CliniClean™ Chlorhexidine Gluconate 4% Solution Clinical Summary

Toxicity

Chlorhexidine Gluconate has very low systemic toxicity because it is negligibly absorbed from the skin and mucous membranes. Chlorhexidine Gluconate has both low oral and local toxicity. Topical application of the 4% Chlorhexidine Solution would not cause systemic intoxication and has been proven from data by various studies conducted. The lethal dose for rats is reported to be 3,000 mg of Chlorhexidine per kilogram of body weight. Dilutions are very well tolerated by the skin and mucous membranes and the risk of skin sensitivity is quite low. Toxicity studies were conducted on rats and rabbits to determine both oral and dermal toxicity of Chlorhexidine Gluconate. A dermal toxicity study conducted on rabbits and were observed for any external signs of toxicity. Blood samples were taken for hematology and clinical chemistry prior to the study and during week 3 and week 12 of dosing. There was slight erythema at the treatment site, but there were no signs of toxicity. In addition, there were no changes in body weight, food consumption, hematological and clinical chemistry of the rabbits. An oncogenicity study was conducted on mice, each mouse was dermally dosed with 0.2mL, and the dosing solution contained 6.5% w/w surfactant and 1% w/w of Chlorhexidine Gluconate. The body weight, food consumption, hematological and clinical chemistry studies were conducted after week 52 and week 78. Overall, this study proved that Chlorhexidine Gluconate did not produce any drug related toxic signs when applied topically. In addition, the pharmacokinetic profile of the surgical scrub shows that it is poorly absorbed orally and would not cause systemic effects.

Skin and Eye Sensitivity

Chlorhexidine Gluconate was applied to the skin of rabbits and mice to test for skin sensitivity and irritation. Based on the clinical study, it does not appear to cause skin irritation when tested on abraded and intact skin of albino rats. There were also several studies conducted on white rabbits to determine skin sensitivity and irritation. Signs for erythema and edema were observed for the animals to determine primary skin irritation. According to the observations it can be considered a moderate irritant based on the sample on rabbit skins. There was slight irritation in the skin of white rabbits but when examined microscopically it was found to be minimal in nature. Primary eye irritation was also measured in rabbits and produced moderate conjunctivitis which gradually subsided after a couple days. Skin sensitivity testing was also conducted on guinea pigs by injecting the test material intradermally in the areas of skin that were clipped of hair. Slight erythema and edema were observed after injecting the guinea pigs, but overall Chlorhexidine Gluconate is not considered a sensitizing material.

Minimum Inhibitory Concentration

The minimum inhibitory concentration of 4% Chlorhexidine Gluconate Solution was determined against clinically isolated strains of medically significant species of microorganisms. The minimum inhibitory concentration is the lowest concentration of Chlorhexidine Gluconate that would yield no growth of the test microorganisms in the broth after 48 hours at 35 degrees Celsius. Several cultures were isolated and prepared in Tryptic Soy Broth, and fungi cultures were grown on Dextrose Agar. Chlorhexidine Gluconate was diluted with sterile water after it was liquefied. The cultures were tested against 1/10, 1/100 and 1/1000 dilutions of 4% Chlorhexidine Gluconate to determine the minimum inhibitory concentration.

The table below summarizes the in vitro Minimum Inhibitory Concentration for the microorganisms tested with 4% Chlorhexidine Gluconate.

Bacterial Species	Number of Strains	Minimum Inhibitory Concentration (mcg/mL)
Staphylococcus Epidermidis	16	0.2- 5.0 mcg/mL
Staphylococcus Albus	3	1.0-2.0 mcg/mL
Staphylococcus Aureus	13	0.2- 4.0 mcg/mL
Micrococcus	3	4.0-5.0 mcg/mL
Sarcina	1	4.0 mcg/mL
Corynebacterium Diptheriae	1	1.0 mcg/mL
Corynebacterium Acnes	5	10-20 mcg/mL
Corynebacterium Xerosis	1	10 mcg/mL
Pseudomonas Aeruginosa	4	20-40 mcg/mL
Pseudomonas Flurorescens	1	10 mcg/mL
Pseudomonas Cepacia	3	10-20 mcg/mL
Streptococcus Pyogenes	3	4.0-6.8 mcg/mL
Klebsiella Pneumoniae	2	4.0-10 mcg/mL
Serratia Marcescens	1	50 mcg/mL
Proteus Vulgaris	1	10 mcg/mL
Escherichia Coli	5	4.0-5.0 mcg/mL
Mycobacterium Smegmatis	1	1.0 mcg/mL
Mycobacterium Phlel	1	2.0 mcg/mL
Bacillus Subtillis	1	4.0 mcg/mL
Neisseria Gonorrhoeae	1	2.0 mcg/mL
Neisseria Catarrhalis	1	4.0 mcg/mL
Allescheria Boydil	1	40 mcg/mL
Aspergillus Niger	2	500 mcg/mL
Candida Albicans	2	10 mcg/mL
Candida Parapsilosis	1	40 mcg/ mL
Cladosporium	1	100 mcg/ mL
Cryptococcus Species	1	20 mcg/ mL
Debaryomyces Species	1	10 mcg/ mL
Microsporum Species	2	10-20 mcg/ mL
Nocardia Species	2	100 mcg/mL
Pityrosporum Species	2	20 mcg/mL
Streptomyces Species	2	40 mcg/ mL
Torulopsis Species	2	10 mcg/ mL
Trichophyton Species	2	20 mcg/ mL
Trichosporon Species	2	20-40 mcg/mL

Glove Juice Study (Surgical Hand Scrub)

Glove Juice study was conducted on 36 subjects to evaluate immediate and persistent effects on five days of use of 4% Chlorhexidine Gluconate to reduce the natural bacterial flora of the hands. Each subject washed their hands for three minutes using a scrub brush and nail cleaner. The excess water was shaken and then the gloves were put on while hands were wet. A stripping solution was added to the gloves and an attendant massaged the hand for one minute. Both hands of test subjects were sampled, the right hand was used to determine the immediate effects and the left hand was sampled after 1, 2, 3, 4, 5 and 6 hours of glove wearing. On day 2, a single scrub and sampling was completed followed by two additional scrubs. On days 3 and 4 all the subjects scrubbed three times daily, and on the day 5 scrubbing was done once along with sampling. The table below summarizes the results of the bacterial count for the right hand, log10 reduction per day of sampling when compared to the baseline count of bacteria which was 6.391. Overall, Chlorhexidine Gluconate was effective in reducing microbial growth and was extremely effective in the prevention of the transfer of pathogenic organisms within healthcare settings. The clinical studies conducted showed a substantial initial reduction of the microbial count which was measured by pre and post wash of the subjects. Chlorhexidine Gluconate was proven to be extremely effective as a broad spectrum, fast acting, antimicrobial that can be used for numerous hand washings required in patient care.

	Day 1	Day 2	Day 5
Bacterial Count	4.984	4.636	3.603
Log10 Reduction	1.407	1.755	2.788
Immediate Percent Kill	96.08	98.24	99.8
Delayed Percent Kill (Hour 1)	95.51	97.60	99.72
Delayed Percent Kill (Hour 2)	91.56	97.92	99.75
Delayed Percent Kill (Hour 3)	96.17	97.94	99.74
Delayed Percent Kill (Hour 4)	90.15	95.62	99.66
Delayed Percent Kill (Hour 5)	92.56	96.05	99.32
Delayed Percent Kill (Hour 6)	86.96	89.88	99.14

Persistence

The persistence of activity was also measured within the Glove Juice Study with the left-hand sampling that was completed at each hour on Day 1. The bacterial count of the immediate post wash count was compared to the hourly delayed post wash counts and there was no significant growth per sample time. According to the data above, the delayed bacterial sampling did not reach the baseline level and there was a reduction when compared to the left-hand baseline count. Overall, Chlorhexidine Gluconate significantly reduced the skins microbial flora and inhibited growth when surgical gloves that were worn for six hours. In addition, there were no observations of irritation or sensitization from the subjects.

Healthcare Personnel Handwash

This study was performed to evaluate the efficacy of the 4% CHG solution in reducing microbial contamination on the hands.

The solution was compared with a control material for activity against a Serratia marcenscens contaminant over the course of 10 hand washings. The glove juice method was used for the testing. Results are tabulated below.

	1st wash		4th wash		7th wash		10th wash	
	Log10	Percent	Log 10	Percent	Log10	Percent	Log 10	Percent
CliniClean™	2.050	99.11	2.563	99.73	2.696	99.80	3.289	99.95

Patient Preoperative Prep

This study was designed to test the efficacy of the solution against the normal microbial flora of the skin.

Each subject had normal skin flora populations at the abdomen/groin areas at the onset of the study. These areas were then prepped for 2 minutes with the solution and samples taken (cylinder sampling technique) at 10 minutes, 30 minutes and 4-hours after treatment. Results from baseline at the sampling intervals are tabulated below.

		CliniClean 4% CHG	
Site	Interval	Log10 Reduction	Percent
Abdomen	10 minutes	3.415	99.96
	30 minutes	3.390	99.96
	4 hours	3.243	99.95
Groin	10 minutes	4.037	99.99
	30 minutes	3.953	99.99
	4 hours	3.466	99.97

Time-Kill

Table 1

Initial Count and Test Results for 4% CHG

Expressed as Average CFU per mL Recovered, Percent and Log10 Reduction

Initial Count	Calculation Units	30 seconds		10 minutes	
		Rep 1	Rep 2	Rep 1	Rep 2
S. aureus MRSA ATCC 33592 Initial Count: 2.5 x 106	CFU Recovered	1.2 x 104	1.1 x 104	<5.0 x 100	<5.0 x 100
	Percent Reduction	99.52000	99.56000	99.99980	99.99980
	Log10 Reduction	2.32	2.36	>5.70	>5.70
E. faecalis VRE CI 99564 Initial Count: 1.8 x 107	CFU Recovered	1.4 x 104	1.5 x 104	3.0 x 101	5.0 x 101
	Percent Reduction	99.92222	99.91667	99.99983	99.99972
	Log10 Reduction	3.11	3.08	5.78	5.56
S. aureus ATCC 6538 Initial Count: 1.3 x 106	CFU Recovered	5.0 x 103	4.1 x 103	<5.0 x 100	<5.0 x 100
	Percent Reduction	99.84375	99.87188	99.99984	99.99984
	Log10 Reduction	2.81	2.89	>5.81	>5.81
P. aeruginosa ATCC 15442 Initial Count: 1.5 x 107	CFU Recovered	6.2 x 103	4.9 x 103	<5.0 x 100	<5.0 x 100
	Percent Reduction	99.95867	99.96733	99.99997	99.99997
	Log10 Reduction	3.38	3.49	>6.48	>6.48
S. enterica ATCC 10708 Initial Count: 1.9 x 106	CFU Recovered	<5.0 x 100	<5.0 x 100	<5.0 x 100	<5.0 x 100
	Percent Reduction	>99.99974	>99.99974	>99.99974	>99.99974
	Log10 Reduction	>5.58	>5.58	>5.58	>5.58
E. coli ATCC 11229 Initial Count: 2.0 x 106	CFU Recovered	1.2 x 102	2.3 x 102	<5.0 x 100	<5.0 x 100
	Percent Reduction	99.99900	99.99808	>99.99996	>99.99996
	Log10 Reduction	5.00	4.72	>6.38	>6.38