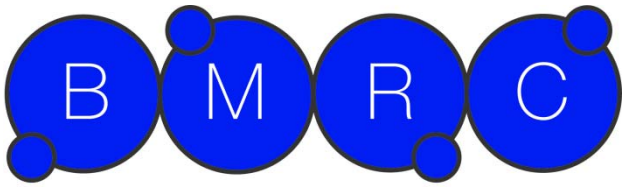

Biomedical Research Centre

Testing of easy-on+ Panels

(easy-on blended with 5% Akacid plus)

augmented for Urban Hygiene Ltd

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Introduction

Hospital acquired infections

Hospital acquired infections also called nosocomial infections, are an infection acquired in hospital by a patient who was admitted for a reason other than that infection, or an infection occurring in a patient in a hospital or other health care facility, where the infection was not present at the time of admission. This includes infections acquired in the hospital but appearing after the patient is discharged and also occupational infections among staff of the facility.

Hospital acquired infections have a great impact not only on patient safety and length of stay in hospital, but also in terms of the financial implications to the hospital in the treatment of the infection. The main financial burden is the increased length of stay, with the average increase being an extra 8.2 days. It is estimated that the rate of hospital acquired infections in Europe is 7.7% of patients currently in hospital, with the most cases being found in intensive care, acute surgical and orthopaedic units.

The most common hospital acquired infection affects the urinary system. Other types of infection include; surgical site infections, hospital acquired pneumonia, and blood infections (bacteraemia) usually introduced via catheters or cannula.

Several bacterial species are responsible for these infections including *E. coli, Staphylococcus aureus* (MRSA), *Klebsiella, Pseudomonas* and uncommonly, *Legionaell.* Fungal species such as *Aspergillius* can also cause infections.

Most regimes put in place to combat the rise of these infections have focussed on the role of patient contact in transmission of infection. Methods employed have ranged from the introduction of alcohol gels, limiting hospital visitor numbers and visitor hours, and increasing the awareness of visitors and staff of infections. Coupled with this, in the UK, there has been a program of deep cleaning of hospital wards, although it is unclear how successful this has been.

There has been little research carried out into the role of the hospital environment in harbouring reservoirs of infection micro-organisms. Most has focussed on objects

coming into direct contact with patients, i.e. chairs, tables, beds, medical apparatus etc. The role of hard surfaces i.e. walls, floors and ceilings is poorly understood.

Most research has looked at the increase in infection rates following construction work in wards and general hospital areas. Both bacterial and fungal infections have been linked to false ceilings, window blind casings and insulation material. In the case of false ceilings any deep cleaning regime would not remove the microorganism reservoir present in these. There has been little or no research carried out on the survival and transmission of micro-organisms in hospital wards and exposed sides of ceilings.

Disinfectants

Traditionally, disinfectants used in hospital have been chlorine/alcohol or phenol based. Each of these has its own disadvantages, for example, phenol based solutions, do not kill bacterial and fungal spores, and chlorine based solutions are easily inactivated by organic matter. Recently, a new form of disinfectants have been introduced and trialled, the polymeric guanidine family of disinfectants.

Akacid plus is a member of this family and was initially developed to increase the antimicrobial activity of this type of disinfectant, while decreasing the toxicity. Standard stock solutions of Akacid contain two polymers I a ratio of 3:1, poly-(hexamethylene-guanidium-chloride) and poly-[2-(2-ethoxy)-ethoxythyl)-guanidinium-chloride]. Guanidinium chloride molecules interact with the surface of bacteria and unfold proteins present. This essentially disrupts the cell surface of the bacteria leading to eventual death.

Akacid was initially tested by the Department of Internal Medicine at the University of Vienna in Austria and was shown to be active against a wide range of bacterial species. Further tests proved Akacid was effective against all bacteria tested at concentrations of 0.5 %. Most bacterial species tested were killed after 60 minutes exposure.

Other research showed that in animal models, skin exposure to Akacid at working concentrations gave no adverse effects.

Overview

easy-on+ panels which includes the biocide Akacid plus® were tested for the survival rates and killing of *Staphylococcus aureus* (Methicillin resistant strain, MRSA) and *Escherichia coli* (E Coli).

Materials and Methods

All coated panels (7.5 x 5.5 cm) were cleaned with 90 % ethanol (V/V) before testing.

Effect of easy-on+ on bacterial survival

An overnight culture of *Methicillin resistant Staphylococcus aureus* was diluted in Nutrient Broth to give a concentration of 10^7 cells/ml. 500 µl of culture was added to each of three panels coated with easy-on+ incorporating 5% Akacid plus active ingredient. This was spread over the surface and the panels incubated at room temperature in a sterile Petri dish for 1 h. The panels were then flushed with 10 ml of sterile quarter strength Ringer's solution and shaken for 30 minutes to remove bacteria from the surface.

The 10 ml of Ringer's solution was then removed, a dilution series created and 200 µl of each dilution was plated out onto Nutrient Agar plates. These plates were then incubated overnight and bacterial colonies counted. This experiment was carried out in triplicate, and average values taken.

The arithmetic mean of each set of replicates was taken and standard deviations calculated

Determination of bacterial survival over a simulated lifespan of easy-on+ coated surfaces

The bacterial coated easy-on+ panels were then cleaned with 1000 ppm Presept solution to simulate a typical hospital cleaning regime. This process was repeated to a total of 28 bacterial additions to represent a life span of the easy-on product in hospital usage, with a cleaning regime employed every 4 months.

The whole experiment was then repeated using *Escherichia coli* as the bacterial species tested.

Results

Effect of easy-on+ on bacterial survival

Figure 1 gives the level of bacteria recovered from both the easy-on and easy-on+ coated panels

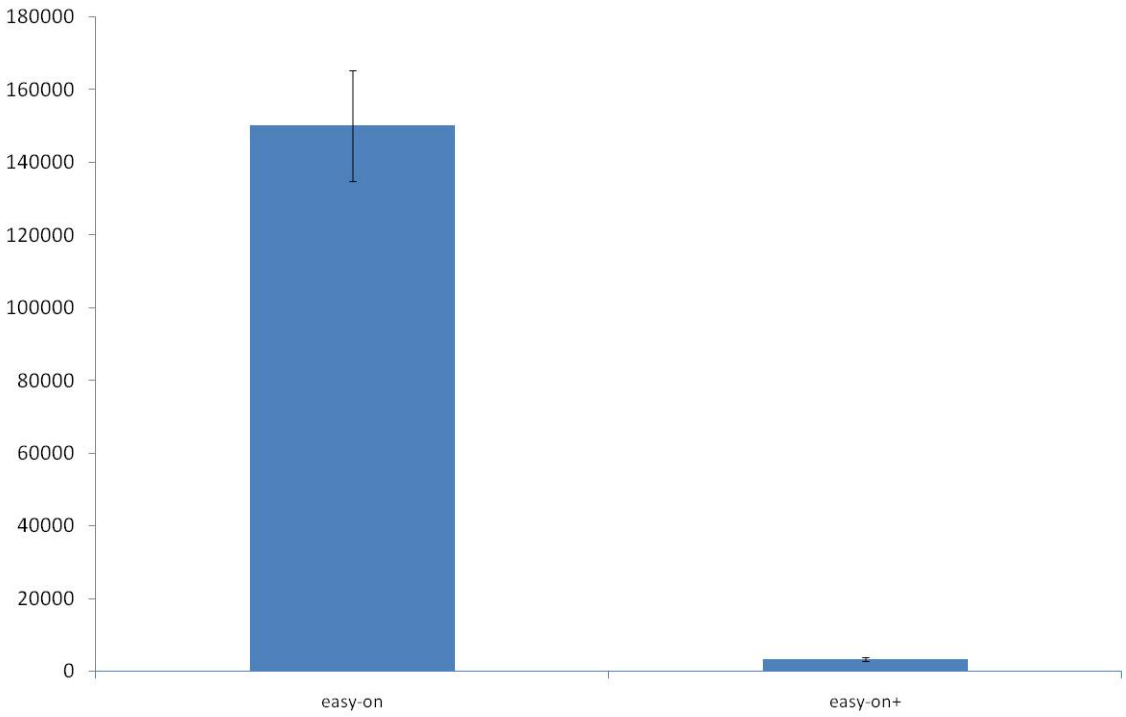

Figure 1 Number of cells recovered from easy-on and easy-on+ panels.

Panels coated in easy-on+ killed the majority of bacteria present resulting in only 3422 bacteria remaining viable.

Taking the figures from easy-on coated panels as a base line which allows for the loss of bacteria resulting from the extraction process, and the effect of easy-on, percentage loss of bacteria is shown in figure 2.

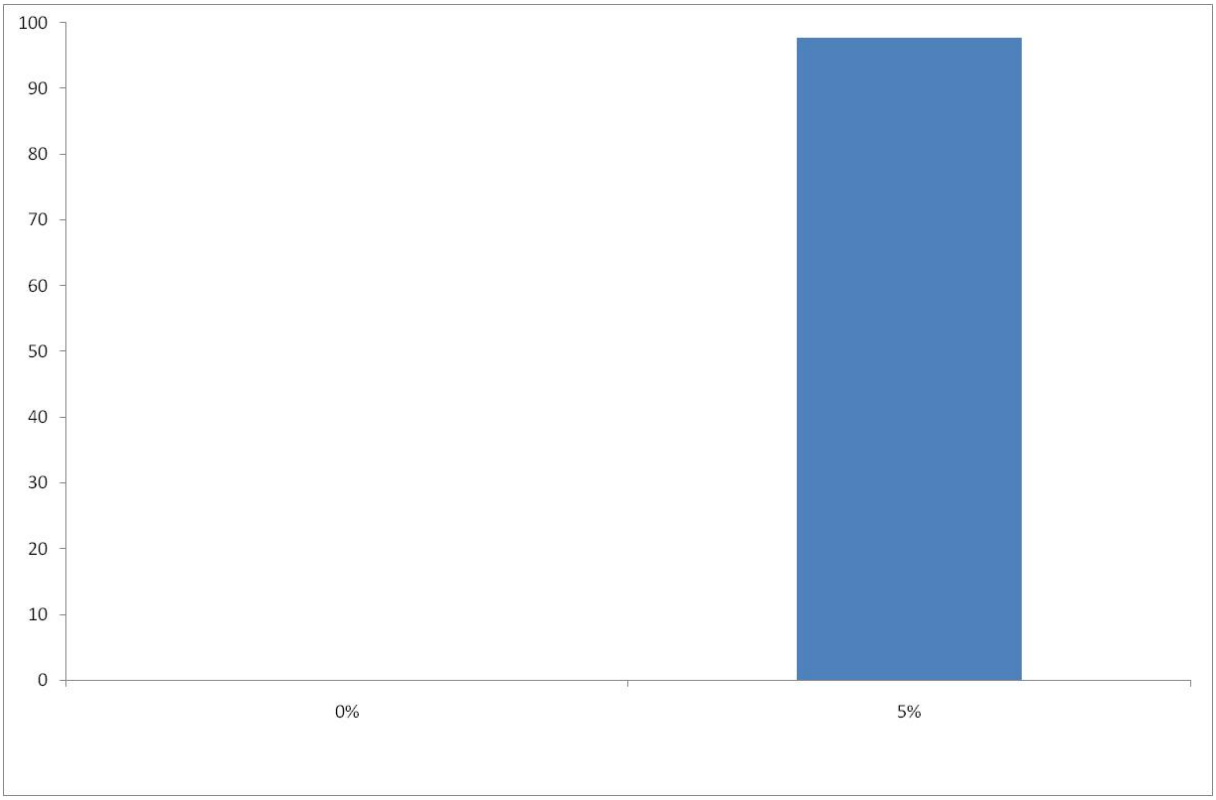

Figure 2. Percentage loss of bacteria from easy-on+ panels only

easy-on+ coated panels removed ~97% of viable bacteria

Determination of bacterial survival over a simulated lifespan of easy-on+ coated surfaces

MRSA results

Only a limited number of bacteria could be recovered at each stage between applications 1 and 14, with numbers on average of 2700 c.f.u./ml compared with the total application of 10^7 c.f.u./ml. After application 14 the number of bacteria recovered steadily rose to reach a level of 52000 c.f.u./ml by application 21. Numbers then remained constant until the end of the experiment (Figure 3)

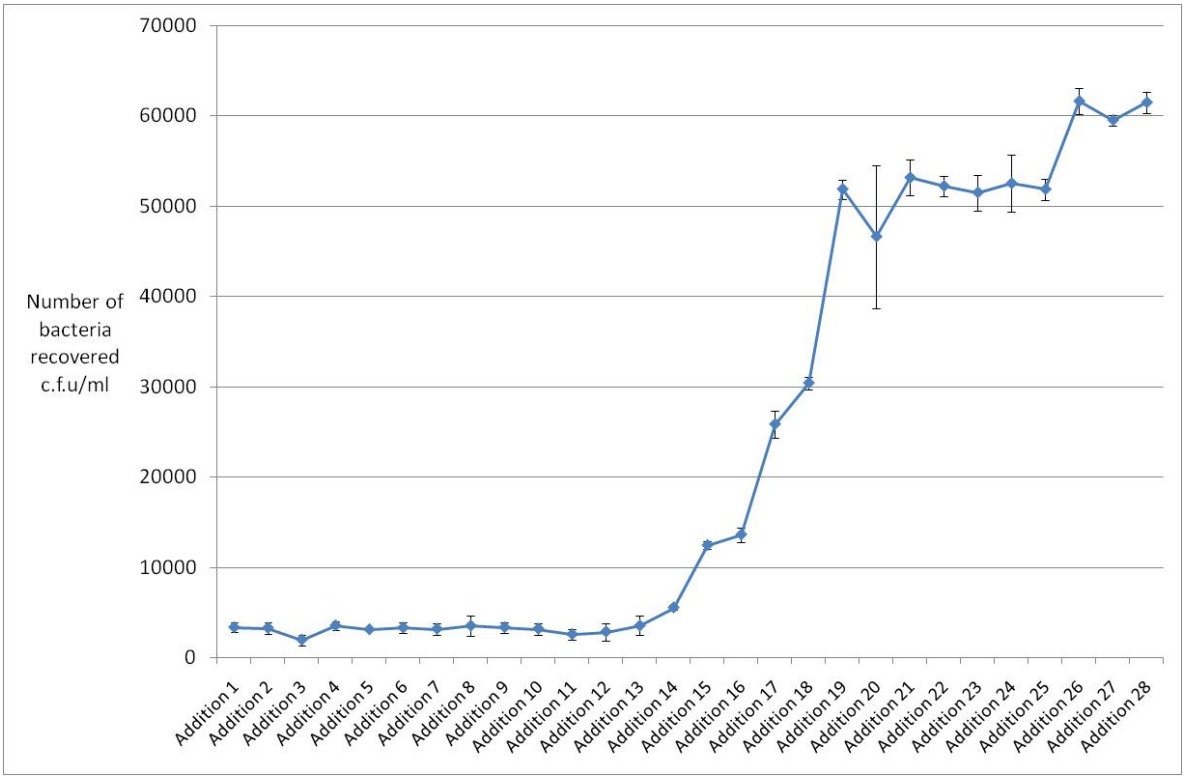

Figure 3. Number of bacteria recovered following each bacterial addition and cleaning step. Error bars represent standard deviations of average bacterial counts

When taking into account the loss of bacteria through easy-on alone and through the removal and extraction process, >97% of bacteria were killed on the coated panels until addition 14, where after the percentage killed gradually dropped to reach an average level of >65% kill from application 19 onwards (Figure 4)

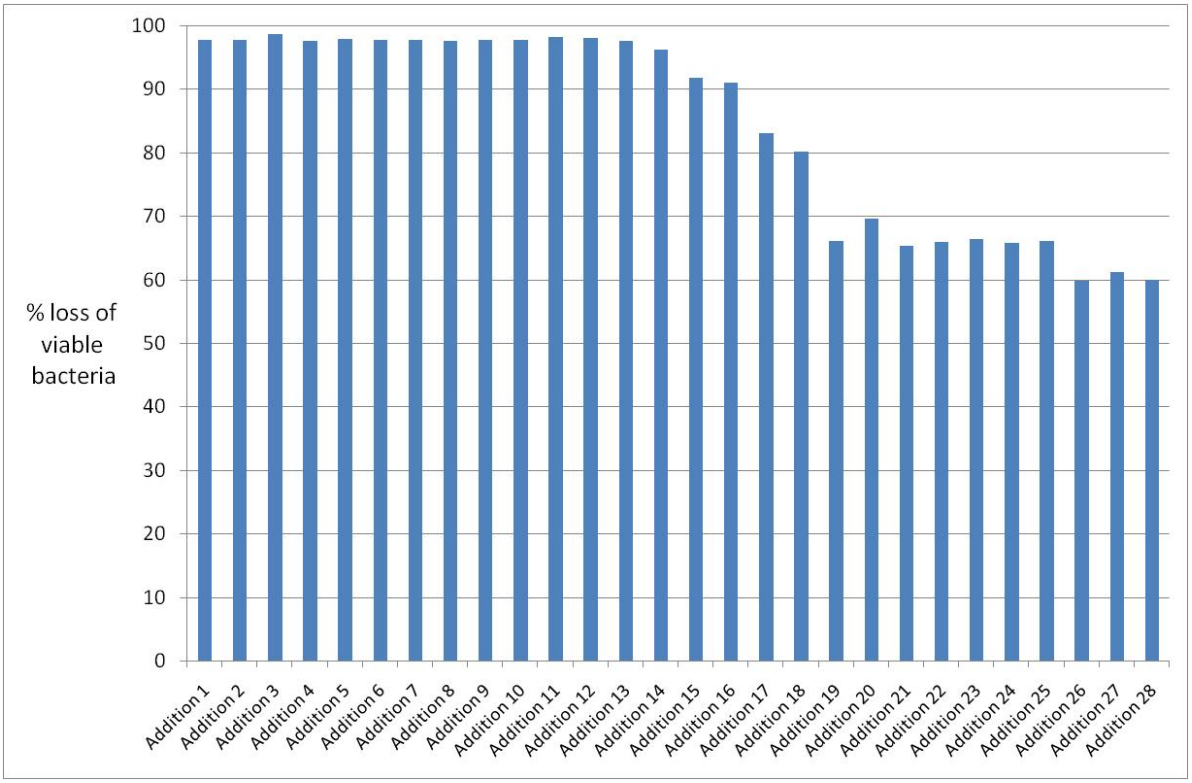

Figure 4. Percentage loss of viable bacteria after each application of bacteria and subsequent washing step

E. Coli Results

Low levels of bacteria were recovered from panels at each stage between applications 1 and 15, with numbers on average of 5000 c.f.u./ml compared with the total application of 10^7 c.f.u./ml. After application 15 the number of bacteria recovered steadily rose to reach a level of 53000 c.f.u./ml by application 20. Numbers then remained constant until the end of the experiment (Figure 5)

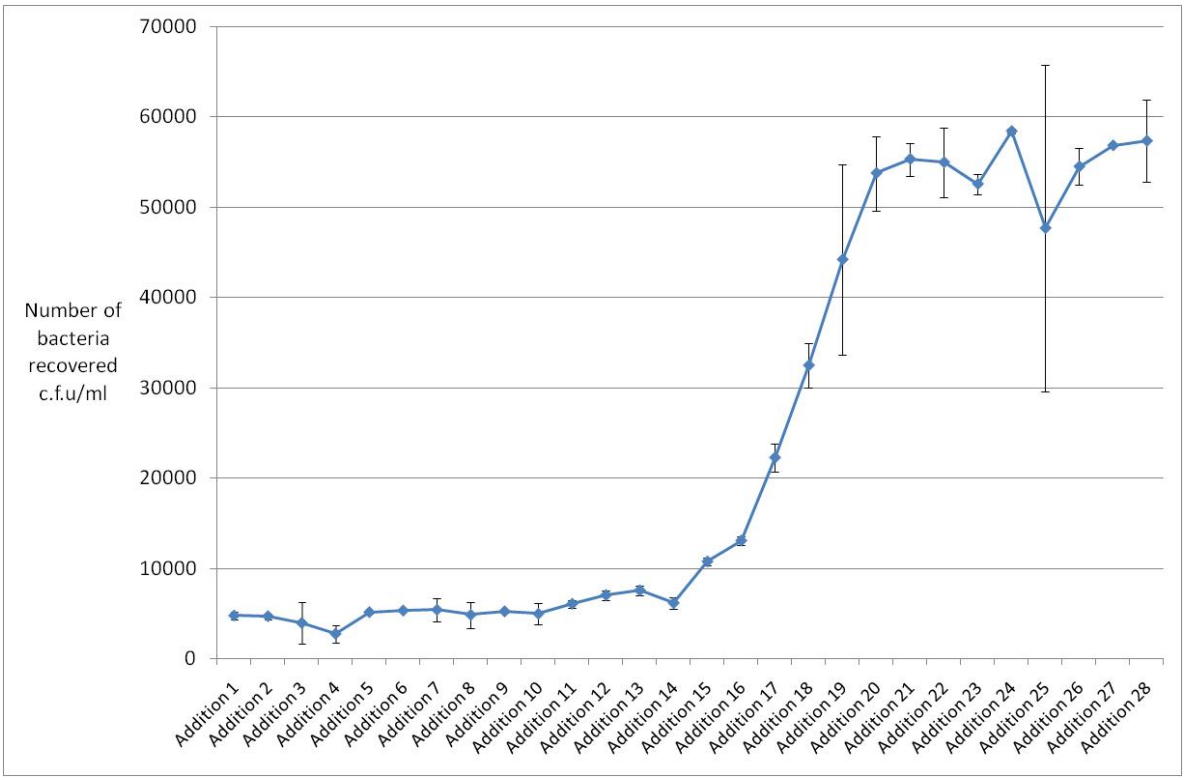

Figure 5. Number of bacteria recovered following each bacterial addition and cleaning step. Error bars represent standard deviations of average bacterial counts

When taking into account the loss of bacteria through easy-on+ alone and through the removal and extraction process, >98% of bacteria were killed on the coated panels until addition 15, where after the percentage killed gradually dropped to reach an average level of >63% kill from application 19 onwards (Figure 6)

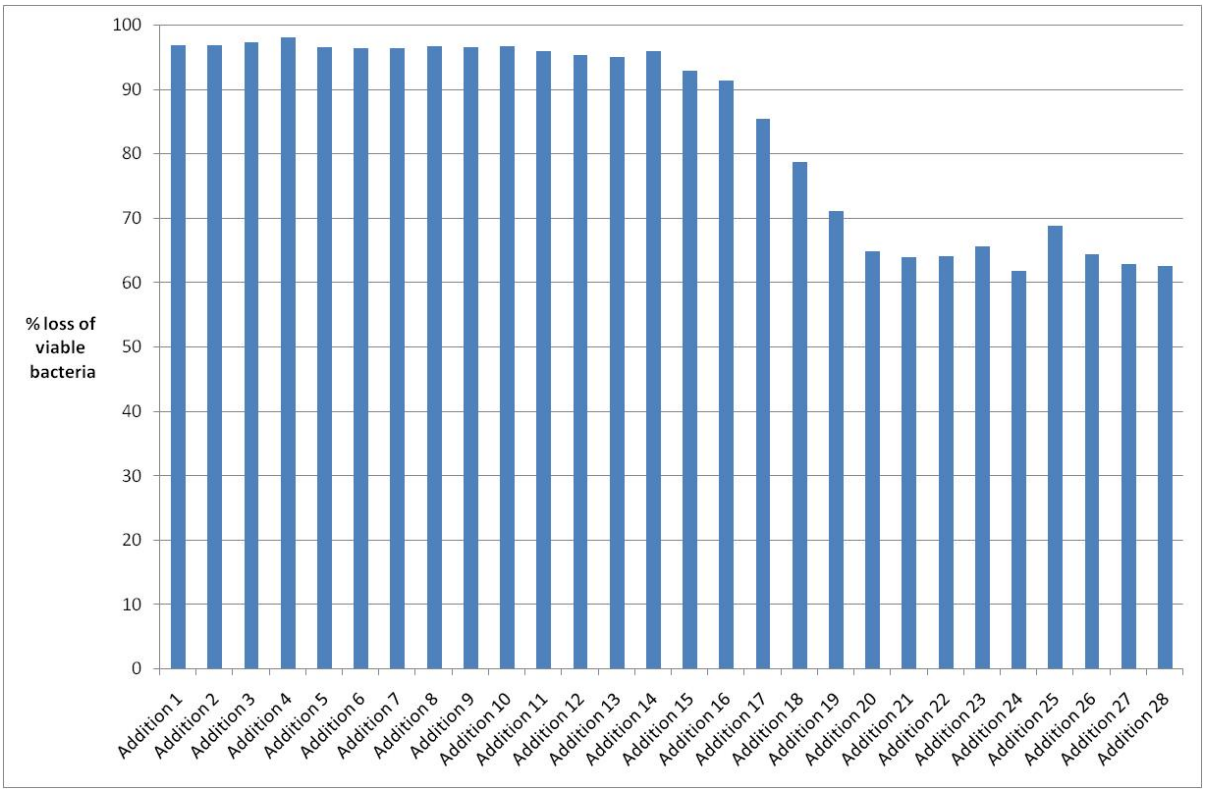

Figure 6. Percentage loss of viable bacteria after each application of bacteria and subsequent washing step

Conclusions

The results presented are based on the testing of easy-on+ coated panels (7.5 x 5.5 cm) tested at room temperature only.

Panels with 5% active ingredient showed bacterial levels dropped to $3.4x10^3$ cells per ml.

When the effect of the extraction process and the easy-on coating was removed the results gave a drop of bacterial units of 97.73% for easy-on+ coated panels

Test results for the life span of easy-on+ coated panels gave a slightly higher level of killing for *Methicillin resistant Staphylococcus aureus* than for *E. coli*.

Even after 28 additions and subsequent cleaning steps, the coated panels were still able to kill >65% of the bacteria added, showing that they are still effective although at a lower rate than prior to multiple cleaning steps.