



# MICROBIOLOGICAL EVALUATION OF THE BIOBURDEN AND PRESERVATIVE EFFICACY OF TWO ACIDIC MOIST WOUND DRESSING FORMULATIONS

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## ABSTRACT

The purpose of these studies was to determine the ability of acidic wound management formulations to effect the growth of various microbiological agents. Two formulations were tested, an aqueous solution and a topical cream. The solution was preservative free and the cream contained a preservative. Both formulations contained low amounts of metallic salts such as Zinc Chloride, Iron Chloride, Manganese Chloride, Copper Chloride, Chromium Chloride, Sodium Fluoride, Cobalt Chloride, Molybdenum Chloride, Potassium Iodide, Sodium Selenite at a pH 2.9-3.4. Assays were conducted to determine the ability of organisms to contaminate these formulations (bioburden) and the ability of organisms to sustain growth (preservative efficacy). Microorganisms were selected to include aerobic bacteria, spore-formers, yeast, fungi and normal flora that might be expected to infiltrate and infect a wound site. Formulations were inoculated with test organisms and incubated. Colony counts were conducted out to 28 days post incubation. Results indicate that both formulations, with and without preservatives were bacteriostatic and in some cases bactericidal.

## INTRODUCTION

The prevention of wound infections should be a preeminent consideration when managing either acute or chronic wounds. Contaminants can be introduced through injury itself, the immediate wound environment or from materials used to clean or dress the wound. It is difficult at best to project which wound may become infected since many factors are associated with this potential: the patient's immune system, the contaminating organisms' virulence and, proper wound management are but a few predisposing factors one must consider. The routine use of antibiotics as prophylaxis is not justified and may contribute to the emergence of antibiotic resistance strains. Hospital acquired infections of surgical wounds may have an incidence as high as 10% and certainly complicate recovery as well as cause a dramatic increase in post surgical care costs. It has been estimated that each patient with a surgical site infection will require additional days in hospital, which may result in the doubling of hospital costs associated with that patient.

Management of a wound infection must also include an emphasis on reducing the risk of cross infection. The caregiver must be assured that the materials used in managing the wound does not introduce further contamination, nor be likely to sustain the growth of pathogens. Although, silver and iodine wound management products play an important role in the treatment and prevention of infection when used appropriately, each has its drawbacks.

We present here data to support the use of two acidic wound care formulations that do not support the growth of a variety of microorganisms, and in fact are bactericidal in some cases, yet do not contain antibiotics nor heavy metals at inhibitory concentrations.

## METHODS & MATERIALS

Manufacturers of non-sterile topical products are required to demonstrate that their products are not contaminated with microorganisms during production (bioburden) and do not support the growth of organisms over time (preservative efficacy). Both of these tests are described in the United States Pharmacopoeia (USP), specifically USP 28<61> Microbiological Examination of non-sterile Products, and USP<51> "Efficacy Test for Antimicrobial Preservation". Samples from product formulations, BioDerm Sciences Wound Spray, and BioDerm Sciences Wound Cream were used to validate the procedures in these test methods. As described, these formulations are relatively simple containing low levels of metallic salts at a pH 2.9-3.4, and the cream containing an accepted preservative. Actual testing was then performed on production samples obtained immediately following production and samples that had been stored as stability samples for up to three years. All testing was performed by a qualified microbiological testing facility (Dr. Manfred Jacobs Mikrobiol. Laboratorium, Dillingen, Germany).

## RESULTS

As shown in Table 1 both formulations when tested for bacteria, fungus or yeast yielded undetectable amounts when tested according to the validated test procedure USP 28<61>.

TABLE 1: BIOBURDEN TESTING OF 1 YR SAMPLES OF THE SPRAY SOLUTION & THE WOUND CREAM

TESTED FOR:	TESTING REQUIREMENTS	TEST RESULTS
Staphylococcus aureus	Not detectable/ 10g	Not detectable
Pseudomonas aeruginosa	Not detectable/ 10g	Not detectable
Escherichia coli	Not detectable/ 10g	Not detectable
Salmonella sp.	Not detectable/ 10g	Not detectable
Yeasts and Fungi	< 100 cfu/g	< 100 cfu/g
Aerobic bacteria	< 100 cfu/g	< 100 cfu/g

A more stringent test of the capabilities of these formulations lay in the preservative efficacy tests with time. Three year stability samples from both formulations were tested according to USP <51> where microorganisms were added to each of the formulations and incubated for 28 days. A successful test is one in which there is no increase in the number of colonies at days 14 and 28. Neither sample supported growth of bacteria, fungus or yeast with the spray showing clear bactericidal activity. It should be noted that only the Wound Cream formulation contained a preservative. Tables 2a and 2b illustrate these laboratory results.

TABLE 2A: PRESERVATIVE EFFICACY OF 3 YR SAMPLES OF SPRAY SOLUTION

SPECIES	S. AUREUS	E. COLI	P.AERUGINOSA	C. ALBICANS	A. NIGER
Inoculum (cfu)	6.7 X 10 <sup>5</sup>	6.5 X 10 <sup>5</sup>	5.4 X 10 <sup>5</sup>	9.0 X 10 <sup>5</sup>	7.3 X 10 <sup>5</sup>
T = 0	9.1 X 10 <sup>2</sup>	1.6 X 10 <sup>2</sup>	2.6 X 10 <sup>2</sup>	8.8 X 10 <sup>5</sup>	5.1 X 10 <sup>5</sup>
T = 14 d	<10	< 10	<10	9.0 X 10 <sup>4</sup>	3.2 X 10 <sup>5</sup>
T = 28 d	None detected	None detected	None detected	1 X 10 <sup>4</sup>	3.1 X 10 <sup>5</sup>

TABLE 2B: PRESERVATIVE EFFICACY OF 3 YR SAMPLES OF WOUND CREAM

SPECIES	S. AUREUS	E. COLI	P.AERUGINOSA	C. ALBICANS	A. NIGER
Inoculum (cfu)	6.7 X 10 <sup>5</sup>	6.5 X 10 <sup>5</sup>	5.4 X 10 <sup>5</sup>	9.0 X 10 <sup>5</sup>	7.3 X 10 <sup>5</sup>
T = 0	5.6 X 10 <sup>4</sup>	9.0 X 10 <sup>2</sup>	9.8 X 10 <sup>3</sup>	6.3 X 10 <sup>5</sup>	5.4 X 10 <sup>5</sup>
T = 14 d	< 100	< 100	<100	1.0 X 10 <sup>5</sup>	4.1 X 10 <sup>5</sup>
T = 28 d	< 100	< 100	<100	<1 X 10 <sup>3</sup>	4.1 X 10 <sup>5</sup>

## DISCUSSION

Bacterial contamination of the wound site as a nosocomial or direct infection remains a major threat to the proper healing of surgical and other wounds and increases the cost of wound care management exponentially. Agents designed to work in concert with the physiologic processes of wound healing must be considered as alternatives to antibiotics when infections are predicted or present. These studies describe two acidic wound management formulations that have demonstrated a unique property to decrease the viable colony counts of organisms by keeping the wound environment moist, at a low pH and with proper nutrition.

Although it might be expected that the inclusion of a preservative in the Wound Cream would lend itself to the antimicrobial activity shown, no such inference can be derived from the data obtained with the Wound Solution. There appears to be an immediate 3 log decrease in the number of viable colonies with most bacteria killed by day 14 and all by day 28. The concentrations of the metallic salts are too low to account for this observation. It remains that the low pH (2.9-3.4) must account for the bactericidal action demonstrated, since this was observed both in the presence (Cream) and absence (Spray) of a preservative. Should the mechanism of killing be related to such a physical aspect of these formulations, it would be interesting to examine whether this effect could also be demonstrated against antibiotic resistant strains of microorganisms. Further, one must be concerned as to the potential toxicity to open wounds associated with a formulation at acidic pH containing metallic salts. The Wound Spray has thus been tested for cytotoxicity, delayed type hypersensitivity, sensitization and acute lethality with no untoward reactions occurring (data not shown). Finally, data presented in an adjacent poster indicate that an acidic zinc/iron wound solution, when used in a moist wound dressing, not only prevented infection following surgical procedure to the chest, but also decreased the time to wound closure and decreased crust and scar formation.

## CONCLUSIONS

- Acidic wound formulations offer remarkable protection against bacterial growth
- The spray solution can thus be used as an effective wound cleansing agent
- The anti microbial properties are achieved without silver, antibiotics or preservatives
- No toxicity associated with Wound Spray
- Acidic formulation prevented infection in clinical setting