07	7.70	
	3	2.87E+08	7.91	
	4	1.50E+08	7.79	STDV
Mean	1.55E+08	7.80	0.08	

48 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Untreated Control	1	2.32E+08	8.16	
	2	8.03E+07	7.83	
	3	1.82E+08	8.14	
	4	1.41E+08	7.95	STDV
Mean	1.59E+08	8.02	0.16	

72 hours				
Treatment	Wounds	CFU/ml	Log CFU/ml	
Untreated Control	1	4.72E+07	7.52	
	2	8.84E+07	7.77	
	3	2.87E+08	8.09	
	4	1.41E+08	7.73	STDV
Mean	1.41E+08	7.78	0.23	

Table 5 Statistical analysis (ANOVA)

Day		(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
48 hours	Tukey HSD	Prontosan	Ringer's	-.67583(*)	.23738	.033	-1.3096	-.0420
			Saline	-.95333(*)	.23738	.00125	-1.5871	-.3195
			Untreated	-1.10583(*)	.23738	.000168	-1.7396	-.4720
		Ringer's	Prontosan	.67583(*)	.23738	.033	.0420	1.3096
			Saline	-.27750	.23738	.649	-.9113	.3563
			Untreated	-.43000	.23738	.282	-1.0638	.2038
		Saline	Prontosan	.95333(*)	.23738	.00125	.3195	1.5871
			Ringer's	.27750	.23738	.649	-.3563	.9113
			Untreated	-.15250	.23738	.918	-.7863	.4813
		Untreated	Prontosan	1.10583(*)	.23738	.000168	.4720	1.7396
			Ringer's	.43000	.23738	.282	-.2038	1.0638
			Saline	.15250	.23738	.918	-.4813	.7863
	Bonferroni	Prontosan	Ringer's	-.67583(*)	.23738	.040	-1.3317	-.0200
			Saline	-.95333(*)	.23738	.001364	-1.6092	-.2975
			Untreated	-1.10583(*)	.23738	.000177	-1.7617	-.4500
		Ringer's	Prontosan	.67583(*)	.23738	.040	.0200	1.3317
Saline			-.27750	.23738	1.000	-.9333	.3783	
Untreated			-.43000	.23738	.461	-1.0858	.2258	
Saline		Prontosan	.95333(*)	.23738	.001364	.2975	1.6092	
		Ringer's	.27750	.23738	1.000	-.3783	.9333	
	Untreated	-.15250	.23738	1.000	-.8083	.5033		
Untreated	Prontosan	1.10583(*)	.23738	.000177	.4500	1.7617		
	Ringer's	.43000	.23738	.461	-.2258	1.0858		
	Saline	.15250	.23738	1.000	-.5033	.8083		

Day		(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
72 hours	Tukey HSD	Prontosan	Ringer's	-1.03250(*)	.23005	.000290	-1.6467	-.4183
			Saline	-1.18833(*)	.23005	.0000322	-1.8026	-.5741
			Untreated	-1.13083(*)	.23005	.00007329	-1.7451	-.5166
		Ringer's	Prontosan	1.03250(*)	.23005	.0002902	.4183	1.6467
			Saline	-.15583	.23005	.905	-.7701	.4584
			Untreated	-.09833	.23005	.973	-.7126	.5159
		Saline	Prontosan	1.18833(*)	.23005	.0000322	.5741	1.8026
			Ringer's	.15583	.23005	.905	-.4584	.7701
			Untreated	.05750	.23005	.994	-.5567	.6717
		Untreated	Prontosan	1.13083(*)	.23005	.00007329	.5166	1.7451
			Ringer's	.09833	.23005	.973	-.5159	.7126
			Saline	-.05750	.23005	.994	-.6717	.5567
	Bonferroni	Prontosan	Ringer's	-1.03250(*)	.23005	0.0003071	-1.6681	-.3969
			Saline	-1.18833(*)	.23005	0.0000334	-1.8239	-.5527
			Untreated	-1.13083(*)	.23005	0.0000764	-1.7664	-.4952
		Ringer's	Prontosan	1.03250(*)	.23005	0.0003071	.3969	1.6681
Saline			-.15583	.23005	1.000	-.7914	.4798	
Untreated			-.09833	.23005	1.000	-.7339	.5373	
Saline		Prontosan	1.18833(*)	.23005	.0000334	.5527	1.8239	
		Ringer's	.15583	.23005	1.000	-.4798	.7914	
		Untreated	.05750	.23005	1.000	-.5781	.6931	
Untreated		Prontosan	1.13083(*)	.23005	.0000764	.4952	1.7664	
		Ringer's	.09833	.23005	1.000	-.5373	.7339	
		Saline	-.05750	.23005	1.000	-.6931	.5781	

Table 5 Statistical Analysis (ANOVA) Comparison Between Treatments and Times.

Dependent Variable: LogCfu

	(I) HoursTreatment	(J) HoursTreatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	48hours Prontosan	48hours Ringer	-.67583	.23374	.087	-1.4016	.0500
		48hours Saline	-.95333(*)	.23374	.002	-1.6791	-.2275
		48hours Untreatment	-1.10583(*)	.23374	.000	-1.8316	-.3800
		72hours Prontosan	.24000	.23374	.969	-.4858	.9658
		72hours Ringer	-.79250(*)	.23374	.022	-1.5183	-.0667
		72hours Saline	-.94833(*)	.23374	.003	-1.6741	-.2225
	48hours Ringer	72hours Untreatment	-.89083(*)	.23374	.006	-1.6166	-.1650
		48hours Prontosan	.67583	.23374	.087	-.0500	1.4016
		48hours Saline	-.27750	.23374	.934	-1.0033	.4483
		48hours Untreatment	-.43000	.23374	.595	-1.1558	.2958
		72hours Prontosan	.91583(*)	.23374	.004	.1900	1.6416
		72hours Ringer	-.11667	.23374	1.000	-.8425	.6091
	48hours Saline	72hours Saline	-.27250	.23374	.939	-.9983	.4533
		72hours Untreatment	-.21500	.23374	.983	-.9408	.5108
		48hours Prontosan	.95333(*)	.23374	.002	.2275	1.6791
		48hours Ringer	.27750	.23374	.934	-.4483	1.0033
		48hours Untreatment	-.15250	.23374	.998	-.8783	.5733
		72hours Prontosan	1.19333(*)	.23374	.000	.4675	1.9191
	48hours Untreatment	72hours Ringer	.16083	.23374	.997	-.5650	.8866
		72hours Saline	.00500	.23374	1.000	-.7208	.7308
		72hours Untreatment	.06250	.23374	1.000	-.6633	.7883
		48hours Prontosan	1.10583(*)	.23374	.000	.3800	1.8316
		48hours Ringer	.43000	.23374	.595	-.2958	1.1558
		48hours Saline	.15250	.23374	.998	-.5733	.8783
	72hours Prontosan	72hours Prontosan	1.34583(*)	.23374	.000	.6200	2.0716
		72hours Ringer	.31333	.23374	.881	-.4125	1.0391
		72hours Saline	.15750	.23374	.997	-.5683	.8833
		72hours Untreatment	.21500	.23374	.983	-.5108	.9408
		48hours Prontosan	-.24000	.23374	.969	-.9658	.4858
		48hours Ringer	-.91583(*)	.23374	.004	-1.6416	-.1900
	72hours Ringer	48hours Saline	-1.19333(*)	.23374	.000	-1.9191	-.4675
		48hours Untreatment	-1.34583(*)	.23374	.000	-2.0716	-.6200
		72hours Ringer	-1.03250(*)	.23374	.001	-1.7583	-.3067
		72hours Saline	-1.18833(*)	.23374	.000	-1.9141	-.4625
		72hours Untreatment	-1.13083(*)	.23374	.000	-1.8566	-.4050
		48hours Prontosan	.79250(*)	.23374	.022	.0667	1.5183
	48hours Saline	48hours Ringer	.11667	.23374	1.000	-.6091	.8425
		48hours Untreatment	-.16083	.23374	.997	-.8866	.5650
		48hours Untreatment	-.31333	.23374	.881	-1.0391	.4125
		72hours Prontosan	1.03250(*)	.23374	.001	.3067	1.7583

		72hours Saline		-1.15583	.23374	.998	-.8816	.5700
		72hours Untreatment		-.09833	.23374	1.000	-.8241	.6275
	72hours Saline	48hours Prontosan		.94833(*)	.23374	.003	.2225	1.6741
		48hours Ringer		.27250	.23374	.939	-.4533	.9983
		48hours Saline		-.00500	.23374	1.000	-.7308	.7208
		48hours Untreatment		-.15750	.23374	.997	-.8833	.5683
		72hours Prontosan		1.18833(*)	.23374	.000	.4625	1.9141
		72hours Ringer		.15583	.23374	.998	-.5700	.8816
		72hours Untreatment		.05750	.23374	1.000	-.6683	.7833
	72hours Untreatment	48hours Prontosan		.89083(*)	.23374	.006	.1650	1.6166
		48hours Ringer		.21500	.23374	.983	-.5108	.9408
		48hours Saline		-.06250	.23374	1.000	-.7883	.6633
		48hours Untreatment		-.21500	.23374	.983	-.9408	.5108
		72hours Prontosan		1.13083(*)	.23374	.000	.4050	1.8566
		72hours Ringer		.09833	.23374	1.000	-.6275	.8241
		72hours Saline		-.05750	.23374	1.000	-.7833	.6683
Bonferroni	48hours Prontosan	48hours Ringer		-.67583	.23374	.135	-1.4289	.0773
		48hours Saline		-.95333(*)	.23374	.003	-1.7064	-.2002
		48hours Untreatment		-1.10583(*)	.23374	.000	-1.8589	-.3527
		72hours Prontosan		.24000	.23374	1.000	-.5131	.9931
		72hours Ringer		-.79250(*)	.23374	.029	-1.5456	-.0394
		72hours Saline		-.94833(*)	.23374	.003	-1.7014	-.1952
		72hours Untreatment		-.89083(*)	.23374	.007	-1.6439	-.1377
	48hours Ringer	48hours Prontosan		.67583	.23374	.135	-.0773	1.4289
		48hours Saline		-.27750	.23374	1.000	-1.0306	.4756
		48hours Untreatment		-.43000	.23374	1.000	-1.1831	.3231
		72hours Prontosan		.91583(*)	.23374	.005	.1627	1.6689
		72hours Ringer		-.11667	.23374	1.000	-.8698	.6364
		72hours Saline		-.27250	.23374	1.000	-1.0256	.4806
		72hours Untreatment		-.21500	.23374	1.000	-.9681	.5381
	48hours Saline	48hours Prontosan		.95333(*)	.23374	.003	.2002	1.7064
		48hours Ringer		.27750	.23374	1.000	-.4756	1.0306
		48hours Untreatment		-.15250	.23374	1.000	-.9056	.6006
		72hours Prontosan		1.19333(*)	.23374	.000	.4402	1.9464
		72hours Ringer		.16083	.23374	1.000	-.5923	.9139
		72hours Saline		.00500	.23374	1.000	-.7481	.7581
		72hours Untreatment		.06250	.23374	1.000	-.6906	.8156
	48hours Untreatment	48hours Prontosan		1.10583(*)	.23374	.000	.3527	1.8589
		48hours Ringer		.43000	.23374	1.000	-.3231	1.1831
		48hours Saline		.15250	.23374	1.000	-.6006	.9056
		72hours Prontosan		1.34583(*)	.23374	.000	.5927	2.0989
		72hours Ringer		.31333	.23374	1.000	-.4398	1.0664
		72hours Saline		.15750	.23374	1.000	-.5956	.9106
		72hours Untreatment		.21500	.23374	1.000	-.5381	.9681
	72hours Prontosan	48hours Prontosan		-.24000	.23374	1.000	-.9931	.5131
		48hours Ringer		-.91583(*)	.23374	.005	-1.6689	-.1627
		48hours Saline		-1.19333(*)	.23374	.000	-1.9464	-.4402

	48hours Untreatment	-1.34583(*)	.23374	.000	-2.0989	-.5927
	72hours Ringer	-1.03250(*)	.23374	.001	-1.7856	-.2794
	72hours Saline	-1.18833(*)	.23374	.000	-1.9414	-.4352
	72hours Untreatment	-1.13083(*)	.23374	.000	-1.8839	-.3777
72hours Ringer	48hours Prontosan	.79250(*)	.23374	.029	.0394	1.5456
	48hours Ringer	.11667	.23374	1.000	-.6364	.8698
	48hours Saline	-.16083	.23374	1.000	-.9139	.5923
	48hours Untreatment	-.31333	.23374	1.000	-1.0664	.4398
	72hours Prontosan	1.03250(*)	.23374	.001	.2794	1.7856
	72hours Saline	-.15583	.23374	1.000	-.9089	.5973
	72hours Untreatment	-.09833	.23374	1.000	-.8514	.6548
72hours Saline	48hours Prontosan	.94833(*)	.23374	.003	.1952	1.7014
	48hours Ringer	.27250	.23374	1.000	-.4806	1.0256
	48hours Saline	-.00500	.23374	1.000	-.7581	.7481
	48hours Untreatment	-.15750	.23374	1.000	-.9106	.5956
	72hours Prontosan	1.18833(*)	.23374	.000	.4352	1.9414
	72hours Ringer	.15583	.23374	1.000	-.5973	.9089
	72hours Untreatment	.05750	.23374	1.000	-.6956	.8106
72hours Untreatment	48hours Prontosan	.89083(*)	.23374	.007	.1377	1.6439
	48hours Ringer	.21500	.23374	1.000	-.5381	.9681
	48hours Saline	-.06250	.23374	1.000	-.8156	.6906
	48hours Untreatment	-.21500	.23374	1.000	-.9681	.5381
	72hours Prontosan	1.13083(*)	.23374	.000	.3777	1.8839
	72hours Ringer	.09833	.23374	1.000	-.6548	.8514
	72hours Saline	-.05750	.23374	1.000	-.8106	.6956

* The mean difference is significant at the .05 level.

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