“COMBAT WOUND DISINFECTION AND HEALING / INVESTIGATION OF A TOPICAL ANTISEPTIC”

Report of Studies Funded and Conducted by the United States Army under the Direction of Colonel Robert Vandre
Director, Combat Casualty Care
Fort Detrick, Maryland

Grant Log #04054001

Research Studies to Determine the Efficacy of EXXCL Oral Blue Antimicrobial Technology for Use in Disinfecting Wounds on the Battlefield
List of Materials Used

Microbes

Staph Aureus ATCC# 25923 and 43300
Staph Aureus clinical isolate
E. coli ATCC# 25922
Pseudomonas Aeruginosa ATCC# 27853
Enterococcus Faecalis ATCC# 29212
Acinetobacter baumanii ATCC#19606
Methicillin resistant Staph aureus ATCC# 43300
Vancomycin resistant Enterococcus Faecalis ATCC# 51299
Candida Albicans ATCC# 28838
Actinomyces Naeslandi ATCC# 19039
Bacillus subtilis spore suspension ATCC# 12084
Bacillus atrophaeus 1062383 106/0.1ml Raven Biologics
Bacillus stearothermophilus spores. ATCC# 7953- Raven
Strep pyogenes ATCC# 12384
Strep sanguis ATCC# 10556
Serratia marscens ATCC# 8100
Clostridium sporogenes ATCC# 3584
Mycobacterium morganii ATCC# 25829

Materials

Technicloth wipers blend of cellulose/polyester, no chemical binders. 7x604
Sporocidal phenol 1.56%
Sodium phenate 0.6%
Vitaphene 0- phenylphenol 9%
benzyl p chlorophenol
Cidex: 2.4% gluteraldehyde solution
0-benzyl-p-chlorophenol 1%
Combat Wound Disinfection and Healing/Investigation of a Topical Antiseptic Agent. Award # W81XWH-4-1-0727

P. I., Jack Beierle, Ph. D.
Associate Professor

Progress Report on the Subcontract: Cytotoxicity and Genotoxicity of Formula 5 in Cultured C3H/10T1/2 Cl 8 Mouse Embryo Fibroblasts

Hemant Vekaria, B. S., David Castillo, B. S., Marni Scheiner, B. S., and Joseph R. Landolph, Jr., Ph. D.

P. I. of the Subcontract:
Joseph R. Landolph, Jr., Ph. D.

Background, Objectives of This Subcontract, and Description of the Cell System Used to Study the Cytotoxicity and Genotoxicity of Formula 5

On this subcontract to the main grant of Dr. Jack Beierle, we have begun to study the cytotoxicity and genotoxicity of Formula 5, a novel antimicrobial agent. We were specifically interested to determine whether formula 5 exerted a uniquely high, intermediate, or low cytotoxicity to these murine cells. For our testing system, we utilized the well-known cell culture system of C3H/10T1/2 Cl 8 (10T1/2) mouse embryo fibroblasts. These cells are contact-inhibited, have a very low saturation density (approximately 800,000 cells/60 mm dish), and have a plating efficiency of approximately 25% - 35%. The cells are grown in humidified incubators at 37 degrees Centigrade in an atmosphere of 5% carbon dioxide/air (v/v). 10T1/2 cells are thought to be a spontaneously immortalized, primitive mesenchymal cell line. These cells can be converted into adipocytes, myocytes, and chondrocytes when treated with the differentiation-inducing agent, 5-azacytidine. When treated with chemical carcinogens such as 3-methylcholanthrene, foci of transformed cells arise. When these foci are cloned and injected into nude mice, they form invasive progressively growing, fibrosarcomas. Dr. Joseph R. Landolph’s laboratory has twenty-eight years’ experience with this cell system.

Progress Studying the Cytotoxicity of Formula 5

We first asked the question, “How cytotoxic is Formula 5 to these non-transformed murine fibroblasts in cell culture?” We conducted our cytotoxicity assays by standard methods in which we plated 200 cells/60 mm dish, five dishes per each concentration of Formula 5 tested. Formula 5 was added one day after the cells were seeded, and remained in contact with the cells for forty-eight hours in the first set of cytotoxicity assays. We first tested Formula 5 in a wide dilution series to determine the concentration ranges over which it was cytotoxic to 10T1/2 cells. To date, we have conducted six cytotoxicity assays with Formula 5, using a forty-eight hour exposure of
cells to Formula 5. In these first experiments, we did not have the information on the concentration of Formula 5. Hence, we expressed all concentrations in the form of the dilution of Formula 5 stock solution, of which we added 25 ul to each cell culture dish containing 5 mls of medium that bathed the cells. In these experiments, we added 25 ul of each dilution of Formula 5 to 5 mls of cell culture medium bathing the cells. In the first cytotoxicity experiment, Formula 5 at a dilution of 1/2000 reduced the plating efficiency of cells to (92.8 ± 3.4%) of the plating efficiency of control (phosphate-buffered saline, PBS) treated cells. At a 1/200 dilution, formula 5 caused the relative plating efficiency to actually increase to (109.6 ± 10.2) %, which was a slight hormetic effect that is often seen in these and other mammalian cells exposed to low levels of toxin. The molecular basis of this effect is not well understood. At a much higher concentration, a dilution of only 1/20, Formula 5 reduced the plating of the cells to (77.8 ± 5.3)%. At the very highest concentration of Formula 65 tested, a 1/2 dilution, the plating efficiency of the cells was reduced to 0% (all cells were killed). Hence, in this initial range-finding cytotoxicity experiment, the cytotoxicity of Formula 5 was dose-dependent at 1/20 and 1/2 dilutions. We therefore refined the concentrations of Formula we tested in successive experiments to be dilutions of 1/10 and lower.

In experiment 2, we tested dilutions of 1/10, 1/100, 1/10000, and 1/10,000 of Formula 5 against 10T1/2 cells. We found that dilutions of Formula 5 of 1/10,000, then 1/1,000, then 1/100, then 1/10 caused a reduction in the plating efficiency of 10T1/2 cells to 93%, 85%, 95%, and then to 0% (Assay 2). Hence, there was little or no cytotoxicity up to a dilution of 1/100, and then a precipitous decline in plating efficiency to 1% at a dilution of 1/10 of Formula 5.

In a third assay, we tested concentrations of a 1/20 dilution and a 1/10 dilution of Formula 5, and found that the plating of 10T12 cells was reduced to 65% and to 1.6%, respectively.

In a fourth assay, we next decided to test concentrations of Formula 5 of 1/20 and greater. In this experiment, a concentration of 1/20 of Formula 5 reduced the plating efficiency of 10T1/2 cells to 62.8%. Next, we found that concentrations of 2/20, 3/20, 4/20, 5/20, and higher than this killed all the 10T1/2 cells; the plating efficiency of treated cells divided by that of control cells was 0%. Hence, the cytotoxicity of Formula 5 was clearly dose-dependent and occurred at concentrations greater than 1/20 of Formula 5. We repeated this experiment exactly in a new experiment (#5), and found that a 1/20 concentration of Formula 5 reduced the cytotoxicity of 10T12 cells to 74.7%, and that a concentration of a 2/20 dilution of Formula 5 and higher concentrations reduced the plating of 10T1/2 cells to 0%. Hence, the data in experiments 4 and 5 were fairly consistent.

In experiment #6, we tested concentrations of Formula 5 flanking the 1/20 dilution, both higher and lower concentrations, to define a cytotoxicity curve. In this experiment, concentrations of 1/100 dilution, 1/50, 1/33.3, 1/25, 1/20, and 1/10 dilutions caused reductions in the plating efficiency of 10t1/2 cells to 93%, 85.4%, 83.3%, 67.4%,
89.2%, and 62.9%. Hence, with the exception of the 1/20 dilution, the cytotoxicity of Formula 5 in this experiment was dose-dependent.

We next cumulated all this data in tabular form, and averaged the results of all the experiments. As shown in this table, when the results so fall the experiments are averaged, concentrations of 1/10,000, 1/2000, 1/1000, and 1/200 cause little or no cytotoxicity. At concentrations of 1/100, 1/50, 1/33.3, 1/25, 1/20, 1/10, 3/20, there are reductions in the plating efficiency of 10T1/2 cells to 94%, 85.4%, 83.3%, 67.7%, 74.0%, 16.1%, and 0%, respectively. Hence, the cytotoxicity of Formula 5 is dose-dependent in this concentration range. The LC50 value (concentration that reduces the plating efficiency to 50% of that of control cells), is estimated to be between a 1/25 and a 1/10 concentration of Formula 5. After completing these experiments, we received information that the concentration of the Formula 5 solution we were provided was 63.5 mg/ml of solids. Hence, we estimated that the actual LC50 value was: 12.7 ug/ml < LC50 < 31.8 ug/ml. We have additional experiments ongoing to determine the LC50 value precisely for a 48 hour exposure of 10T1/2 cells to this compound. However, as listed in the table below, we can say at this point in time that Formula 5 is certainly not as cytotoxic as adriamycin, whose LC50 is 0.0158 ug/ml, nor as cytotoxic as the metabolite of the fungus Aspergillus, whose LC50 value is 1.50 ug/ml. The LC50 value of Formula 5, between 13 and 32 ug/ml, ranks it slightly above the carcinogenic polycyclic aromatic hydrocarbon, 3-methyloctanol. Similarly, Formula 5 is 5 and 70 times more cytotoxic to 10T1/2 cells than acetoaminophen, aspirin, and borax, whose LC50 values are 1,000 ug/ml, 1,500 ug/ml, and 2,000 ug/ml, respectively. Hence, Formula 5 has intermediate cytotoxicity as constituted.

Table 2. Cytotoxicity of Various Chemicals to 10T1/2 Cells Measured in the Laboratory of Dr. Joseph R. Landolph.*

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<th>Chemical</th>
<th>LC50 value, ug/ml</th>
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<th>micrograms per mL</th>
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</table>
3-methylcholante 10.74 ug/ml 40.0

Formula 5 12.7 – 31.8 ug/ml

Ouabain 500. ug/ml

Acetaminophen 500. ug/ml

Phenacetin 1,000. ug/ml

Aspirin 1,500. ug/ml

Refined Borax 2,000 ± 1,200 ug/ml

* These values were derived in the papers listed below.

We are also in the process of determining the cytotoxicity curves and the LC50 values for a nine day treatment of 10T1/2 cells with Formula 5, which is the situation that would be encountered when Formula 5 is applied to humans and left on the tissues. These experiments are currently in progress.

References


Patierno, S. R. Banh, D. and Landolph, J. R. Transformation of C3H/10T1/2 Mouse Embryo Cells to Focus Formation and Anchorage Independence by Insoluble Lead

**CYTOTOXICITY DATA**

**Hemant Vekaria, Dave Castillo, Marni Schiner**

**Compound: Solution Number 5 (At a 1:5 dilution)**

**Assay 1**
5 dishes per point / 6 points

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| Mean   | 51.8 | 47.8  | 44.4  | 52.4 | 37.2 | 0   |
| Std Dev| 6.978539 | 5.1672 | 3.435113 | 10.2516 | 5.2631 | 0 |
| %      | At 100% | 92.88703 | 109.623 | 77.824 | 0 |

PBS Effect: 47.8/51.8=.92  Plating Efficiency = 25.9%

**Assay 2**
5 dishes per point / 8 points

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| Mean   | 49.5 | 48.4  | 45     | 45.8  | 46   | 0   |
| Std Dev| 4.654742 | 5.41295 | 3.316625 | 4.96991 | 6.892 | 0 |
| %      | At 100% | 92.97521 | 94.6281 | 95.041 | 0 |

PBS Effect: 48.4/49.5=.98  Plating Efficiency = %24.8

**Assay 3**
5 dishes per point / 4 points

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| Mean   | 111.6 | 101.8 | 66.6 | 1.6 |
| Std Dev| 17.09678 | 7.15542 | 8.648699 | 3.04959 |
| %      | At 100% | 65.4224 | 1.57171 |
PBS Effect: 101.8/111.6 = .91  Plating Efficiency = %55.8

Note: Due to the abnormally high Plating efficiency, it is likely that there was an error in dilution calculations and the experiment was repeated with new points.

**Assay 4**  
5 Dishes per point / 12 points

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Mean: 52.8  46.8  29.4

Std Dev: 9.782638  4.20714  5.88662

% At 100%  62.82051

**Assay 5**  
5 Dishes per point / 12 points

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Mean: 20.75  19  14.2

Std Dev: 1.707825  3.3665  11.25611

% At 100%  74.73684

**Assay 6**  
5 Dishes per point / 8 points

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Mean: 31.4  37.2  34.6  31.8  31  25.2  33.2  23.4


% At 100%  93.01075  85.4839  83.333  67.7419  89.247  62.9032

For the next assays, we will continue to use these points but add another stronger concentration to reach 100% Cell toxicity.

The points we will use are 1:100, 1:50, 1:33.3, 1:25, 1:20, 1:10, and 4:5 3:20, 4:1:5
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</tr>
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</tbody>
</table>
2. Animal Studies
Wound Healing and Infection Prevention in Rabbits and Guinea Pigs
June 30, 2005

To Col. Vandre

From: Stephen Swenson Ph.D.,
Univ. of Southern California
Keck School of Medicine

Colonel Vandre,
Attached is the report on the *in vivo* studies involved with Grant log #04054001 in the evaluation of Formula 5 as an antiseptic agent. If there are any problems with the file or you need further information please let me know.

Thank You,

Stephen Swenson Ph.D.
Progress Report “Evaluation of a Topical Antiseptic Agent”

Antiseptic care of incisional and burn wounds is an important part of infection control. Wound contamination and the subsequent decontamination of wounds are of interest in a combat care setting. A number of methods are currently in use in wound and instrument decontamination including sterilization, disinfection, and antisepsis. Contamination is defined as the introduction of microorganisms into tissues or sterile materials, whereas decontamination is defined as the reverse; disinfection or sterilization of infected wounds to an acceptable level (noninfectious level). Disinfection is defined as the selective elimination of selected undesirable microorganisms to prevent their transmission (the reduction of the number of infectious organisms to a level below that necessary to cause infection), sterilization is defined as the complete killing of all foreign organisms while, antisepsis is the application of a liquid antimicrobial to skin or other living tissue to inhibit the growth of and or destroy microorganisms. Examples of antisepsis include hand washing with germicidal solutions or swabbing skin before an injection. A number of different product types are available for skin antisepsis including, hand washes, body washes, solutions (in a variety of physical forms) and all of these can be used in a wide variety of situations. As a guideline, to be considered must demonstrate a $2 \log_{10}$ reduction of bacterial contamination within five minutes after the first wash and a $3 \log_{10}$ reduction within five minutes of the tenth wash. The project described in this proposal will evaluate and compare the efficacy of Formula 5 with the topical antiseptic agent betadine in preventing contamination of clear skin, surgical wounds and debrided skin with partial thickness burns.

In the in vivo studies reported here we describe the effect of an investigational antiseptic agent, Formula 5, in limiting and eliminating infection in wound models. In the course of this study we have evaluated the efficacy of Formula 5 under in vivo conditions against common human pathogenic bacteria including, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and normal oral flora.

The first study completed was designed for the evaluation of bacterial “kill” on normal uncompromised skin. In these experiments, the bacterial strains shown above ($1 \times 10^9$ CFU/25 μl) were introduced to the shaved backs of rabbits (back of the neck was used to reduce contamination by the cage floor or grooming); the areas for study were outlined defined by outline using a sterile template. The animal backs were then scrubbed with betadine scrub (standard skin preparation for surgery) followed by wiping with isopropanol to remove residual betadine. The two defined sites per rabbit were approximately 6.5 cm² (1 inch²) placed along the spine. Following application of the bacterial the treatments; saline (control), betadine and Formula 5 were introduced via pipette tip (100 μl) and spread over the entire marked area. At 5, 30 minutes, 1, 6 and 24 hours following treatment application (a zero minute point in these studies is technically infeasible), swabs were swabbed with sterile saline wetted sterile cotton swabs. For quantitation the swabs were placed...
into 1ml of sterile saline. In addition, following swabbing of the infected area the animals were sacrificed by administration of Euthasol and the marked contamination skin areas were surgically excised and placed into 5ml of sterile saline. Both the swabs and the skin were vortexed at least three times over the following hour followed by application of dilutions of the saline solutions to bacteria growth plates. CFU's were counted both 24 and 48 hours post plating. This same harvest procedure was followed for all of the animal models; normal skin, incision and burn. In the clear skin model the both Formula 5 and Betadine are effective topical anti-microbial agents. As can be seen in Figure 1 following the initial bactericidal action in the first five minutes both betadine and Formula 5 prevent further bacterial growth as compared to the saline control which exhibits no bactericidal action.

Of interest in all models are the time points in excess of six hours, precise quantitation of the number of bacteria becomes impossible. The reason for this is due to the biology of the study animals as well as animal welfare requirements. Both guinea pigs and rabbits are terrestrial animals and as such have a normal flora on the skin. This bacterial flora is capable of overwhelming other bacteria acting a natural bacterial growth limitation process. While the background from the flora on non-selective culture plates makes it impossible to quantitate the applied bacterial we are able to evaluated using an observational scale the effect of each of the treatments. In the case of the clear skin model the 24 hour time period follows what is observed in the shorter times with Formula 5 and betadine being near equal in "killing: and limiting the growth of the applied bacteria.

In a second set of experiments directly following the clear skin studies were investigated the antiseptic effect of treatment with saline, betadine and Formula 5 in a partial thickness incision model. In these studies a 2.5cm incision extending through the dermal layer was made within a demarked area, as above; on the shaved back of the 2kg New Zealand white rabbits. Rabbits were anesthetized with an IM administration of a mixture of ketamine hydrochloride and Xylazine intramuscularly and prepared for the surgical procedure as described above and following USC IACUC guidelines. Following preparation for surgery, three (or two in evaluation of cross contamination) incisions were made on a shaved area on the back of the neck. As in the clear skin studies the various microbes (10^9 CFU in 25μl) were placed in the incision and the site treated with 100μl of saline, betadine or Formula 5. The incisions were covered with occlusive Hilltop chamber dressings held in place with patches of tegaderm. At 0 30 min, 6 hours, 1,3,5,7 days different groups of animals were be sacrificed and necropsied and the wounds swabbed for quantitative microbiology, as described above. In each experiment three animals were evaluated per group with the three treatments on each animal. Initially, cross contamination was a concern in this study, but through controls it was observed that the hilltop chamber dressing and the tegaderm created a barrier that prevented bacterial cross contamination between the three wound sites.

![Figure 2: Effect of Antiseptic Treatment in a Purposely Contaminated Rabbit Incision Model.](image)
Quantitatively the data for the early time period in the incision model mimics that observed in the studies on clear skin (Figure 2). There did not appear to be a promotion or inhibition of bacterial growth or “kill” when the culture was placed in the wound as compared to being placed on clear skin. The later time points shown in Figure 3 show the same trend observed at the short time points that both betadine and Formula 5 limit the growth of the applied bacteria, but quantitation is difficult due to the intrinsic bacterial flora present on the animals (Table 1).

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Treatment</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3 Day</td>
<td>0</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5 Day</td>
<td>0</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>7 Day</td>
<td>0</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Efficacy of treatment on growth of *Pseudomonas aeruginosa* applied to a 2.5cm incisional wound on a rabbit back. Due to natural background bacteria found on rabbit skin numerical quantitation is impossible at these longer time points. In qualitative (and semi quantitative) evaluation: +++ indicates highly effective treatment, ++ lesser efficacy, + limited efficacy and 0 indicates no antiseptic effect as compared to no-treatment.

Figure 3  Average bacterial count in a partial thickness burn model of the effectiveness of a topical antiseptic agent.
Following burn, debridement and treatment, skin from the guinea pig back is surgically harvested. The tissue is placed in a sterile saline solution and agitated for 1 hour. The resultant solution is then plated on non-selective agar plates and allowed to grow for 48 hours. Bacterial counts are made of visible bacterial at this point. Data presented is the average effectiveness of each treatment against 3 bacterial isolates (mixed oral, pseudomonas and staphylococcus). The data includes the averages of four treatment periods including 30 min., 60 min., 6 hours and 24 hours. There is no appreciable difference between the data (Betadine and Formula 5) at any period. Each treatment was repeated and reported as the average of triplicates.

Finally, male Hartley guinea pigs were used in the evaluation of the antiseptic propeties of Formula 5 in a model of partial thickness burns. The burn model is a much better example of what may occur in the field. In the previous studies wounds were surgically made and the bacteria were forced into the wound in an attempt to create an infectious condition. In the burn model the wound is created and the bacteria are applied to the healing wound as could be the case in the field. In these studies following shaving and depilatory application to the backs of 400g guinea pigs burns are made with a 20mm diameter brass rod heated to 70C. The rod is applied for a period of 55 seconds and removed. The burn is then covered with an occlusive hiltop chamber dressing held in place with a Tegaderm bandage. Twenty four hours after the burn the wound is debrided and intentionally infected with bacteria as with the rabbits described above. Following application of the bacteria the wound is treated with saline (control), Betadine or Formula 5. As is evident from Figure 3 Betadine and
Formula 5 both significantly limit bacterial infection in this wound model. Limitations imposed by an inability to maintain the animals under sterile conditions limits the acquisition of quantitative data at the longer time points, but the data clearly shows that in the guinea pig model a single application of a topical antiseptic agent (either Formula 5 or Betadine) limits the infective process over a 24 hour period. In the longer time points the same pattern observed with the clear skin and incision and the qualitative data is shown in Table 2. Of note in these longer term observation is the any animal that lost the occlusive dressing during the course of the experiment the applied bacterial count dropped to zero as the normal bacteria on the animal skin overwhelmed those applied. Due to this any animals with loosened or missing bandages were eliminated from the study.

Statistically the data represented here is based on >200 guinea pigs in the burn model, and >400 rabbits in the clear skin and incision models. For each time point and each condition a minimum of five animals were evaluated. Through sheer number of animals and repeats at each point the data shows with a significance the efficacy of Formula 5 as compared to saline, and at least equivalency to betadine in limiting contamination and preventing bacterial growth.

Formula 5 is as effective as betadine in the decontamination of intentionally contaminated clear skin, incisions and partial thickness burns. The efficacy of this agent in the “kill” and limitation of growth of a panel of bacteria shows that Formula 5 is a broad spectrum efficient anti-microbial agent.

<table>
<thead>
<tr>
<th>Table 1: Effect of Treatment on Normal Oral Flora in a Partial Thickness Burn Model (Long Time Points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1 Day</td>
</tr>
<tr>
<td>3 Day</td>
</tr>
<tr>
<td>5 Day</td>
</tr>
<tr>
<td>7 Day</td>
</tr>
</tbody>
</table>

Efficacy of treatment on growth on normal oral flora applied to a partial thickness burn on a guinea pig back. Due to natural background bacteria found on guinea pig skin numerical quantitation is impossible at these longer time points. In qualitative (and semi quantitative evaluation: +++ indicates highly effective treatment, ++ lesser efficacy, + limited efficacy and 0 indicates no antiseptic effect as compared to no-treatment.
Other Animal Studies

To test additional microbes of particular interest further rabbit studies were initiated. The test microbes included

1. Methicillin Resistant Staph aureus (MRSA).
2. Strep pyogenes.
3. Vancomycin Resistant Enterococci faecalis (VRE).
4. E. coli.
5. Pseudomonas Aeruginosa

After animals were anaesthetized and shaved as previously performed, deep wounds (one wound on each side of the backs of the animals, one wound was for Betadyne and one was for Formula 5) were initiated by scalpel and microbial cultures grown on blood agar were inoculated heavily on cotton swabs directly from large colonies and rubbed into the wound sites. Massive inoculums were therefore achieved. After three days, when reddening and some pus was noted, samples were swabbed from the wound sites to determine levels of inoculum remaining. Distinctive colonies were stained for morphology and gram staining characteristics. The following day Betadyne and Formula 5 were applied with cotton balls and swabs were taken after one minute, five minutes and one hour to determine cfu’s remaining on the wound. This was followed by a three day waiting period with no additional disinfectant applied.
Swabs taken from the animal were placed in 3ml saline and vortexed for 30 seconds to remove bacteria. Samples were spread by plastic spreaders on blood agar and incubated for 48 hrs for cfu analysis. Results are as follows.

<table>
<thead>
<tr>
<th></th>
<th>MRSA</th>
<th>Strep Pyogenes</th>
<th>VRE</th>
<th>E. coli</th>
<th>Pseudomonas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>378</td>
<td>217</td>
<td>212</td>
<td>406</td>
<td>397</td>
</tr>
<tr>
<td>1 min Betadyne</td>
<td>335</td>
<td>220</td>
<td>243</td>
<td>356</td>
<td>350</td>
</tr>
<tr>
<td>1 min Formula 5</td>
<td>135</td>
<td>117</td>
<td>36</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td>5 min Betadyne</td>
<td>286</td>
<td>65</td>
<td>60</td>
<td>165</td>
<td>112</td>
</tr>
<tr>
<td>5 min Formula 5</td>
<td>36</td>
<td>17</td>
<td>10</td>
<td>40</td>
<td>56</td>
</tr>
<tr>
<td>24 hrs with no further treatment</td>
<td>585</td>
<td>305</td>
<td>393</td>
<td>375</td>
<td>428</td>
</tr>
</tbody>
</table>

There is a pattern that appears in the infections that results in the microbes remaining in the wound after three days. Treatments with Formula 5 after one minute show some microbe reduction, there is, however, little effect (if any) from Betadyne. After five minutes, good reduction of all five microbes tested is found with Formula 5. Fair to good reduction is also found with Betadyne after this passage of time. After 24 hours, microbes re-establish themselves. Our belief is that a one-day study every four hours would indicate a critical eight-hours-relief-period, especially with Formula 5 applications. Obviously multiple applications or continuous contact with Formula 5 and its low toxicity level would keep the wound in an excellent stage for healing and/or subsequent surgery.
Photos

Photos 1 and 2: Dr Steve Swenson and crew at work with Guinea Pigs.

3. Guinea Pigs with hilltop chambers and sterile transparent covers.

4. A burn wound in healing with hair re-growing around it. Betadyne surrounds the wound for protection.

5. Shaved Guinea Pig with two burn wounds.

6. Hair growth around healing wound. Top treated with Formula 5 and bottom with Betadyne.
Photos: Rabbit

1. Deep wounds. Right side treated with Formula 5, left side treated with Betadyne.

2. A healing back wound 13 days.
3. Summary
The question of combat casualty care revolves around the ability of rapid treatments that are effective in the prevention of infection. The critical first eight hours are of extreme importance to prevent the microbes from first establishing a point of invasion. The rapidly multiplying and spreading of bacteria, either through local invasion or by systemic metastatic means, is the crux of the situation.

One can regulate, diminish, or prevent infection through the use of antibiotics, topical or systemic, or with chemical disinfectants and antiseptics. The limitations with antibiotics have been amplified by mutational resistance and resultant infectious drug resistance. Biofilm formation and the costly search for new antibiotics are additional problems. The limitations in chemical disinfection include toxicity of such compounds which results in delayed wound healing and tissue death, as well as mutational chemical resistance by bacteria.

The purpose of this study was to enhance our understanding of Formula 5, a new solution with disinfecting properties and of very low toxicity. These studies furthered our in vitro studies on microbial kill, examined wounds and burns in rabbit and guinea pigs infected with various microbes and subsequently treated with Formula 5. Cytotoxicity studies were also advanced in vitro on the new, advanced Formula 5 solution to ensure low toxicity. Blood and skin tests were also tested to check other sources of potential cytotoxicity or irritation.
Hygiene and environmental studies were initiated to determine if Formula 5 has possible abilities to disinfect in health care, floors, surfaces, instrument decontamination, and if it had any positive cleaning effects on hair, skin, hands, and other body components. This report summarizes those studies.

Since combat is currently taking place in desert and mountainous conditions, our first trial with the new Formula 5 was designed around the kill of microbes from desert, mountain and shoreline soils.

The following soil samples we obtained from:

1. The Mojave Desert, California, 60 miles north of the City of Mojave and 20 miles south of Adelanto. Altitude 2, 300 feet.

2. The Sierra Nevada Mountain Range, McGee Canyon, California, 13 miles south of the town of Mammoth Lakes at 7,600 feet altitude.

3. A breach in the island of Kauai, Hawaii, 12 miles north of the airport at sea level elevation.
**METHODS**

Collected soil samples were taken back to the laboratory in plastic bags and weighed out in 2 gram aliquots. They were suspended in 15 ml of sterile water, shaken into suspension and 1 ml water suspension removed. A 1.0 ml aliquot was pipetted and a dilution series made two-fold. Enriched agar media was poured into petri plates and counted after three days incubation at ambient temperature. Some 1.0 ml aliquots had a 0.1 ml aliquot of Formula 5 (l: 10) added to test for microbial kill. At various time increments, 0.5 ml aliquots were pipetted into Petri plates and then 12 ml of enriched nutrient agar was added, allowed to solidify and measured after three days for colony forming units (cfu’s). The data collected was recorded and plotted.

**Table 1**

*Kill Curves of Microbes Isolated from Desert, Mountain and Beach Soils, Treated with Formula 5*

<table>
<thead>
<tr>
<th>Time in Minutes</th>
<th>Soil: Desert</th>
<th>Desert</th>
<th>Mountain</th>
<th>Beach</th>
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<tbody>
<tr>
<td>0</td>
<td>850</td>
<td>1,100</td>
<td>6778</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>76</td>
<td>52</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
The results showed that Formula 5 killed all microbes isolated from soil samples obtained from desert, mountain and beach soils or sands. The complete kills were obtained within 2 minutes, whereas, ca. 90% kill or better was obtained in the first minute of contact with Formula 5.
4. Formula 5 Destruction of Microbial Biofilms
Picture 1 is of a Staph biofilm after three minutes’ treatment with Formula 5 as seen at 100x magnification.

Picture 2 is of a Pseudomonas biofilm after three minutes’ treatment with Formula five as seen at 2,000x magnification.

Picture 3 is of the damage Formula 5 has done to a Pseudomonas biofilm after 15 minutes’ treatment with Formula 5 as seen at 2,000x magnification.

Picture 4 is of the complete destruction of bacteria in a MRSA biofilm colony after 15 minutes’ treatment as seen at 5,000x magnification. The matter in the picture is the leftover slime that once covered this colony.

Picture 5 is of a Mixed Oral biofilm colony that has been broken, with parts reduced to a planktonic form after three minutes’ treatment as seen at 1,000x magnification.

Picture 6 is of a Mixed Oral biofilm colony after 15 minutes’ treatment demonstrating that a large part of the colony has been unaffected by Formula 5 as seen at 5,000x magnification.

Picture 7 is also of the Mixed Oral biofilm at 15 minutes treatment but is only taken from 100x magnification to give a broader view.

Picture 8 is of the remains of an Enterococci biofilm (the object seen is the slime of a biofilm devoid of any bacteria) after three minutes’ treatment with Formula 5 as seen at 5,000x magnification.

Picture 9 is of another part of the remains of the same biofilm colony as seen at 1,100x magnification.
Picture 10 is of a completely destroyed Enterococci biofilm colony after 15 minutes' treatment (the objects seen in the photograph are debris (dust particles etc.) as seen at 100x magnification.

Picture 11 is of an E. Coli colony that has been taken out of its biofilm state after 15 minutes of treatment as seen at 5,000x magnification.

Picture 12 is of a destroyed area of the E. Coli colony after 15 minutes of treatment as seen at 2,000x magnification.
5. Body and Hair Cleaning with Formula 5
THE POTENTIAL OF FORMULA 5 AS A SOAP WASH AND HAIR SHAMPOO

Formula 5 was placed in a plastic pump bottle, 35 ml size. The finger pump generates foam. The foam was used as a shampoo or general body soap. Men in the field are often on duty in long term situations. These tests were performed to determine the efficacy of Formula 5 in cleansing numerous body parts. The values (cfu reduction) were compared to starting points at the beginning or end of a work day. Formula 5 was compared to soaps and shampoos in order to ascertain if Formula 5 could successfully reduce microbes in key body areas, such as head, face, legs, arms, feet and so on. Test individuals ranged from 17 to 66 years of age and were males. Five test subjects were used. One woman was tested for hand cleaning.
### Head Region

<table>
<thead>
<tr>
<th></th>
<th>Start (Baseline)</th>
<th>After Soap/Shampoo</th>
<th>After Formula 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheek</td>
<td>489</td>
<td>312</td>
<td>67</td>
</tr>
<tr>
<td>Nose</td>
<td>3,027</td>
<td>2,905</td>
<td>190</td>
</tr>
<tr>
<td>Chin</td>
<td>110</td>
<td>80</td>
<td>56</td>
</tr>
<tr>
<td>Hair</td>
<td>212</td>
<td></td>
<td>57</td>
</tr>
<tr>
<td>Scalp</td>
<td>2,100</td>
<td></td>
<td>222</td>
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</table>

### Upper Body Region

<p>| | | | |</p>
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<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Forearm</td>
<td>57</td>
<td>32</td>
<td>11</td>
</tr>
<tr>
<td>Bicep</td>
<td>25</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Underarm</td>
<td>TNTC</td>
<td>4,050</td>
<td>3,450</td>
</tr>
</tbody>
</table>
EFFECT OF FORMULA 5 AS A CLEANING AGENT ON HAIR
AND BODY PARTS

<table>
<thead>
<tr>
<th>CFU's</th>
<th>Start (Baseline)</th>
<th>After Soap/Shampoo</th>
<th>After Formula 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest</td>
<td>1,125</td>
<td>385</td>
<td>101</td>
</tr>
<tr>
<td>Back</td>
<td>127</td>
<td>96</td>
<td>55</td>
</tr>
<tr>
<td>Buttocks</td>
<td>17</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Thigh</td>
<td>121</td>
<td>26</td>
<td>11</td>
</tr>
<tr>
<td>Calf</td>
<td>47</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>Foot</td>
<td>98</td>
<td>80</td>
<td>48</td>
</tr>
<tr>
<td>Between toes</td>
<td>TNTC</td>
<td>TNTC</td>
<td>3,080</td>
</tr>
</tbody>
</table>

Hand

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Back</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>Palm</td>
<td>212</td>
<td>2</td>
</tr>
<tr>
<td>Knuckle</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Nail</td>
<td>1,600</td>
<td>960</td>
</tr>
<tr>
<td>Thumb</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Forefinger</td>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>
**Wash Discussion**

There is much variability on a person to person basis. A woman had the cleanest hands and reduced the microbial load best. Actually, not unexpected, older men were more casual in the clean-up. The best specimen was a 17 year old with a full bodied head of hair. This individual reduced his hair load to 1 microbe/swab and a photo is enclosed to demonstrate that.

Basically, Formula 5 reduced microbial levels from every site tested. The sites of highest microbe loads were on hairy areas such as the chest, underarms and groin. Highest numbers of microbes were detected at the end of the work day. Persons undergoing physical labor were noted to have the highest counts. Areas as found between toes and under fingernails are difficult to clean and subjected to high cfu levels. Swabbing of toes with Formula 5 produced the best results. Anecdotal responses have suggested that Formula 5 works against atheletes’ foot. We did not have the opportunity to test that supposition.

The head and face are also crucial areas. The scalp is found to contain higher cfu’s than hair. The face is generally high in most areas, such as nose and forehead.

To all appearances, Formula 5 could be used as a foaming soap and has the potential to be incorporated into a soap. The question of water availability suggest that a foam drying on a body part having a residue may have a sustained killing and protective action. We will continue to explore that question. The potential is there.
Baselines were established by swabbing at the end of a workday or in the morning.

Swabs were inoculated directly on blood agar plates, or in the case of high counts, swabs were broken off in test tubes with 5 ml sterile saline, mixed in a vortex mixer 2 minutes. Aliquots were then measured by dilutions and 0.5 ml was added to a blood agar plate. The mixture was spread by a plastic plate spreader and incubated for 48 hours prior to plate counts and cfu determinations.
Formula 5 and Hair Cleansing

The photo of the blood agar plate below is a before (top) swab of hair and a bottom view of a hair swab after hair shampoo with Formula 5. The upper segment is a Too Numerous to Count (TNCT) view of the microbial load prior to cleaning. The lower half, after an eight day incubation period, revealed several colonies. The growth on the edges is the result of spread by moisture contamination by the top load. At present we don’t know how long the disinfecting process lasts nor how rapidly the microbial load increases. It should be noted that not all individual achieved a 99+% reduction. That reduction is a function of intensity and thoroughness of cleaning and training. That the level of cleaning was approachable to perfection was startling in itself and was accomplished on two separate occasions.
6. Formula 5 as a Medical/Dental Instrument Sterilant or Antiseptic
Instrument Sterilization

One potential use of importance for Formula 5 is its potential as an instrument sterilizer in the field. We have examined the kill rate of bacteria when fresh serum or blood is added to a chemical-bacteria-organic mixture and found that a less than 5% reduction in kill or kill time is noted. To test contaminated instruments we placed instruments in tubes sufficiently large enough to hold sterile media, instruments and various controls.

Instruments tested included scissors, forceps, tweezers, dental burs, probes, explorers and clamps. Serrated edges, hinged devices and knurled ends were examined to determine whether sequestered areas could be disinfected. Instruments were placed in trays containing $10^8$ bacteria per milliliter and allowed to remain in contact for 45 minutes. They were removed, air-dried, and placed in sterile tubes with various dilutions of Formula 5, e.g. 1:5, 1:10, 1:20, and 1:40. After incubating with Formula 5 for various times they were removed, dipped in sterile saline, and placed aseptically in sterile tubes of appropriate sizes which contained sterile media and incubated at 35° for up to 8 days. Tubes and positive controls could be visually detected by turbidity. Media containing purple base could be detected by observing a purple to yellow color shift via pH change by acid production indicating microbial growth. Growth was surveyed at room temperature and at 35°C incubator temperature under aerobic conditions.

Positive control tubes showed turbidity at 24 hours and extensive turbidity at 48 hours. Under proper conditions no growth was observed at 8 days. In some conditions of lower level kill at 8 days, very few microbes per milliliter were detected. Under the sterilization conditions, no turbidity or pH change is detected, nor are any Cfu’s noted.
when 1 milliliter of test media was inoculated and spread on the surface of blood agar plates. The following table summarizes the results.

**Table 1**

Day 8 of Test after 5 minute exposure to Formula 5
Reduction of Strep Pyogenes after exposure to Formula 5

<table>
<thead>
<tr>
<th>Turbidity</th>
<th>pH Shift</th>
<th>Cfu's</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Control</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>+ Control</td>
<td>Heavy</td>
<td>Yes</td>
</tr>
<tr>
<td>1:5 dilution of F5</td>
<td>0/3</td>
<td>Slight</td>
</tr>
<tr>
<td>1:10</td>
<td>2/3</td>
<td>Moderate</td>
</tr>
<tr>
<td>1:20</td>
<td>3/3</td>
<td>Heavy</td>
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**Table 2**

Day 8 of Test after 10 minute exposure to Formula 5
Reduction of Strep Pyogenes after exposure to Formula 5

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<tr>
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</tr>
</thead>
<tbody>
<tr>
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<td>None</td>
</tr>
<tr>
<td>+ Control</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>1:5 dilution of F5</td>
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<td>None</td>
</tr>
<tr>
<td>1:10</td>
<td>Slight</td>
<td>Yes</td>
</tr>
<tr>
<td>1:20</td>
<td>Yes</td>
<td>Yes</td>
</tr>
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</table>
Table 3

Day 8 of Test after 15 minute exposure to Formula 5
Reduction of Strept Pyogenes after exposure to Formula 5

<table>
<thead>
<tr>
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<th>Turbidity</th>
<th>pH Shift</th>
<th>Cfu's</th>
</tr>
</thead>
<tbody>
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<td>TNTC</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:10</td>
<td>±</td>
<td>Slight</td>
<td>16</td>
</tr>
<tr>
<td>1:20</td>
<td>+</td>
<td>+</td>
<td>1720</td>
</tr>
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</table>

The analyses have shown that microbes do remain on the instruments up to test
time and that they quickly grow out as a positive or growth result that are quickly and
easily detected. Formula 5 is capable of disinfecting so long as sufficient time elapses for
contact of the chemical with the contaminated instrument. With the data to date, it appears
that a minimum of 15 minutes is required for complete disinfection to occur.

Development of a device impregnated with Formula 5 may be necessary for
constant moist contact during such a procedure. As field conditions are varied and arduous
such procedures need be discussed further to define parameters to achieve expected results.
The use of chemical field sterilization remains a strong possibility.

Following

Figure 1 shows from left to right, a growth control: a 10 minute Formula 5 test; a 15
minute test, a 10 minute test showing slight growth, and a failed test. All samples held for
eight days.

Figure 2 is a close-up of negative growth.
7. In Vitro Kill Curves of Bacteria by Formula 5
IN VITRO KILL CURVES

The testing of formula 5 in in vitro kill curves revealed that the killing power of Formula 5 was rapid and complete in every microbe tested. Whether the microbe was gram positive or negative, rod or cocci, a mycobacterium member, Pseudomonas or drug resistant made no difference. Extremely high numbers are rapidly killed and blood or serum only slightly interfere with Formula 4’s killing potential on microbes. Dilutions of 1:200 of Formula 5 still showed antimicrobial activity. Usually an addition of dilution 1:5 and by 100-200 microliters is sufficient to provide such kill.

The general protocol was to take a 2 ml aliquot of bacteria containing a $10^9$ ml of the test microbe and adding a 200 microliter aliquot of 1:5 Formula 5. After mixing quickly aliquots of 0.5ml of the mixture were added to a petri plate and 12ml of media were instantly added and mixed. Plates were incubated aerobically at 35 degrees Celsius or other appropriate temperatures. Cfu’s were measured at time points ranging from seconds to minutes. After 48 hours of incubation the cfu’s were counted and recorded. Experiments were all run a minimum of three times to assure reproducibility. A range of times, volumes and concentrations of bacteria and Formula 5 were examined, but the basic procedure used was that discussed above. The following list of Bacteria was examined. The range tested included drug resistant strains spore formers, yeast, streptococci and staphylococci, and a mycobacterium species.
Graph one indicates rapid kill of Staph aureus. This microbe is killed in 20 seconds. The scale is set for 10,000 ml arbitrarily for the sake of demonstrating a reduction but at actual zero time there was one billion.

Graph two is a mixed oral preparation containing numerous species. The oral cavity is known to contain in excess of 500 species. This complex milieu containing clusters of plaque biofilm microbes takes about a minute to achieve a 99% kill and up to ten minutes for complete eradication. Diluted to 1:60, Formula 5 is still very effective. G

Graph three: The spore former B stearothermophilus, the gold standard for autoclave testing takes up to 15 minutes for total kill but again reaches excellent kill at one minute.

Graph four: Demonstrates some resistance to rapid kill by Candida Albicans, taking up to 15 minutes for complete kill. High kill rates are noted at 1-5 minutes.

Table one provides data on a mixed oral population versus several disinfectants. Formula 5 and Betadyne are comparable in kill with Formula 5 having a slight edge in kill at thirty seconds. Vitaphene, a phenolic, has good kill as well, trailing Betadyne slightly. Aerocide has a moderate kill with the remaining two not particularly effective.

Graph five shows a kill of streptococcus pyogenes. This group of strep is particularly virulent and is commonly found in various wounds. Formula 5 has complete kill in 900 seconds.

Graph six: A strep pyogenes kill curve versus various disinfectants. In this case, Vitaphene was most effective followed by Betadyne and Formula 5.

Graph seven: A kill curve of mixed oral bacteria versus various disinfectants. Betadyne, Formula 5 and Sporocidin all showed excellent kill in thirty seconds.
Graph eight: Formula 5 was tested against Acinetobacter baumannii and revealed excellent kill at one minute.

Graph nine: Formula 5 shows excellent kill against Vancomycin resistant Enterococci (MRE) in one minute.

Graph ten: Formula 5 reveals excellent kill versus Methicillin resistant Staph aureus (MRSA).
Solution 5 vs. Mixed Oral

Graph #

Time in Minutes

Relative Number of Colonies

000 5
000 10
000 15
000 20
000 25
000 30
000 35
000 40
000 45
000 50
000 55
000 60

Time in min: 0 1 5 10 15 20 25 30 40 45 50 55 60

1:10 5000
1:15 450
1:21 421
1:25 281
1:30 0

Dil sol 1:10 — Dil sol 5:1:60
Effect of Sol 5 on B. Stearo Spores

Time in Minutes

Relative Number of Colonies

0 5,000 10,000 15,000
0 5,000 10,000 15,000
0 5,000 10,000 15,000
0

Time in min: 0 1 5 10 15

Dil Sol 5 1:10 — Dil Sol 5 1:60
### Mixed Oral Bacteria Kill Curve vs. Various Disinfectants

**June 2005**

<table>
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<th>5 min.</th>
<th>10 min.</th>
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<td>0</td>
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<td>TNTC</td>
<td>TNTC</td>
<td>TNTC</td>
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June 2005

Various Distinctants.

Serratococcus Pyogenes Kill Curve vs.
June '2005

Mixed Oral Bacteria Kill Curve vs. Various Distinctions.
solids treated with Formula 5.

Kill curves of Microbes isolated from desert, mountain and beach.

Table:
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<th></th>
<th>0 min</th>
<th>1 min</th>
<th>5 min</th>
<th>10 min</th>
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**Formula: 5 vs. A. baumitii: Kill curve**
Resistant Enterococci: Kill curve
Formula 5 vs. Vancomycin
8. Human Wounds and Formula 5
Photos - Human Wounds

1. Hand wound day three, an inflamed gouge

2. A close-up showing pus.
   A pump spray was applied three times daily and within two days, inflammation subsided. In ten days the wound healed completely.

3. A glass cut on an arm. Formula 5 swab within ten minutes, only slight inflammation was noted. The wound healed rapidly with a two-times-daily-swab.

4. The same wound one day earlier.

5. A lesion left after a mole removal on a temple; no stitches. Day three inflamed and swollen.

6. Foam application of Formula 5 reduces inflammation after three days.

7. A second day view of showing reduced inflammation.
9. Health Care Facility Cleanup by Formula 5
Health Care Facility Cleanup

Graph 1 illustrates a medical facility exam room, the pulmonary machine of almost 200 cfu’s/swab.

Graph 2 illustrates a Pediatric office with a baby scale with over 100 Beta Hemolytic pathogenic colonies/swab. This would represent an ideal site for transfer of disease.

Graph 3: A medical office exam room with the sink harboring the highest cfu’s.

Graph 4: A hospital nurses’ station which was a relatively clean area with the floor showing minor contaminants.

Graph 5: A rather clean dental laboratory.

Graph 6: A hospital based lab facility, which was fairly clean due to it being maintained by trained lab personnel.

Graph 7: A dental operatory: a fairly clean facility.

Graph 8: A medical lab which had a floor with a moderate level of cfu’s and an extremely contaminated sink.

Graph 9: An aerosol fallout in a Dental facility. This graph illustrates a lack of consistency in cleaning procedures and the variance one finds in dental air. The variables contributing to this include level of contamination in dental water lines, oral condition, use of vacuum lines, and rubber dams.

Graph 10: Another aerosol bioload in a dental operatory, in this case a number of Beta-hemolytic pathogens are detected throughout the facility—not something one should inhale.
Graph 11: A third aerosol fallout in a dental clinic. In this case all nine work sites had Beta Hemolytic microbes in the air.

Graph 12: Medical facility, a before and after Formula 5 cleanup.

Graph 13: Laboratory and surroundings; disinfection of areas with a 1:10 dilution of Formula 5: Spray bottle and wipe followed by swab after 10 minutes.

Graph 14: Formula 5 wipe down before and after shows enhanced cleanup.

Graph 15: Formula 5 wipe down of a laboratory… all cfu counts are under 10.

Graph 16: Dental operatory comparison of Formula 5 vs. disinfectant used (sani-wipes), Formula 5 shows most kill by far.
Cleaning Potential of Formula 5; 
in Operatories, Labs and Medical Facilities

Formula 5 used in foams, sprays and in liquid forms as a wipe with 4x4 gauzes were tested for the efficacy in killing and cleaning over thirty medical, dental and laboratory facilities. Formula 5 showed excellent antimicrobial/cleaning powers at least equaling, but usually exceeding, other standard disinfectants.

The areas of highest contamination in dentistry were sinks, floors, high power evacuation lines, and counter tops. Aerosol studies indicated that the higher the microbial count in waterlines, the higher the surface count. Aerosol fallout is the source of surface contamination. Patients with high oral microbial counts also add greatly to the aerosol bioload during operative procedures.
FORMULA 5 VS. VARIOUS DISINFECTANTS

Numerous disinfectants are used in health care facilities. In addition to Formula 5 and Betadyne, four other commonly used disinfectants were used. Their chemical make-up is listed in the page labeled "List of Materials and Bacteria Used." Formula 5, Betadyne and Vitaphene are all good disinfectants. Povidone Iodide and Vitaphene (9% phenyl phenol) are rather toxic and harmful to tissues. In all instances, Formula 5 was as good, if not better, than the other compounds.

The two graphs of Strep pyogenes are duplicates, one being a line graph, the other a bar graph. The bar graph best demonstrates the kill from 30 seconds. Formula 5 has the earliest complete kill of the culture. A 2 ml dose of $10^8$ Strep/ml was tested vs. 200 microliters of Formula 5 and time points measured in seconds up to 900 seconds were measured. The line graph excluded Aerocide as the slow action kill of this compound could not easily be recorded with test.

The kill curves of the remainder of the microbes provided the same basic pattern as Strep pyogenes give or take a few seconds or minutes. I have provided a sampling of kill curves rather than the entire 18 tested.

Table A shows mixed oral bacteria vs. Formula 5 and other disinfectants. At 30 seconds, Formula 5 has killed all bacteria, whereas, Betadyne takes 60 seconds, still a very adequate kill. Only Vitaphene approached Betadyne and Iodine.
April 21, 2005

EXAM ROOM # 1

KEY

1. Exam table
2. Sink
3. Pulmonary machine
4. Floor
5. EKG machine
6. Counter top

![Graph showing the relative number of colonies for different locations in Exam Room #1. The key indicates the types of organisms identified: Microbes, Molds, Beta Hemolytic Pathogens.](image-url)
December 21, 2004

**KEY**

1. Exam table
2. Baby scale numbers
3. Baby scale
4. Forms holder inside
5. Outer door handle
6. Counter top
7. Floor
8. Sink

**EXAMINATION ROOM #1**

![Graph showing relative numbers of colonies for different locations in examination room #1.](image)
January 31, 2005

**EXAMINATION ROOM # 5**

**KEY**

1. Exam table
2. Sink
3. Floor
4. Telephone
5. Table base
6. Counter top

![Graph](image)

**KEY**

- Microbes
- Molds
- Beta Hemolytic Pathogens
April 21, 2005

**KEY**

1. Sink
2. Counter top
3. Plastic trays
4. Floor
5. Stethoscope
6. Chart

*Numbers 5 and 6 are not reflected on the graph because the colonies were zero (0).*
Solution 5 A. Untreated

KEY

1. Lab 102 floor
2. Lab 102 sink
3. Lab 102 incubator door
4. Lab 102 refrigerator door
5. Lab 102 side workbench
6. Lab 102 keyboard
7. Lab 102 media shelf
8. Lab 102 hot plate
9. Lab 102 rear workbench
10. Conf. Room table
11. Conf. Room water cooler
12. Conf. Room refrigerator
13. Bathroom door handle
14. Bathroom flush handle
15. Bathroom floor
16. Room 123 sink
17. Room 123 table
18. Room 123 microscope
19. Room 124 autoclave handle (TNTC)
20. Room 124 refrigerator
21. Room 124 biohazardous waste container

<table>
<thead>
<tr>
<th>Relative Number of Colonies</th>
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</thead>
<tbody>
<tr>
<td>20</td>
</tr>
<tr>
<td>40</td>
</tr>
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KEY

- Microbes
- Molds
- Beta Hemolytic Pathogens
January 25, 2005

OPERATORY #10

KEY

1. Chair arm
2. Egg timer
3. Lamp switch
4. Bracket table
5. Chair base
6. Counter top
7. Hand mirror
8. Floor
9. X-ray viewer
10. Sink
11. High Power evac
12. Saliva ejector

KEY

□ Microbes □ Molds □ Beta Hemolytic Pathogens
March 9, 2005

KEY

1. Counter top
2. Floor
3. Telephone
4. Inside centrifuge
5. Stethoscope
6. Sink*

* Number 6 (Sink) is not reflected on the graph because the colonies were too numerous to count (TNMC).

Laboratory

- Microbes
- Molds
- Beta Hemolytic Pathogens
January 19, 2005

**AEROSOL FALLOUT**

**KEY**

1. Operatory #1
2. Operatory #2
3. Operatory #3
4. Operatory #4
5. Operatory #5
6. Operatory #6
7. Operatory #7
8. Sterilization Area
9. Laboratory

**KEY**

- □ Microbes
- □ Molds
- □ Beta Hemolytic Pathogens
AEROSOL FALLOUT

KEY

1. Operatory #1
2. Operatory #2
3. Operatory #3
4. Operatory #4
5. Operatory #7

Relative Number of Colonies

1  2  3  4  5

KEY
- Microbes
- Molds
- Beta Hemolytic Pathogens
October 21, 2004

AEROSOL FALLOUT

KEY

1. Operatory #1
2. Operatory #2
3. Operatory #3
4. Operatory #4
5. Operatory #5
6. Operatory #6
7. Operatory #7
8. Sterilization Area
9. Laboratory

KEY

Microbes  Molds  Beta Hemolytic Pathogens
Efficacy of Solution 5 in Disinfection

April 21, 2005

Office:

Key

1. Exam table
2. Sink
3. Magnifying lamp
4. Floor
5. Microscope
6. Counter top

Relative Number of Colonies

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<tr>
<th></th>
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</thead>
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</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0</td>
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</table>

Surgery
Solution 5  D. 1:10, Spray 1 min.  

February 1, 2005

KEY

1. Lab 102 floor
2. Lab 102 sink
3. Lab 102 incubator door
4. Lab 102 refrigerator door
5. Lab 102 side workbench
6. Lab 102 keyboard
7. Lab 102 media shelf
8. Lab 102 hot plate
9. Lab 102 rear workbench
10. Conf. Room table
11. Conf. Room water cooler
12. Conf. Room refrigerator
13. Bathroom door handle
14. Bathroom flush handle
15. Bathroom floor
16. Room 123 sink
17. Room 123 table
18. Room 123 microscope
19. Room 124 autoclave handle
20. Room 124 refrigerator
21. Room 124 biohazardous waste container

KEY

□ Microbes  □ Molds  □ Beta Hemolytic Pathogens
Efficacy of Solution 5 in Disinfection

April 21, 2005
Office:

Key:

1. Stainless tray
2. Sink
3. Exam table
4. Light switch
5. Not cleaned
6. Counter top

Relative Number of Colonies

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Exam Room #2

Key:

- Before Solution 5
- After Solution 5
Solution 5  C. 1:5, 5 min.

February 1, 2005

KEY

1. Lab 102 floor
2. Lab 102 sink
3. Lab 102 incubator door
4. Lab 102 refrigerator door
5. Lab 102 side workbench
6. Lab 102 keyboard
7. Lab 102 media shelf
8. Lab 102 hot plate
9. Lab 102 rear workbench
10. Conf. Room table
11. Conf. Room water cooler
12. Conf. Room refrigerator
13. Bathroom door handle
14. Bathroom flush handle
15. Bathroom floor
16. Room 123 sink
17. Room 123 table
18. Room 123 microscope
19. Room 124 autoclave handle
20. Room 124 refrigerator
21. Room 124 biohazardous waste container
Examination Room # 3
OPERATORY LAB: 1. UNCLEAN  2. FORMULA 5  3. BLEACH
JAN 18, 2005

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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pathogens</td>
<td>0</td>
<td>0</td>
<td>54</td>
</tr>
</tbody>
</table>

**OPERATORY 6: 1.UNCLEAN  2. FORMULA 3  3. BLEACH  Oct 26, 2004**

- **Microbes**: 5000
- **Molds**: 0
- **Pathogens**: 54