

Chlorhexidine Gluconate 4% Antiseptic / Antimicrobial Solution Clinical Summary

1.0 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 was conducted on rabbits which 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 0.2 ml, 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.

2.0 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 of erythema and edema were observed in 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 of 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.

3.0 Minimum Inhibitory Concentration:

The in-vitro 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 which 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.

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

4.0 Clinical Studies

Chlorhexidine Gluconate 4% Solution includes three (3) indications for use which occur in hospital settings; Surgical Hand Scrub, Healthcare Personnel Handwash, and Patient Preoperative Prep. Therefore, to evaluate the effectiveness of this solution in a hospital setting, Clinical Studies were initiated for each indication in accordance with standard test methods.

4.1 Surgical Hand Scrub

A Glove Juice Study was conducted on 36 subjects to evaluate the immediate and persistent effects of Chlorhexidine Gluconate 4% Solution on the reduction of the natural bacterial flora of the hands. Each subject washed their hands for three (3) minutes with a scrub brush and nail cleaner. Excess water was shaken from the hands, and the damp hands were immediately gloved. A stripping solution was added to the gloves and an attendant massaged each hand for one (1) minute.

Both hands of the test subjects were sampled, with the right hand being used to determine the immediate effects of the solution, and the left hand sampling the persistent or long-term effects of the solution. On day 1, the left hand was scrubbed once, and sampled after 1, 2, 3, 4, 5, or 6 hours of glove wearing. On day 2, a single scrub was conducted, and samples were taken at the 1, 2, 3, 4, 5, or 6-hour markers of glove wearing, followed by two (2) additional hand scrubbing and no sampling. On days 3 and 4, subjects scrubbed three (3) times with no sampling, and on day 5, hands were scrubbed once before sampling.

Right Hand – Immediate Testing

The following table summarizes the data, which was taken from the right hand, involving immediate testing of the glove juice environment. The data was evaluated on a scale of Log_{10} to better compare larger numerical values against the later measured smaller values.

Parameter	Baseline	Day 1	Day 2	Day 5
Bacterial Count (Right Hand)	N/A	4.984	4.636	3.603
Log_{10} Reduction Bacterial Count	6.391	1.407	1.755	2.788
Immediate Percent Bacteria Killed	N/A	96.08%	98.24%	99.80%

Left Hand – Persistence Testing

The following table summarizes the data, which was taken from the left hand, involving long-term or “persistence” testing of the glove juice environment.

Time (Hours)	Percent Bacteria Killed		
	Day 1	Day 2	Day 5
Hour 1	95.51%	97.60%	99.72%
Hour 2	91.56%	97.92%	99.75%
Hour 3	96.17%	97.94%	99.74%
Hour 4	90.15%	95.62%	99.66%
Hour 5	92.56%	96.05%	99.32%
Hour 6	86.96%	89.88%	99.14%

Conclusions

Overall, Chlorhexidine Gluconate 4% Solution was effective in reducing microbial growth and was extremely effective in the prevention of the transfer of pathogenic organisms within healthcare settings. Evaluation in a glove juice environment illustrated a substantial initial reduction of the microbial count, which was measured by both pre- and post-wash sampling of the subjects. Additionally, Chlorhexidine Gluconate was proven to be an extremely effective broad-spectrum, fast-acting, antimicrobial, which can be used for the numerous hand washings required in patient care.

The persistence of antimicrobial activity was measured during the glove juice study as well. When the immediate bacterial count was compared to the post-wash hourly counts, there was no significant growth between the time samples. According to the data, the delayed bacterial sampling as part of the persistence testing remained significantly reduced when compared to the initial baselines recorded. Additionally, bacterial counts did not return to baseline during the duration of sampling. In conclusion, Chlorhexidine Gluconate significantly reduced the skin's microbial flora and further inhibited growth over a period of six (6) hours.

There were no reports of subjects experiencing irritation or sensitization during the time of the study.

4.2 Healthcare Personnel Handwash

To evaluate the effectiveness of Chlorhexidine Gluconate 4% Solution in reducing microbial contamination of the hands, a Glove Juice Study was initiated. Following the pre-test period, 5mL of *Serratia marcescens* were pipetted into the cupped hands of the subjects and thoroughly distributed over all surfaces of the hands. After a 1-minute drying period, sterile gloves were donned, and samples were procured using the glove juice sampling procedure. This constituted the baseline sample.

Following the baseline count, the hands were again contaminated with *Serratia* and allowed to air dry. After drying, subjects washed both hands according to the test product label directions. This first wash was followed by nine more washes. Contamination proceeded and sampling followed the 4th, 7th, and 10th washes.

The following table illustrates the results of the washes below:

Wash Cycle	Microbial Count (Log ₁₀)	Percent Decrease
1st Wash	2.050	99.11%
4th Wash	2.563	99.73%
7th Wash	2.696	99.80%
10th Wash	3.289	99.95%

Conclusions

Overall, Chlorhexidine Gluconate 4% solution demonstrated a kill rate with good residual activity throughout the course of the study. The percentage decrease is noted as substantial, and therefore, the solution is effective as an antiseptic.

4.3 Patient Preoperative Prep

To evaluate the effectiveness of Chlorhexidine Gluconate 4% Solution as a preoperative preparation, testing was conducted on areas of the skin in which large populations of normal skin flora accumulate. Areas of skin around the abdomen and groin areas, where sweat and body heat propagate microorganisms, were evaluated, and the activity after a single use of the test product was tested against the microbial flora of the skin over the course of four (4) hours.

Each subject of the test had their normal skin flora populations measured at the onset of the study. Afterward, the abdomen and groin areas of skin were prepped for two (2) minutes, and samples were taken using the cylinder sampling technique. Samples were then taken ten (10) minutes, thirty (30) minutes, and four (4) hours after the treatment. The following table illustrates the results compared from the baseline at the aforementioned sampling intervals below:

Site	Interval	Log ₁₀ Reduction	Percent Reduction
Abdomen	10 Minutes	3.415	99.96%
	30 Minutes	3.390	99.96%
	4 Hours	3.243	99.95%

Site	Interval	Log ₁₀ Reduction	Percent Reduction
Groin	10 Minutes	4.037	99.99%
	30 Minutes	3.953	99.99%
	4 Hours	3.466	99.97%

Conclusions

Over the course of the study, Chlorhexidine Gluconate 4% solution demonstrated accurate residual activity based on the recommended use of the product. According to the data, there were no statistically significant differences between the product and the reference solution. Based on the results of this study, the reductions achieved by the product prove that it is effective at its desired antiseptic properties.

5.0 Time-Kill Study

Time-Kill studies evaluate the activity of an antimicrobial agent against a bacterial strain over a period of time. To aid in the evaluation of the efficacy of the solution, specific microorganisms of interest were tested against the solution. Solution was tested as two (2) repetitions, each at thirty (30) seconds and at ten (10) minutes. The following data reflects this study, separated by the organism of interest:

Microorganism of Interest	Initial Count	Calculation Units	30 seconds		10 minutes	
			Rep 1	Rep 2	Rep 1	Rep 2
<i>S. aureus</i> MRSA	ATCC 33592 Initial Count: 2.5 x 10 ⁶	CFU Recovered	1.2 x 10 ⁴	1.1 x 10 ⁴	<5.0 x 10 ⁰	<5.0 x 10 ⁰
		Percent Reduction	99.52000	99.56000	99.99980	99.99980
		Log ₁₀ Reduction	2.32	2.36	>5.70	>5.70
<i>E. faecalis</i> VRE	CI 99564 Initial Count: 1.8 x 10 ⁷	CFU Recovered	1.4 x 10 ⁴	1.5 x 10 ⁴	3.0 x 10 ¹	5.0 x 10 ¹
		Percent Reduction	99.92222	99.91667	99.99983	99.99972
		Log ₁₀ Reduction	3.11	3.08	5.78	5.56
<i>S. aureus</i>	ATCC 6538 Initial Count: 1.3 x 10 ⁶	CFU Recovered	5.0 x 10 ³	4.1 x 10 ³	<5.0 x 10 ⁰	<5.0 x 10 ⁰
		Percent Reduction	99.84375	99.87188	99.99984	99.99984
		Log ₁₀ Reduction	2.81	2.89	>5.81	>5.81
<i>P. aeruginosa</i>	ATCC 15442 Initial Count: 1.5 x 10 ⁷	CFU Recovered	6.2 x 10 ³	4.9 x 10 ³	<5.0 x 10 ⁰	<5.0 x 10 ⁰
		Percent Reduction	99.95867	99.96733	99.99997	99.99997
		Log ₁₀ Reduction	3.38	3.49	>6.48	>6.48
<i>S. enterica</i>	ATCC 10708 Initial Count: 1.9 x 10 ⁶	CFU Recovered	<5.0 x 10 ⁰	<5.0 x 10 ⁰	<5.0 x 10 ⁰	<5.0 x 10 ⁰
		Percent Reduction	>99.99974	>99.99974	>99.99974	>99.99974
		Log ₁₀ Reduction	>5.58	>5.58	>5.58	>5.58
<i>E. coli</i>	ATCC 11229 Initial Count: 2.0 x 10 ⁶	CFU Recovered	1.2 x 10 ²	2.3 x 10 ²	<5.0 x 10 ⁰	<5.0 x 10 ⁰
		Percent Reduction	99.99900	99.99808	>99.99996	>99.99996
		Log ₁₀ Reduction	5.00	4.72	>6.38	>6.38

Conclusions

Overall, this study evaluated the effectiveness of Chlorhexidine Gluconate 4% solution as an antimicrobial agent against specific strains of organisms of interest. The test concluded that the reduction times and percent bacteria killed were substantial, and the solution is effective as an antiseptic.

HR HealthCare
2600 Eastern Blvd., Suite 201
York, PA 17402 USA
hrhealthcare.com

CliniClean is a trademark of HR HealthCare.
©2024 HR HealthCare. All rights reserved.

REC-0011 Rev 2 (06/2024)