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**Assessment of degree of bacterial colonisation
of surfaces coated with easy-on.**

Project Report on behalf of:

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Section A: Colonisation of Coated surfaces

Summary

20 mm discs coated with easy-on durability coating were tested for their resistance to bacterial colonization over a period of 5 days. The growth of seven pathogenic bacterial species was monitored over time, with bacteria added to the discs in both growth medium and also in a simple salt-buffered solution. Easy-on coated discs were shown to prevent the colonization and growth of *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica* subs *enterica* and *Pseudomonas aeruginosa* after a period of 3 days. The survival of *Klebsiella pneumoniae* was also severely reduced.

Materials and Methods

Bacterial Species

Species	American Type Culture Collection (ATCC) identification number	Description	Growth Media
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	ATCC 12600	Gram +ive, common skin organism	Nutrient Broth
<i>Escherichia coli</i>	ATCC 11775	Gram -ive, common gut organism, some strains are pathogenic	Nutrient Broth
<i>Listeria monocytogenes</i>	ATCC 15313	Gram +ive rod, 10% of human population are carriers, can cause severe infection in some patients	Brain Heart Infusion
<i>Salmonella enterica</i> subsp. <i>enterica</i>	ATCC 43971	Gram -ive rod, common cause of salmonella food poisoning	CASO broth
<i>Bacillus cereus</i>	ATCC 14579	Gram +ive rod, implicated in food poisoning. Forms spores which are resistant to heat and some disinfectants	Nutrient Broth
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	ATCC 13883	Gram -ive, common cause of hospital acquired pneumonia	Nutrient Broth
<i>Pseudomonas aeruginosa</i>	ATCC 10145	Gram -ive, fourth most common cause of hospital acquired infections. Very simple growth requirements, and can easy colonise surfaces	Nutrient Broth

All bacterial species used were supplied as vacuum dried cultures by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany.)

Media

Nutrient agar/broth, Caso agar/broth and brain heart infusion agar/broth were supplied by Sigma-Aldrich (UK)

Nutrient Broth/Agar

Peptone	5.0 g
Meat extract	3.0 g
Agar, if necessary	15.0 g
Distilled water	1000.0 ml
Adjust pH to 7.0.	

Caso Broth/Agar

Peptone from casein	15.0 g
Peptone from soymeal	5.0 g
NaCl	5.0 g
Agar, if necessary	15.0 g
Distilled water	1000.0 ml
Adjust pH to 7.3.	

BHI Broth Agar

Brain Heart Infusion	37.0 g
Agar, if necessary	15.0 g
Distilled water	1000.0 ml

Buffers and solutions

Quarter strength Ringer's solution

Sodium chloride	2.25 g/l
Potassium chloride	0.105 g/l
Calcium chloride 6H ₂ O	0.12 g/l
Sodium bicarbonate	0.05 g/l
Distilled water	to 1000.00 ml
pH 7.0	

All Chemicals and reagents used in this experiment were supplied by Sigma-Aldrich (UK) unless otherwise stated

Methods

Sterilisation of coated discs

22mm discs coated with easy-on were placed in 90 % ethanol for 30 min. Following sterilisation discs were air-dried in a sterile flow hood.

Growth of bacterial cultures

Dried cultures were resuspended for 30 min in the appropriate media, and then added to culture flasks containing 250 ml of nutrient broth, Caso broth or BHI broth. Flasks were incubated overnight at 37 °C. Following incubation cultures were centrifuged and the medium removed. Cells were then re-suspended in quarter strength Ringer's solution and centrifuged again. The supernatant was removed and cells suspended in either culture media or Ringer's solution to give a working concentration of 10^7 cells/ml. Cell numbers were determined using a haemocytometer and light microscopy.

Addition of bacteria to discs

200 µl of washed and resuspended cells were added to each coated disc. As a negative control 200 µl of either sterile growth media or sterile Ringer's solution was added to separate discs. A set of discs was also used with no addition of either bacterial cells or media/Ringer's solution. Discs were then placed in sterile Petri dishes and incubated at 22 °C.

Sampling of coated discs

Discs were sampled at Day 0, 1, 3 and 5, following initial addition of bacterial cultures. Each disc was placed in a sterile Universal tube and 5ml of Ringer's solution was added. Tubes were agitated for 10 min using a Griffin shaker to remove bacteria from the coated surface. A dilution series was created in Ringer's solution. After this 200 µl of the 10^{-3} , 10^{-4} and 10^{-5} dilutions was plated out onto the appropriate agar plates. Plates were incubated overnight at 37 °C and cell counts taken the following morning.

Each experiment was carried out on triplicate discs for reproducibility.

Analysis

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

Results

Staphylococcus aureus

Complete loss of culturable cells was noted at day 3 in bacteria suspended in Ringer's solution, and by day 5 in bacteria suspended in growth media. Loss of bacterial cells appears to be quite rapid with a drop from 10^7 cfu/ml to 2×10^4 cfu/ml in Ringer's solution and 7×10^4 cfu/ml in growth media by day 1 (Fig. 1)

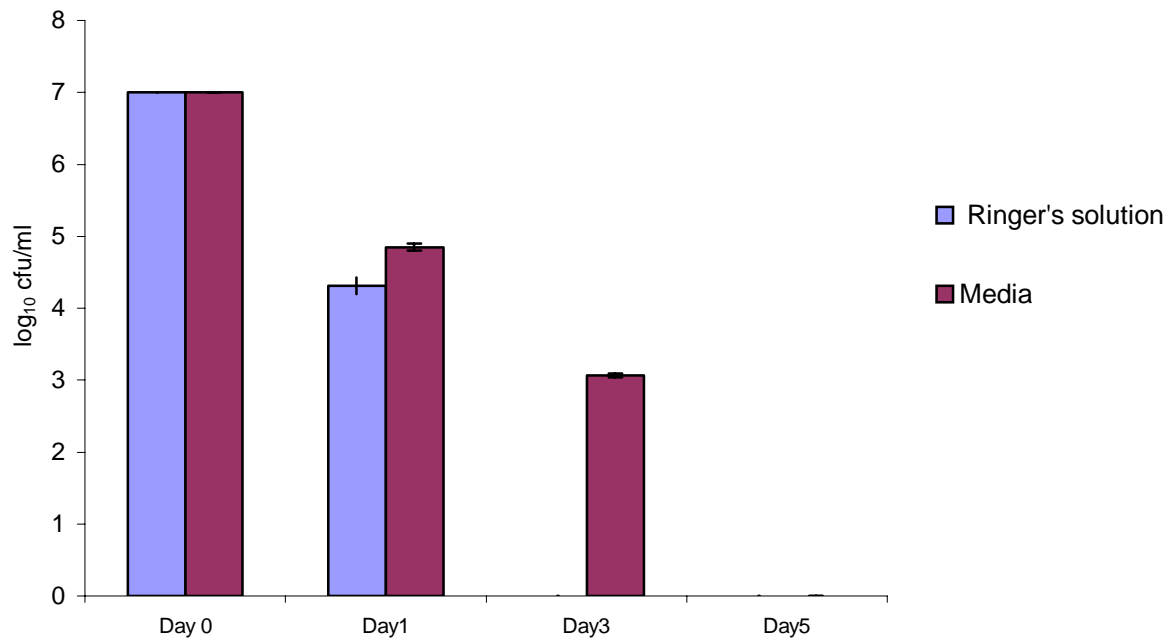


Figure 1. Cell concentration of *Staphylococcus aureus* added to coated discs over time. Error bars indicate standard deviation

Escherichia coli

Viable *E. coli* cells were detected at day 5. Cell numbers did reduce over time, with no significant difference between media and Ringer's solution. Final cell concentrations were 3.9×10^3 cfu/ml for Ringer's solution and 2.5×10^3 cfu/ml in media (Fig 2).

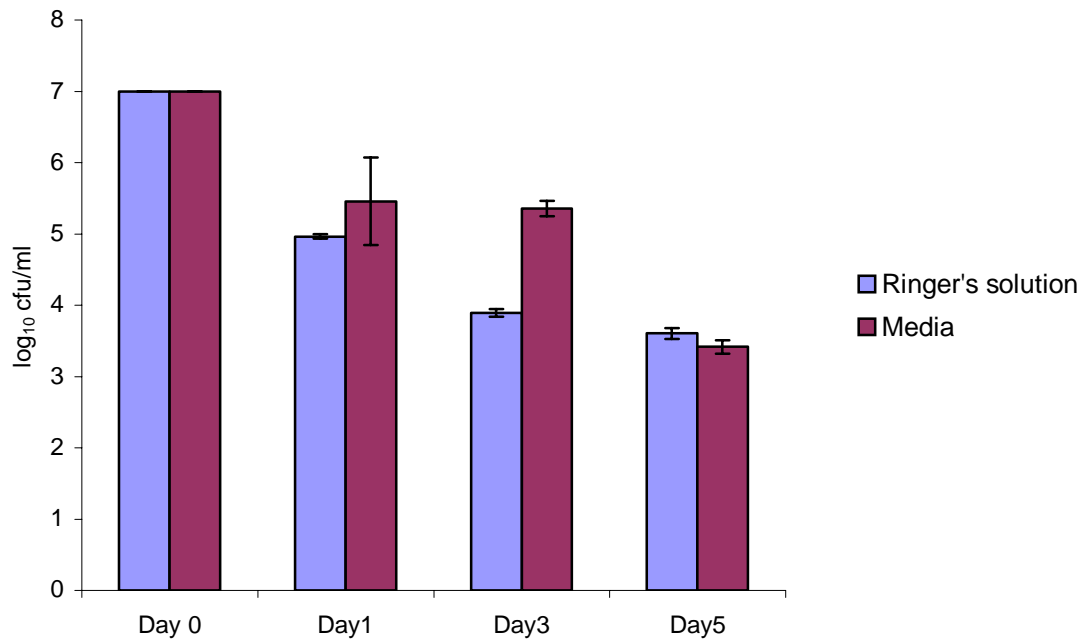


Figure 2. Cell concentration of *Escherichia coli* added to coated discs over time. Error bars indicate standard deviation.

Listeria monocytogenes

Complete loss of cells in Ringer's solution was noted by day 3, and by Day 5 in media. The drop in cell numbers was much more rapid in bacteria suspended in Ringer's solution, falling to 2.5×10^3 cfu/ml by day 1. This compares to a bacterial count of 7.9×10^4 cfu/ml in cells suspended in growth media (Fig. 3).

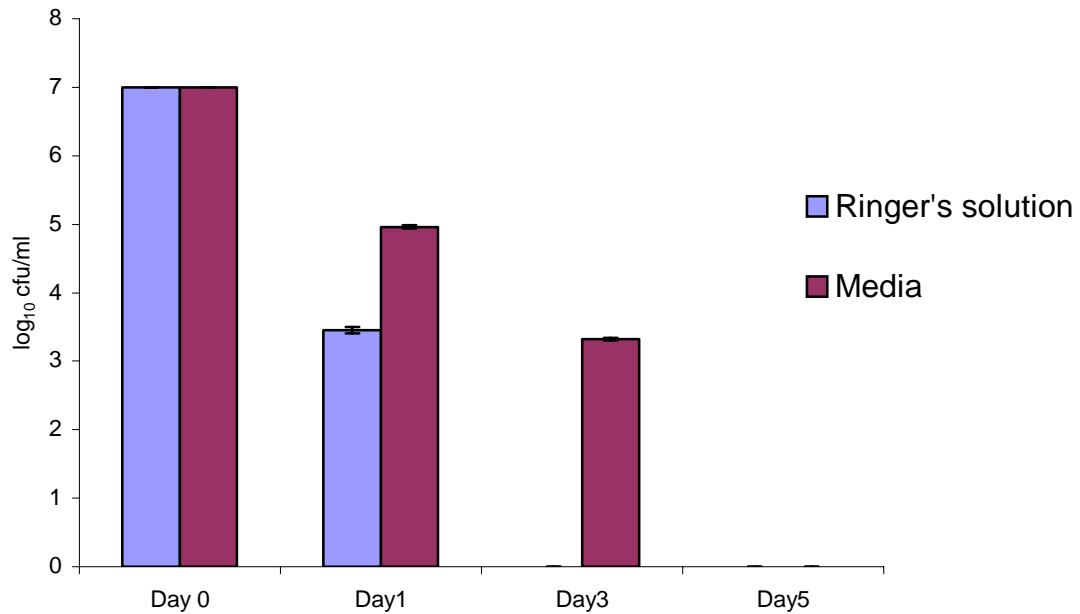


Figure 3. Cell concentration of *Listeria monocytogenes* added to coated discs over time. Error bars indicate standard deviation.

Salmonella enterica subsp. *enterica*

Total loss of bacterial cells was noted by day 3 in both cell suspensions. There appeared to be a faster loss of cell numbers when bacteria were resuspended in Ringer's solution, compared to growth media, with cell numbers falling to 7.6×10^4 cfu/ml and 1.8×10^5 cfu/ml respectively by day 1 (Fig. 4).

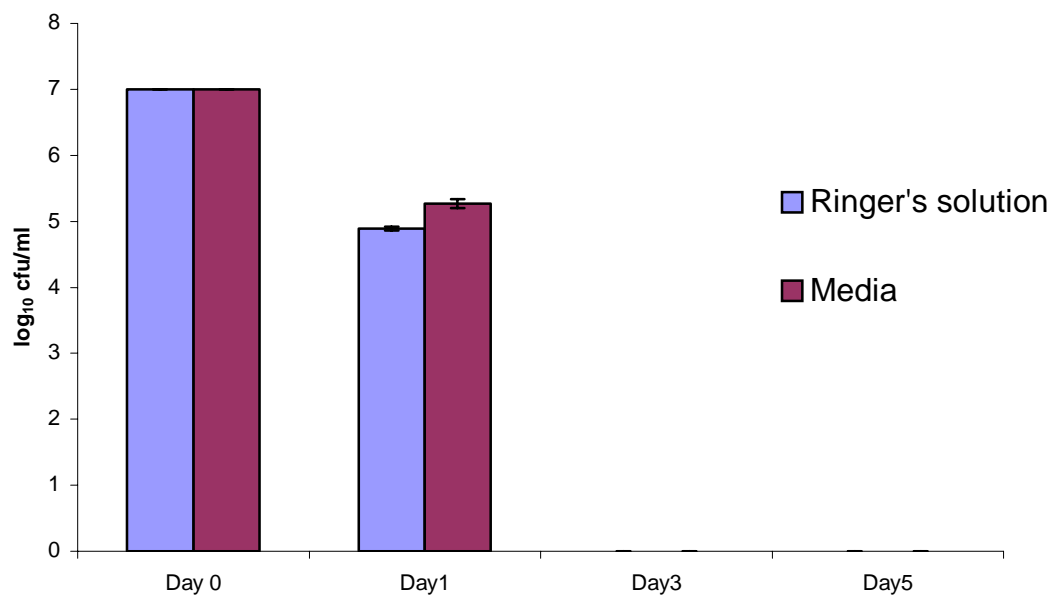


Figure 4. Cell concentration of *Salmonella enterica* subsp. *enterica* added to coated discs over time. Error bars indicate standard deviation.

Bacillus cereus

Bacterial cells were still viable at a high rate in both Ringer's solution and Growth media. Cell numbers did fall but appeared to reach a stable level of 3.1×10^4 cfu/ml in Ringer's solution and 1.2×10^4 cfu/ml in growth media by day 3. There was no significant difference between the cell concentrations in Ringer's solution and media at day 5 (Fig. 5).

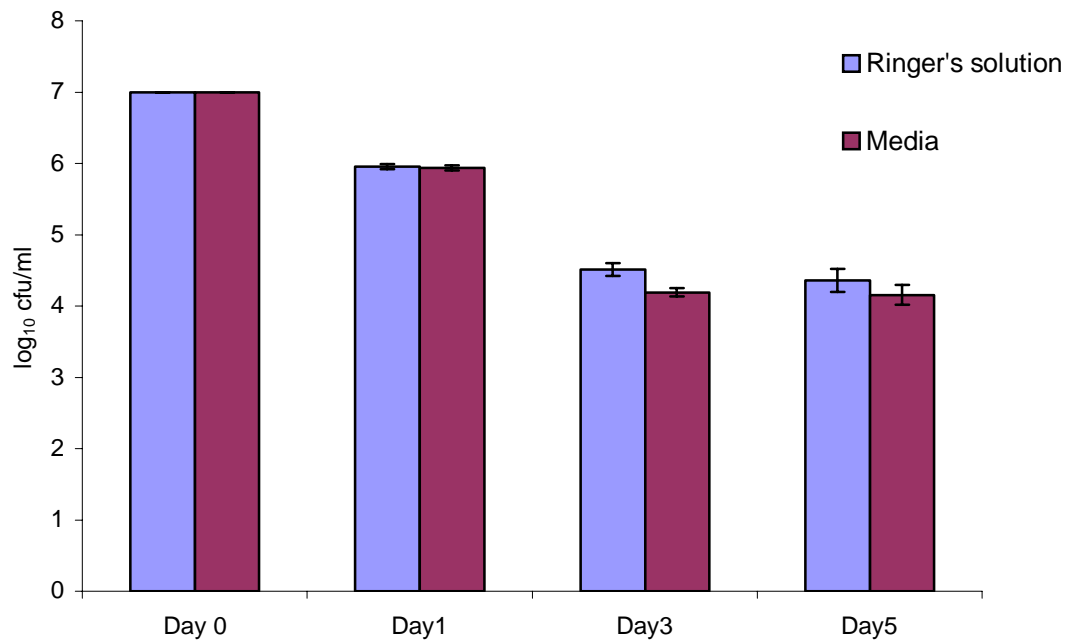


Figure 5. Cell concentration of *Bacillus cereus* added to coated discs over time. Error bars indicate standard deviation.

Klebsiella pneumoniae

A complete loss of cell numbers was noted when bacteria were resuspended in Ringer's solution; however, cell numbers only fell to a level of 2.7×10^4 cfu/ml by day5 when bacteria were resuspended in media (Fig. 6).

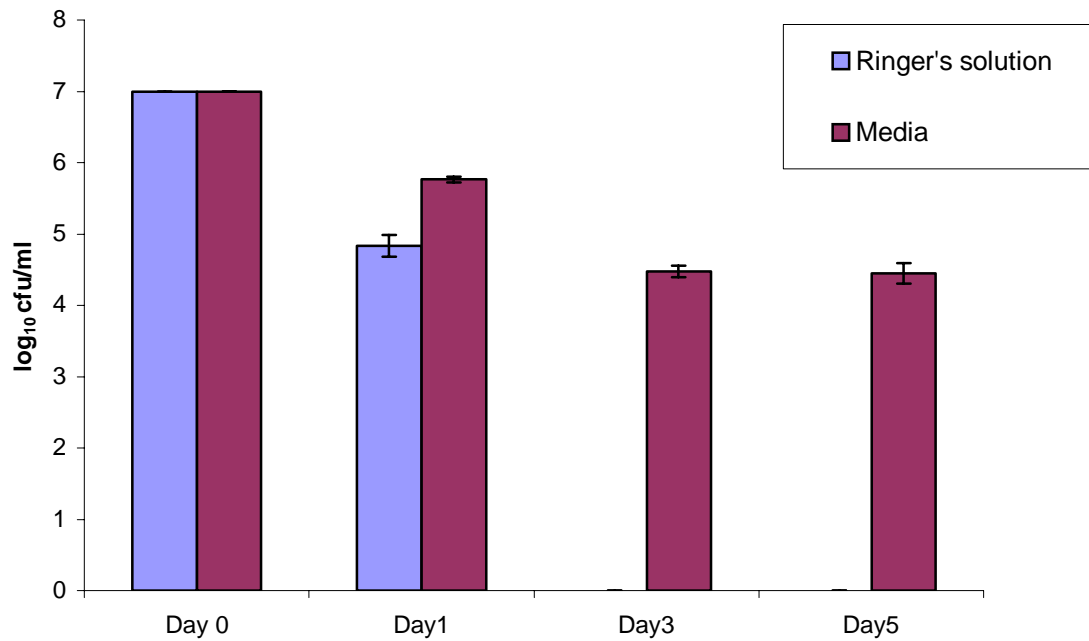


Figure 6. Cell concentration of *Klebsiella pneumoniae* added to coated discs over time. Error bars indicate standard deviation.

Pseudomonas aeruginosa

Total loss of viable cells was noted at day 3 in cells suspended in Ringer's solution and by day 5 in cells suspended in media. A slower drop in cell numbers occurred in bacteria in media, compared to bacteria in Ringer's solution (Fig. 7).

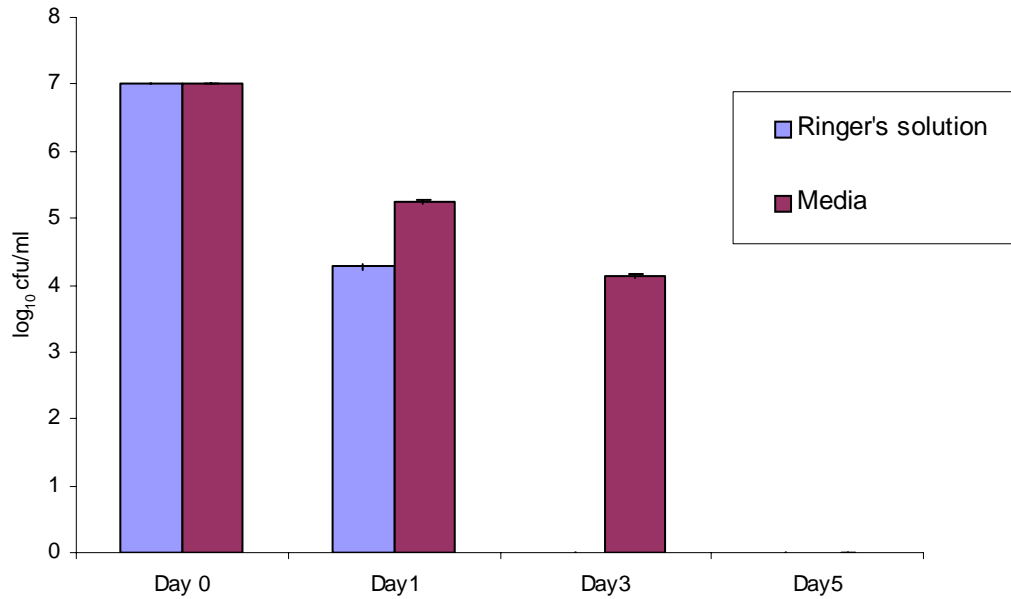


Figure 7. Cell concentration of *Pseudomonas aeruginosa* added to coated discs over time. Error bars indicate standard deviation.

Negative controls

No bacterial growth was seen on any negative controls used in this experiment.

Conclusions

- easy-on coating prevents the colonisation of *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica* subs *enterica* and *Pseudomonas aeruginosa* after a period of 3 days on treated surfaces.
- easy-on coating significantly reduces the survival of *Klebsiella pneumoniae* in a buffered solution when added to the treated surface.
- easy-on coating does not inhibit the colonisation of *E. coli* or *Bacillus cereus*. A drop in cell numbers however is seen.
- Where loss of cell numbers is noted it is usually higher in cells resuspended in a simple salt-buffered solution.

Section B

Bacterial survival on surfaces coated with easy-on durability coating after disinfection with Presept (Dichloroisocyanurate)

Summary

Wooden panels were coated with vinyl emulsion paint, acrylic paint or easy-on durability coating. Bacterial cultures were added and subsequently cleaned using a 1000 ppm solution of Presept (Johnson and Johnson, US). Panels were incubated on agar plates for 1 and 3 days following cleaning. Results show that when panels coated with easy-on a simple cleaning procedure removes the bacteria. Panels coated with emulsion and acrylic paints show survival of bacteria after cleaning.

Materials and Methods

Bacterial Strains

The seven bacterial species used in Section A were used in this experiment.

Preparation of Panels

Wooden panels were coated with vinyl matt emulsion, vinyl matt emulsion and over-coated with easy-on coating, and with acrylic paint. Panels were cleaned thoroughly with 1000 ppm of Presept solution prior to addition of bacterial cultures

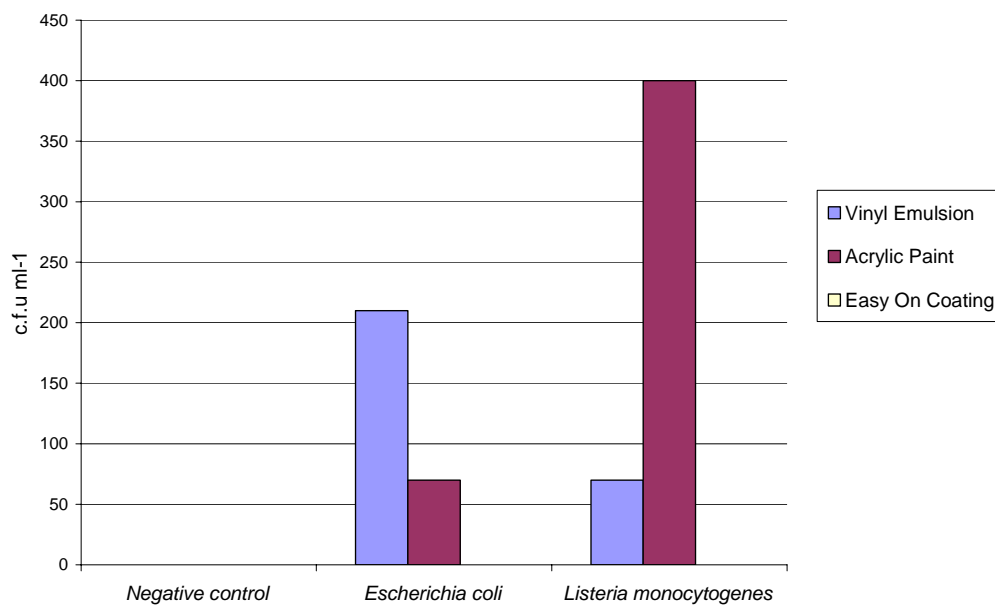
Addition of Bacteria

Cultures of bacteria were incubated overnight at 37 °C and then diluted in culture media to achieve a bacterial concentration of 10^7 cells ml⁻¹. 100 µl of bacterial cultures were then added to each panel and incubated at room temperature for 30 min. Following incubation wooden panels were cleaned twice with sterile tissue soaked in 1000 ppm Presept solution. A general cleaning action was simulated with the panels being wiped vertically twice in each cleaning step. Panels were then placed face down on nutrient agar plates for 30 minutes to allow transfer of bacteria to the agar surface. Panels were then removed and the plates incubated at 37 °C overnight. Bacterial colonies were then counted. The process was then repeated with the bacteria incubated on the panels for 3 days prior to cleaning.

Results

Panels coated with easy-on and subsequently cleaned with Presept solution showed no survival of bacterial species after 30 min and three days incubation.

After 30 minutes incubation followed by cleaning, *E.coli*, *L. monocytogenes*, *Staph. aureus* and *B. cereus* all exhibited survival on both cleaned acrylic and emulsion coated panels (Fig 1). *Salm. enterica*, *K. pneumoniae* and *Ps .aeruginosa* only showed survival on acrylic coated surfaces. Results after 3 days incubation were identical, with the same survival patterns present.



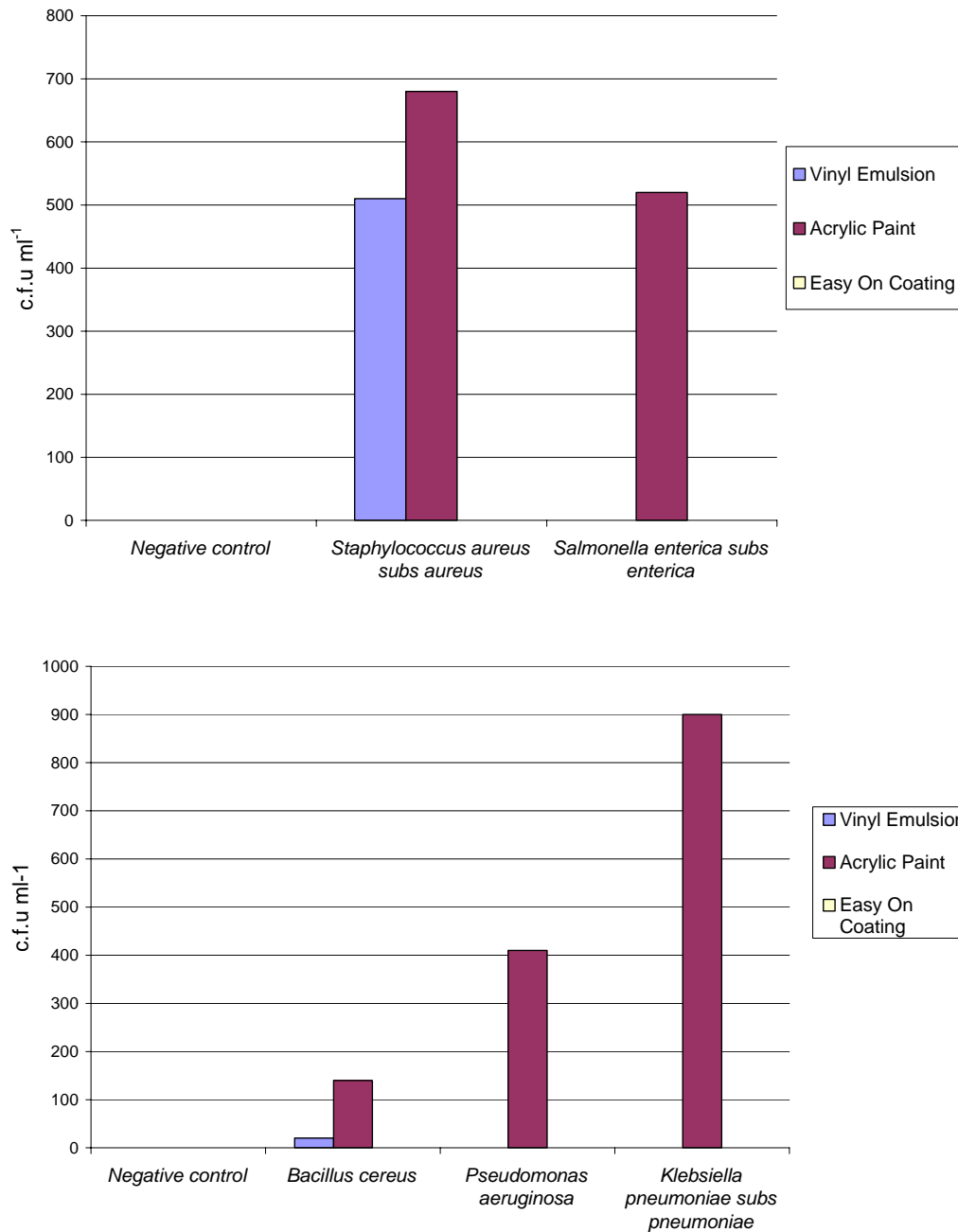


Figure 1. Survival of bacterial species on wooden panels with different coated surfaces, after 30 minutes of incubation followed by cleaning.

Conclusions

Following a basic cleaning regime, all bacteria were removed from surfaces coated with easy-on.

Surfaces coated with acrylic paint allowed survival of all seven bacterial species tested after cleaning

Surfaces coated with emulsion paint allowed survival of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*.

Section C

Overall Summary

- easy-on coating prevents the colonisation of several pathogenic bacterial species including *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica* and *Pseudomonas aeruginosa* on treated surfaces.
- easy-on coated surfaces are easier to clean than untreated surfaces with simple paint finishes.
- The durable coating allows for decontamination of surfaces using simple cleaning solutions with no damage to the surface
- easy-on coated surfaces contaminated with bacteria can be thoroughly cleaned with a simple solution with a 100% bacterial removal rate.
- *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were all completely removed from coated surfaces after cleaning. This compares with surfaces with simple paint finishes, where bacteria remained on the surface after cleaning.