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TITLE: SURGICAL INSTRUMENT DECONTAMINATION UNIT

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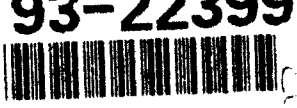

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Results of our studies during this Phase have demonstrated that a simple 2-bath system utilizing the synergistic effect between hydrogen peroxide and sonication is effective in achieving total sterilization when preceded by a precleaning treatment in a proteolytic enzyme/detergent cleaner. Furthermore, we have observed that using this two step procedure, the total time required for both cleaning and sterilizing the instruments is appreciably shorter than any other method presently in use.

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U.S. DEPARTMENT OF DEFENSE

SMALL BUSINESS INNOVATION RESEARCH PROGRAM
PHASE I - FY 1988
PROJECT SUMMARY

Topic No. A88-184 Military Department/Agency = ARMY

Name and Address of Proposing Small Business Firm

The ABEL Company
SR 774 Box 192-A
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Name and Title of Principal Investigator

Kenneth Abel, Research Director

Proposal Title

Surgical Instrument Decontamination Unit

Technical Abstract (Limit your abstract to 200 words with no classified or proprietary information/data)

During times of high surgical patient inputs, it would be desirable to have a simple and rapid method to clean and resterilize surgical instruments without the delays associated with conventional routine cleaning, sorting, packaging, and resterilization procedures. Ideally, such a system would be sufficiently small, safe, and easy to use so that it could be located within the operating rooms themselves.

Results of our studies during Phase have demonstrated that a simple 2-bath system utilizing the synergistic effect between hydrogen peroxide and sonication is effective in achieving total sterilization when preceded by a precleaning treatment in a proteolytic enzyme/detergent cleaner. Furthermore, we have observed that using this two step procedure, the total time required for both cleaning and sterilizing the instruments is appreciably shorter than any other method presently in use.

Anticipated Benefits/Potential Commercial Applications of the Research or Development

There is currently considerable interest throughout the world toward identifying and perfecting new approaches to medical product sterilization. With the almost total certainty that ethylene oxide sterilizers will be forbidden within the next half decade, alternative approaches to sterilizing heat- or radiation-sensitive medical products are being investigated both by major companies and by many small entrepreneurial organizations. A multi-million dollar market is forecast worldwide.

List a maximum of 8 Key Words that describe the Project

Sterilization, Ultrasonics, Hydrogen Peroxide, Proteolytic Enzymes

Nothing on this page is classified or proprietary information/data

Table of Contents

1. Introduction.

In civilian hospitals or in peacetime military surgical facilities, current methods of cleaning and resterilizing surgical instruments are perfectly adequate with the possible exception of certain specialized optical or electronic instruments or other devices which may not be capable of withstanding automated cleaning cycles and steam autoclaving. Normally the hospital or surgical staff can schedule patients to allow sufficient time between procedures for proper cleaning, packaging, resterilization, and re-storage of instruments. During hostilities or in times of major local catastrophes, it is not possible to treat severely injured or wounded patients on predetermined schedules: injuries tend to come in very large batches which must be sorted by triage and treated as rapidly as possible. To meet these requirements, the surgical facilities must maintain large numbers of basic instrument trays which presents both cost and storage problems.

During times of high surgical patient inputs, it would be desirable to have a simple and rapid method to clean and resterilize surgical instruments without the delays associated with conventional routine cleaning, sorting, packaging, and resterilization procedures. Ideally, such a system would be sufficiently small, safe, and easy to use so that it could be located within the operating rooms themselves.

Chemical methods have been utilized for decades to sterilize heat sensitive medical devices. Ethylene oxide gas sterilizers became the sterilization method of choice by medical device and materials manufacturers for heat sensitive materials until radiation sterilization facilities were shown to be safer and more cost effective. Although ethylene oxide is still used extensively by medical device manufacturers and within hospitals, it is generally conceded that ethylene oxide will soon be banned from use. One of the earliest water-based chemical disinfectants utilized was formaldehyde which combines chemically with critical bacterial cell components to prevent their replication. As the health effects of formaldehyde vapors on the clinical staff became more widely recognized, a number of other chemicals were adopted. None of these chemicals are fully safe for the user. Today, the most popular chemical sterilant for "cold" sterilization is glutaraldehyde. Chemically, glutaraldehyde could be expected to produce *in vivo* effects on staff personnel similar to formaldehyde. A chemical widely utilized for in gnotobiotic animal studies is peracetic acid. Peracetic acid is reported to be the most active of 14 disinfectants tested against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris,* and *Pseudomonas aeroginosa* in use-dilution tests (Krzywicka; 1975). Unfortunately, peracetic acid is known to be a respiratory depressant and (as with formaldehyde and glutaraldehyde) a primary skin and eye irritant (Heneghan; 1966). It has been shown to have potent tumor-promoting ability in mice at the 1% aqueous solution level but is a weak carcinogen (Brock; 1975).

Currently there is no chemical cleaning/sterilization system for surgical and dental instruments which is faster than the presently used multi-step cleaning, steam autoclaving cycle. There is, however, considerable interest throughout the world toward identifying and perfecting new approaches to medical product sterilization. With the almost total certainty that ethylene oxide sterilizers will be forbidden within the next half decade, alternative approaches to sterilizing heat- or radiation-sensitive medical products are being investigated both by major companies and by many small entrepreneurial

organizations. In the past few months, we have become aware of a number of these. American Sterilizer Company (Erie, PA) has developed a prototype "cold" sterilization process based on H_2O_2 vapors at 60°C. The system requires an 80 minute cycle and has been granted EPA approval. Surgikos (a division of Johnson and Johnson) has a prototype cold sterilizer that operates at room temperature and utilizes H_2O_2 vapor introduced into a low pressure radio frequency discharge chamber. The process requires a vacuum of under 2 torr and a radio apparently requires approximately 60 minutes for a sterilization cycle. The Surgikos system has received EPA registration approval and FDA marketing clearance was expected by late January, 1989. International Dioxcide (Clark, NJ) is developing a cold sterilizer based on ClO_2 (chlorine dioxide). Preliminary reports indicate exposure times of 48 hours are required to achieve biocidal activity. Steris Labs (Phoenix, AZ) is apparently now marketing a desk-top enclosed unit based on peracetic acid which claims a 20 minute sterilization time. Karlson Medical Laboratories (Erie, PA) has reported a similarly sized desk top sterilizer using ozone. Sterilizing cycles depend on the product with sterilization cycles up to almost 9 hours being required.

2. Experimental Procedures.

Organism cultivation:

Two spore forming test organisms were utilized, *Bacillus subtilis* and a heat resistant organism, *Bacillus stearothermophilus*. Spore formers were chosen over the more likely vegetative organisms in order to provide a more rigorous test condition. The organisms were cultivated by aseptically transferring 3 loops of each from freshly obtain and refrigerated commercially-obtained slants to 200 ml flasks of trypticase soy both and incubated for 5 days at their respective optimum temperatures of 37°C and 55°C. During cultivation, turbidity measurements and microscopic slide examinations were recorded periodically to check the progress of sporulation. By the fifth day, spores numbering approximately 10^6 per cm^2 were evident on the slides. At this point, flasks were transferred to refrigerated storage at 4°C until needed.

Simulant preparation:

Once a suitable concentration of spores was obtained, an inoculum was prepared by adding the spore culture to sterile defibrinated equine blood at a ratio of 1 part culture to 2 parts blood. Test slides were inoculated with approximately 0.2 ml of inoculum to provide a total surface spore count of approximately 10^6 to 10^7 spores per slide. Instrument simulant test strips (tightly woven stainless steel wire mesh screens cut to the same dimensions as the test slides) were inserted into the inoculum and manually swirled to ensure thorough contamination within the wire mesh matrix. Based on dried weight increase, we estimated the spore count on the wire mesh test strips to be between 10^8 and 10^{10} spores. To simulate even more heavily contaminated instruments, the wire mesh strips were at first dipped in the above manner for 3 consecutive times with 24 hour drying periods between each dip. Estimated spore concentrations per strip for this more heavily contaminated test was 10^9 to 10^{11} spores. Later, even more heavily contaminated strips were prepared by placing each of the strips horizontally within individual

inoculum-filled depressions in an aluminum tray and allowing the entire tray to dry before removing the strips. Estimated spore concentrations in this case were in the range of 10^{10} to 10^{12} spores per strip.

Test procedure:

During treatment in the test system, six (6) 3"x1" simulants were first suspended in a cleaning solution for a fixed time, then transferred to a sterilization bath. A test simulant was removed prior to immersion in the cleaning tank and a second test simulant removed following the cleaning tank but preceding the sterilization tank. These two simulants were incubated as controls. The first tank contained Haemosol[tm] detergent with proteolytic enzyme at the concentration recommended by the manufacturer. It was typically operated at 45°C, the maximum at which the proteolytic enzyme will not be denatured and loose its activity. The second tank contained hydrogen peroxide at concentrations ranging from 1% to 25%. It was operated at temperatures from 20°C to 60°C. The tanks were standard 20 khertz, moderate power Branson ultrasonic cleaning tanks.

The test simulants were held vertically in a brass tray and inserted in the first (cleaning) bath for a prescribed time. The tray was then lifted from the bath, allowed to drain for approximately 30 seconds, then lowered into the second bath for a prescribed time. For tests using sonication, the ultrasonic generator was left on throughout the procedure. Following removal from the second bath, the tray was allowed to drain for another 30 seconds, then aseptically transferred to a sterile drying area where they were left for two (2) hours to insure complete drying with resultant decomposition of any residual hydrogen peroxide. They simulants, including both controls, were then inserted into individual threaded cap specimen jars of sufficient size containing 40 ml each of sterile trypticase soy broth with 0.1% added sodium pyruvate. The sodium pyruvate was included to insure that any residual hydrogen peroxide would be consumed. The jars were then capped loosely, placed in racks at a 30° angle to increase exposed surface area for oxygen diffusion, and incubated for a minimum of 96 hours at the optimum growth temperature for the specific organism utilized. The efficacy of the test protocol was determined by the presence or absence of growth in the culture media.

Since the two spore forming organisms required incubation at substantially different temperatures, we conducted a first series of studies using the mesophilic *Bacillus Subtilis*. After establishing optimum conditions for this first organism, the thermophilic *Bacillus stearothermophilus* was then tested using the test protocols found to be optimum for *B. subtilis*.

3. Test Results.

The first series of tests were directed towards the determination of optimum time, temperature, and hydrogen peroxide concentrations which reproducibly achieve total sterilization in the 2-bath system. This was accomplished by maintaining two of the three conditions constant while varying the third in each test group. Each test was performed first on slides, then on wire mesh strips coated with blood-spore culture inoculum. Seven slides or mesh strips were inoculated to allow for two controls and five test samples.

Tables I through III summarize the results of these studies. For this series of tests, the first (cleaning) bath was maintained at 45°C with a 5 minute immersion time. All results shown are with sonication for the full duration of immersion. Plus (+) indicates growth, (-) indicates no growth.

Table I
Effect of Time
25% H_2O_2 at 60°C

	Time in minutes			
Test	1.0	2.5	5.0	10.0
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-

Table II
Effects of H_2O_2 Concentration
Temperature = 60°C

		Concentration (wt%)				
Time	Test	25.0	10.0	5.0	2.5	1.0
2.5 min						
	1	-	-	-	-	+
	2	-	-	-	-	+
	3	-	-	-	-	+
	4	-	-	-	-	+
	5	-	-	-		
	6	-	-	-		
	7	-	-	-		
	8	-	-	-		
1.0 min						
	1	-	-	-	+	+
	2	-	-	-	+	+
	3	-	-	-	-	+
	4	-	-	-	+	+
	5	-	-	-		
	6	-	-	-		
	7	-	-	-		
	8	-	-	-		

Table III
Effect of Temperature
5% H_2O_2, 1 minute immersion

	Temp in °C			
Test	60	50	40	20
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	+	+

The data demonstrates that the most rapid and reliable sterilization of both the glass and wire mesh simulants was obtained using a 5.0 wt% concentration of hydrogen peroxide at 60°C with 1 minute sonication when preceded by a 5 minute sonication in Haemosol[tm] at 45°C.

Efforts to assess the limitations of these test parameters were then performed by reusing the cleaning and hydrogen peroxide solutions for a total of 4 cycles four times over a 5 day time period using two types of wire mesh simulants. The "simple" mesh was a 50x250 square weave mesh while the "complex" was a 24x110 dutch weave. Results of this study are summarized in Table IV.

Table IV
Solution Sterilizing Efficiency
with Repeat Solution Usage

Test samples held vertically and equally spaced

Mesh Type	Test	Day 1	2	3	5
Simple					
set 1	1	-	-	-	-
set 1	2	-	-	-	-
set 1	3	-	-	-	-
set 1	4	-	-	-	-
set 2	5	-	-	-	-
set 2	6	-	-	-	-
set 2	7	-	-	-	-
set 2	8	-	-	-	-
Complex					
set 3	9	-	-	-	-
set 3	10	-	-	-	-
set 3	11	-	-	-	-
set 3	12	-	-	-	-
set 4	13	-	-	-	-
set 4	14	-	-	-	-
set 4	15	-	-	-	-
set 4	16	-	-	-	-

In all of the tests reported previously, the test strips (whether glass or wire mesh) were held vertically in a carrying tray with 6 mm separation between test strips. Tests were also conducted on test strips with no separation between the strips. Three configurations were utilized: (1). 9 strips were racked side by side (vertical orientation) without separation between strips, (2). 9 strips were placed in a single stack (horizontal orientation) with no separation between strips, (3). 15 strips were racked side by side with no separation between strips. Conditions were: cleaning tank at 45°C, sterilization tank at 60°C with 5 wt% H_2O_2 concentration. These results are shown in Table V.

Table V
Sterilization Efficiency
With no Separation Between Test Strips

Mesh Type	Test	Orientation & Number Vert - 9	Horz - 9	Vert - 15
Simple				
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-
	7	-	-	-
	8	-	-	-
	9	-	-	-
	10			-
	11			-
	12			-
	13			-
	14			-
	15			-
Complex				
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-
	7	-	-	-
	8	-	-	-
	9	-	-	-
	10			-
	11			-
	12			-
	13			-
	14			-
	15			-

As indicated by Tables IV and V, continued usage of the solutions with moderate contamination loads and with contact between test strips did not have an adverse effect on sterilization efficacy.

Effect of Cleaning Time/Temperature Prior to Sterilization:

Tests were run to determine if reduction in cleaning time could be achieved without compromising the subsequent sterilization step. For these tests, the sterilization bath was maintained at 60°C and 5 wt% H_2O_2 while the time and temperature of the cleaning bath were changed. The results are summarized in Table VI.

Table VI
Effect of Time/Temperature
of Cleaning Step on Subsequent Sterilization

Soln Temp	Test	Cleaning Time in min 1.0	2.0	3.0	4.0	5.0
45°C	1	-	-	-	-	-
	2	-	-	-	-	-
	3	+	+	+	+	-
37°C	1	+	-	-	-	-
	2	+	-	-	-	-
	3	+	-	-	-	-

Following these tests, the effect of heavy contamination was examined at two contamination levels. In the first set of tests, both the simple and complex wire mesh screen test strips were contaminated by repeat dipping as noted in the procedures section. For these tests, the temperature of the Haemosol[tm] solution was maintained at 45°C and immersion times of 2 and 4 minutes were utilized. The results are summarized in Table VII.

Table VII
Effect of Time of Cleaning
on Subsequent Sterilization Step
for Moderately High Contamination Level

Mesh Type	Test	Time in min. 2.0	4.0
Simple	1	-	-
	2	-	-
	3	-	-
	4	-	-
Complex			
	1	-	-
	2	-	-
	3	-	-
	4	-	-

Highly contaminated test strips were then prepared by immersion of the test strips in shallow wells filled with culture media and allowing the water to evaporate. These samples were then cleaned for 2, 4, and 7 minutes in Haemosol[tm] at 45°C followed by 1 minute in 5 wt% H_2O_2 at 60°C. Results are shown In Table VIII, following page.

Table VIII
Effect of Time of Cleaning
on Subsequent Sterilization Step
for Very High Contamination Level

Mesh Type	Test	Time in min. 2.0	4.0	2.0	7.0
Simple	1	-	-	-	-
	2	-	+	-	-
	3	+	+	-	-
	4	+	+	+	-
Complex	1	+	-	+	-
	2	+	-	+	-
	3	+	+	+	-
	4	+	+	+	-

As would be expected intuitively, the more heavily contaminated test items require longer cleaning times prior to immersion in the sterilizing solution in order to insure attainment of sterility.

Effect of Increased Temperature and Sonication using Sporicidin[tm]:

During the final month of the Phase I effort, comparison studies were made comparing the cold chemical sterilant, Sporicidin[tm], against hydrogen peroxide. The Sporicidin was used at full strength in accordance with manufacturer's directions, but tested at two temperatures: 24°C and 60°C, and with and without sonication using *B. stearothermophilus* spores. As with the hydrogen peroxide studies, the sterilization step was preceded by a 5 minute cleaning period in Haemosol[tm]. As was expected, the higher temperatures with sonication reduced the sterilization time to as little as 15 minutes; a value which may be suitable for field hospital application. There is, however, a significant difference in sterilization solution costs between Sporicidin[tm] and 5 wt% hydrogen peroxide: at normal commercial pricing, the Sporicidin[tm] solution costs approximately $100.00 per gallon (at the recommended 2% glutaraldehyde concentration level) while hydrogen peroxide costs approximately $0.25 per gallon.

Effect of Liquid Detergents Containing Proteolytic Enzymes:

Haemosol[tm] is obtained as a dry powder with dried-stabilized proteolytic enzyme. It is used at near room temperature solution saturation levels. Several commercial liquid laundry detergents also contain proteolytic enzymes. Because it would facilitate the semi-automatic preparation of the cleaning solution, we investigated the possibility of using the liquid detergent (with enzyme) in place of Haemosol[tm]. During testing, the same parameters were maintained. Results demonstrated that solutions containing up to 30 ml of liquid detergent per gallon showed satisfactory cleaning ability for the first two trays of lightly contaminated simulants, it was insufficient to clean subsequent trays. We assume this marginal cleaning ability is the result of a combination of lower enzyme

concentration and less aggressive detergent formulation. We concluded that Haemosol[tm] should remain the cleaning detergent of choice.

4. Estimate of Technical Feasibility.

We have concluded from the data reported herein that the use of a two step cleaning, sterilization procedure based on a precleaning step utilizing a commercially available proteolytic enzyme detergent solution with sonication at 45°C followed by a very short sterilization step utilizing 5 wt% hydrogen peroxide at 60°C, also with sonication, is technically feasible. Minimum time of exposure to both the cleaning step and the sterilization step is dependent upon the degree of and adherence characteristics of the contaminants. The cleaning step appears to be the most critical and time consuming portion of the cycle: Once the contaminants are dislodged from the surfaces of the item to be sterilized, the hydrogen peroxide-sonication procedure appears to have no difficulty in effecting sterilization with as little as 1 minute of exposure.

The following report sections describe other related work as reported in the open literature and considers safety factors associated with the proposed use of hydrogen peroxide.

5. Related Work.

Chemical methods have been utilized for decades to sterilize heat sensitive medical devices. Ethylene oxide gas sterilizers became the sterilization method of choice by medical device and materials manufacturers for heat sensitive materials until radiation sterilization facilities were shown to be safer and more cost effective. Although ethylene oxide is still used extensively by medical device manufacturers and within hospitals, it is generally conceded that ethylene oxide will soon be banned from use. One of the earliest water-based chemical disinfectants utilized was formaldehyde which combines chemically with critical bacterial cell components to prevent their replication. As the health effects of formaldehyde vapors on the clinical staff became more widely recognized, a number of other chemicals were adopted. None of these chemicals are fully safe for the user. Today, the most popular chemical sterilant for "cold" sterilization is glutaraldehyde. Chemically, glutaraldehyde could be expected to produce *in vivo* effects on staff personnel similar to formaldehyde. A chemical widely utilized for in gnotobiotic animal studies is peracetic acid. Peracetic acid is reported to be the most active of 14 disinfectants tested against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris,* and *Pseudomonas aeroginosa* in use-dilution tests (Krzywicka; 1975). Unfortunately, peracetic acid is known to be a respiratory depressant and (as with formaldehyde and glutaraldehyde) a primary skin and eye irritant (Heneghan; 1966). It has been shown to have potent tumor-promoting ability in mice at the 1% aqueous solution level but is a weak carcinogen (Brock; 1975).

None of the present cold chemical sterilants have rapid action against bacterial spores. For example, the most popular glutaraldehyde formulations (Cidex[tm] and Sporicidin[tm]) recommend 6 3/4 hours soaking at room temperature to insure sporicidal action.

Historical background:

Hydrogen peroxide has been recognized as a germicide for more than a century. However, application of low concentrations of unstable preparations to tissues containing inactivating levels of catalase led to unfavorable results and general abandonment of this agent as an antiseptic. Examination of the early literature reveals that hydrogen peroxide was quite satisfactory when used as a disinfectant for inanimate materials. For example, used in low concentrations, hydrogen peroxide was considered ideal for the preservation of milk and water (Heinemann; 1913) and for the sterilization of cocoa milk beverage (Wilson; 1927).

The literature contains few accounts of the germicidal effectiveness of *stabilized* hydrogen peroxide, although today's product has been available commercially for more than 30 years. By 1950, an electrochemical process had been developed that produced pure preparations in high concentrations that were stable even at elevated temperatures and have extremely long shelf lives with autodecomposition rates substantially below 1% per year. The apparent lack of interest on the part of disinfection microbiologists can be attributed to the fact that they were generally unaware of this development, principally because publications describing the stabilized form were limited almost entirely to specialized chemical literature (Roth; 1953), and until the second edition of Disinfection, Sterilization, and Preservation (Block; 1977), books dealing with hydrogen peroxide as a disinfectant were not appropriately revised.

There has been an upsurge of interest in hydrogen peroxide as a disinfectant during the past two decades, particularly in Europe and the Soviet Union. Hydrogen peroxide at 0.1% concentration and 54°C has been found to reduce the total bacterial count in raw milk by 99.999% with 30 minutes exposure (Naguib; 1972) with coliform, staphylococcal, salmonellae, and clostridial counts were reduced by 100%. In a hospital water contamination control study, concentrations as low as 0.03% killed 10^6 colony-forming units per milliliter of seven bacterial strains overnight, with an 80% kill in 1 hour (Rosensweig; 1978). Hydrogen peroxide was found to have rapid virucidal activity against rhinovirus tested both in suspension and on carriers (Mentel; 1973).

Hydrogen peroxide in relatively high concentrations (10 to 25%) is also an excellent sporicidal agent. At 24°C, D values of 0.8 to 7.3 minutes have been demonstrated with four aerobic spore strains and one anaerobic spore strain (Toledo; 1973). Work in the USSR has indicated the practicability of this agent for sterilization of spacecraft and this was subsequently confirmed in the United States (Wardle; 1975). The US studies demonstrated complete kill of spore suspensions at the level of 10^8 colony-forming units per ml by a 10% concentration at 25°C in 60 minutes, but not in 30 minutes. Metal ions, particularly Cu^{++} enhance the sporicidal activity of hydrogen peroxide (Waites; 1979). A synergism between hydrogen peroxide and ultrasonic waves was demonstrated using *Bacillus subtilis* and *Clostridium sporogenes* spores (Ahmed; 1975). As expected, the sporicidal activity of hydrogen peroxide increases with increasing temperature and concentration (Toledo, 1973, Baylis; 1976).

Hydrogen peroxide has also been reported to destroy pyrogens and was shown to render solutions intended for injection pyrogen-free when used at concentrations as low as 0.1% (Campbell; 1945, Taub; 1948, Menezel; 1951).

Unlike glutaraldehyde or peracetic acid preparations which have a relatively short storage life after mixing (typically less than 30 days), current stabilized hydrogen peroxide has been shown to retain 98% of its original active oxygen after being subjected to 100 C for 24 hours (Technical Bulletin No. 42–Super D Hydrogen Peroxide–FMC Corporation, NY). At room temperature, commercially available solutions from 3% to 70% have rated decomposition rates of less than 1% per year (Schumb; 1955). For military applications, hydrogen peroxide was formerly available at 90 and 98% concentrations, but all US facilities capable of producing these concentrations were dismantled in the late 1960's.

Mode of action:

Most studies suggest that the mode of action of hydrogen peroxide is due to the production of the hydroxyl free radical which occurs on addition of catalytic amounts of Fe^{++} or Cu^{++} ions (Guzmann-Barron; 1952, Yoshpe-Purer; 1968). In the case of low levels of bacterial contamination, Yoshpe-Purer concluded that the bacteria themselves provide the necessary metal ions. King and Gould (1969) found that lysis of bacterial spore cell walls was the result of free radicals formed from hydrogen peroxide in the presence of added Cu^{++} ions. As early as 1934 the superoxide radical (O_2^-) was implicated in the formation of the hydroxyl free radical (Haber; 1934). The hydroxyl radical, in turn then attacks membrane lipids, DNA, and other essential cell components. Based on our observations, however, we believe that the mode of action in the presence of ultrasonic cavitation does not rely solely upon a chemical reaction between the hydroxyl radical and bacterial cell structures or components, although these cavitation effects may play a significant role.

Possibly the best and most detailed study of the practical application of hydrogen peroxide to a tissue contact device is that by Turner, *et al* (1975) on the sterilization of soft contact lenses. Using lenses contaminated with 7×10^5 organisms per lens so as to give zero-hour control counts of 1×10^5 per ml, data were obtained on: (1) the rates of kill [D values]; (2) times required for reduction of the inoculum to a theoretical 0.5 organism per ml; and (3) times required for negative lens cultures. The test strains consisted of 13 bacteria, 4 fungi, and herpes simplex virus. Turner obtained D values for 16 test strains as indicated in Table 1. The addition of organic loads in the form of 10% serum albumin or 1×10^6 yeast cells per ml did not cause a significant reduction in the biocidal activity. Turner found that 3% hydrogen peroxide at room temperature was rapidly biocidal for all except one of the vegetative bacteria as well as for herpes simplex virus and moderately so for *Serratia marcescens* and two of the fungi. Although *Aspergillus niger* and *Candida parapsilosis* were more resistant, lenses were completely disinfected when a precleaning step was incorporated; probably due to a substantial reduction in organism numbers before introduction into the hydrogen peroxide bath. In our experience, a simple, short presoak in a commercial detergent with ultrasonic agitation can reduce the organism loading by a factor of 10^5 prior to introduction into the hydrogen peroxide.

Table IX

Lens Disinfection D Values
3% Hydrogen Peroxide[note 1]

Microorganism	D Value[note 2] Minutes	Standard Error
Neisseria gonorrhoea	note 3	-
Hemophilus influenzae	0.29	0.07
Pseudomonas aeruginosa	0.40	0.05
Bacillus subtilis	0.50	0.15
Escherichia coli	0.57	0.07
Proteus vulgaris	0.58	0.24
Bacillus cereus	1.04	0.12
Proteus mirabilis	1.12	0.33
Streptococcus pyogenes	1.50	0.25
Staphylococcus epidermidis	1.82	0.14
Staphylococcus aureus	2.35	0.18
Herpes simplex	2.42	0.71
Serratia marcescens	3.86	0.53
Candida albicans	3.99	0.54
Fusarium solani	4.92	0.54
Aspergillus niger	8.55	1.32
Candida parapsilosa	18.30	3.44

note 1: As reported by Turner, 1975.
note 2: Contamination level = $7x10^5$ organisms per lens, 7 ml H_2O_2 solution.
note 3: Too rapid to measure.

Although Turner's studies were conclusive on the ability of hydrogen peroxide to effect sterilization of vegetative bacterial cells, a device/procedure based on these studies was rejected for approval by the FDA on the grounds that the manipulations required to remove residual hydrogen peroxide from the lens (the rinsing steps) were too complicated and therefore might not be followed by the wearer. Other investigators have also expressed concern over procedures needed to remove hydrogen peroxide from the lens after disinfection (Hornbrook; 1973, Levine; 1981). This problem was addressed by Gaglia (1975), who developed a device that uses a platinum black catalyst in the rinse chamber. This device has been incorporated into a hydrogen peroxide lens disinfection system that has been used successfully and marketed in Canada and Europe for several years.

Unlike glutaraldehyde, formaldehyde, peracetic acid, and chlorine-based disinfectants, defenses against free radicals have evolved in all respiring cells (Fridovich; 1975). These consist of catalase and peroxidases, which directly inactivate hydrogen peroxide, and superoxide dismutases, which scavenge superoxide radicals, thereby preventing their interaction and resultant formation of the destructive hydroxyl free

radical. In living systems, the natural source of hydrogen peroxide is the reduction of molecular oxygen by aerobic dehydrogenases. Once formed in a living system, hydrogen peroxide reacts or decomposes with a steady state concentration of hydrogen peroxide maintained by the balance between formation and disappearance. In the liver, for example, this steady-state concentration has been estimated to be few micromolar (Chance; 1951). Catalase produced by respiring cells can adequately protect the cell from damage by steady-state levels of metabolically produced hydrogen peroxide and low levels of externally added hydrogen peroxide, but this defense is over-whelmed by concentrations above 3% used for practical disinfection.

The effects of intravenous injection have been studied with several species (Krackow; 1950, Lorincz; 1948, Feinstein; 1950). In sublethal doses, liver catalase and blood methemoglobin levels are affected. The dose of 90 wt% hydrogen peroxide found lethal to 50% of injected rabbits was 0.015 cc/kg body weight. An instance of the death of a person caused by inadvertent injection of hydrogen peroxide has been reported (Licurzi; 1938). The major contributing factor to death of the test subjects appears to have been oxygen gas embolism resulting from the sudden decomposition of the hydrogen peroxide upon introduction into the vascular system.

Availability, Uses, and Hazards of Hydrogen Peroxide:

Hydrogen peroxide is widely used in industry with the result that is readily available at low cost. Current pricing for 50 wt% Food Grade solutions in 30 gallon quantities is under $2.50 per gallon while Industrial Grades sells for as little as $1.00 per gallon making it the least expensive chemical disinfectant available. For this proposed application, dilution of 50% Food Grade solution to 5% sterilization solution may result in a daily chemical cost of under $2.50 (not including the Haemosol™ detergent cost). For comparison purposes, the equivalent quantity of full strength Sporicidin™ prepared fresh daily would have a daily chemical cost close to $500.00.

Hydrogen peroxide is used in the minerals industry as an in-situ leaching oxidant for uranium and copper, to decolorize sulfuric acid recovered from smelting operations, in molybdenite flotation, and in various separation and extraction processes. It is used in food processing, starch modification, treatment of milk and production of cheese, and as a sterilant for polyethylene food containers. It is used extensively as a pulp, paper, and textile bleach where it exhibits excellent bleaching properties without the degradation associated with chlorine-type bleaches. It removes polysaccharide sizing from textiles without damaging the textile structure. It is used as a gas generator for foam products and very large quantities are used annually for oxygen supplementation in waste water disposal systems while dilute H_2O_2 is used to improve water quality in waste or sewer systems.

Hydrogen peroxide is currently available from its manufacturers at 30, 50, and 70 wt% concentrations. The Department of Transportation (DOT) requires that drums carry both an OXIDIZER and a CORROSIVE label for concentrations greater that 52%. For concentrations from 8 to 52 wt%, containers need carry only the OXIDIZER label, the same label required for compressed oxygen. For field hospital usage, the safest and most convenient container would be the 50 wt% 15 gallon (57 liter) high density polyethylene drum.

Hydrogen peroxide solutions available commercially are not explosive. If handled properly to avoid contamination, H_2O_2 may be stored indefinitely in appropriate containers and transported from storage areas to operational areas without difficulty. Although 100% hydrogen peroxide has approximately the same freezing point as water, water solutions containing 50 wt% hydrogen peroxide have a listed freezing point of -52°C but since it supercools readily, it can be left outdoors in far colder climates without danger of freezing. As noted earlier, even the most stable hydrogen peroxide solutions decompose slowly and should never be stored in a nonvented container. Spillage must be considered: some flammable materials in contact with H_2O_2 concentrations greater than 52% may burst into flames upon contact. Hydrogen peroxide itself will not burn. However, H_2O_2 can start fires and increase the severity of fires because it is a source of oxygen. Only water is recommended to fight fires involving hydrogen peroxide.

Hydrogen peroxide does not show human toxicity in the conventional sense. This is due in part to the fact that hydrogen peroxide is a byproduct of cellular aerobic respiration and efficient cellular biological catalysts rapidly decompose the product to water and oxygen as noted earlier. Nevertheless, H_2O_2 concentrations above 10% can cause eye burns. Solutions up to 3% are not eye irritants but solutions of 6% or more are eye irritants. Solutions over 8% but less than 50% are skin irritants. Concentrations over 52% can cause severe burn-like skin blisters. Short term skin contact with 30-50% solutions will result in temporary whitening and a mild stinging sensation. This whitening is the result of H_2O_2 diffusing through the upper non-living dermal layers, then being decomposed into oxygen upon contact with the inner living dermal layers. The outer dermal layers trap the released oxygen producing micro oxygen cavities which scatter light and thereby appear white. This entrapped oxygen results in the stinging sensation which disappears as the excess oxygen is absorbed into the surrounding cells. The effect is most pronounced on the palms and finger pads where the outer dermal layers are thickest. The effect disappears within minutes leaving no residual after effects. If swallowed, H_2O_2 of any concentration may produce a sudden evolution of oxygen which can cause injury by distention of the esophagus or stomach. Inhalation of H_2O_2 vapor or mist may cause irritation and inflammation of the nose and throat. OSHA has ruled that exposure to hydrogen peroxide vapors shall not exceed a time-weighted average of 1 ppm hydrogen peroxide vapor in air for an 8-hr shift. First aid for inhalation of hydrogen peroxide vapor is immediate removal to fresh air with referral to a physician if nose or throat irritation is apparent. First aid for eye exposure to H_2O_2 solutions is to immediately flush with water and referral to a physician. For skin contact with solutions below 52%, first aid is flushing with water. Normally, skin contact is not referred to physician since the effects are transient.

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