# DeWipe antimicrobial formula

## Preliminary investigation report

Assessing the efficacy of DeWipe antimicrobial solution to remove and kill model bacteria (*Escherichia coli*, *Staphylococcus aureus* & *Klebsiella pneumoniae*)

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**Figure 3:** The recovery of culturable (viable/live) bacteria, as colony forming units per millilitre (CFU/ml), following treatment in solution with DeWipe Formula (DWF), water or soap wash. Whiskers represent standard deviations of replicates (duplicates) within a treatment. Percentage reduction in initial microbial number is also indicated for each treatment. Stars represent treatments where viable microbial numbers were below detection.

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## Production history

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## Executive Summary


### Aim

The primary aim of this investigation was to undertake laboratory testing to assess the bacteriocidal effectiveness of DeWipe Formula on common bacterial contaminants which are known human pathogens.

### Approach

We undertook an investigation to compare the performance of the DeWipe Formula (DWF) in solution against other technologies (water and two varieties of soap wash) in a controlled laboratory setting. Three bacterial test organisms were selected; *Escherichia coli* and *Klebsiella pneomoniae*, organisms found in mammalian guts and commonly found in contaminated water/unsanitary conditions, and *Staphylococcus aureus*, a bacterium common in skin microflora. Furthermore, we investigated the performance of a DWF-soaked DeWipe against a water-soaked DeWipe, selecting *S. aureus* and *E. coli* as test organisms. In all cases the antibacterial efficacy of the DWF solution or DWF-soaked DeWipe was determined by attempting to culture the target microorganisms post-treatment.

### Results

The results of this preliminary investigation indicated that when applied directly and under laboratory conditions, the DWF solution was effective at killing *E. coli* and *K. pneumonia,* but less effective at killing *S. aureus*. When used in a DeWipe, the DWF was effective at reducing the number of viable bacteria on a non-porous plastic (polyethylene) surface, achieving greater than the standard 3-log reduction (>99.9%) in bacterial load. Furthermore, while significant numbers of culturable and therefore viable bacteria were recovered from the control (water-soaked) DeWipe, none were recovered from the DWF-soaked DeWipe, further indicating the

effective antibacterial activity of the DeWipe Formula. We recomend caution in the extrapolation of the above results, given the limited organisms tested thus far and the potential for a variation in efficacy based on surface texture and composition.

**Conclusions**

The wipe was effective at reducing the viable number of microorganisms tested by >99.9%. These results are encouraging and it is recommended that further investigation is undertaken to validate efficacy of the DeWipe Formula-containing DeWipe on other bacteria and viruses and to assess its effectiveness of the in further laboratory and field studies.

## (1) Introduction

In everyday life we are constantly exposed to microorganisms, the vast majority of which are relatively benign for the healthy individual. Under certain conditions however, for example immunosuppression, ill health, injury or allergies, exposure to some of these organisms, known as opportunistic pathogens, becomes a cause for concern [1,2]. One of the best known examples is *Staphylococcus aureus*, a bacterium which resides naturally on our skin without detriment to the healthy. In recent years it has evolved to include strains, including the MRSA 'superbug', which are resistant to common antibiotics and are becoming more prevalent in the community, care homes and healthcare settings alike [3-5].

The risk of individual exposure to both pathogens and opportunistic pathogens depends upon occupation. There is extensive potential for occupational exposure in both indoor and outdoor work environments, encompassing professions from healthcare workers and emergency responders, to cleaners, tradespersons, tattooists and those working outdoors in general [6,7], while the sources of contamination may include humans or their bodily fluids, animals, refuse, contaminated water (human or animal faeces), ventilation systems, soil etc.

While in an occupational setting personal protective equipment (PPE) protects the worker, a simple, safe, reliable and effective means of microbial decontamination of the surfaces of reusable PPE (e.g. firefighter turnout gear) other equipment and vehicles is vital, to protect not only the worker but to prevent the spreading of the pathogen elsewhere [8-12].

In this study we tested the antibacterial efficacy of DeWipe Formula against selected opportunistic bacterial pathogens. The efficacy of the DWF was tested in two formats; as a stand-alone solution added to bacterial cultures, and as a DWF-soaked wipe used to decontaminate a non-porous plastic surface inoculated with bacteria. The efficacy of the DWF-soaked wipe was tested post-treatment by comparing the recovery of culturable bacterial numbers to the control (water treatment) treatments.

## (2) Methods

### 2A. Bactericidal activity of DeWipe Formula in solution format

A schematic overview of the protocol for testing the bactericidal activity of DWF against *E. coli*, *S. aureus* and *K. pneumoniae* is illustrated in Figure 1. The protocol followed a modified version of the standardised British Standards Institution (BSI) BS EN13727:2012 testing method. The following steps, repeated twice for each microorganism, describe the procedure:

(1) A freshly-grown bacterial culture was inoculated into one of the following solutions; water, soap wash (two varieties) or DWF, giving a final density of approximately 1 x $10^8$ colony forming units per ml (CFU/ml).

(2) Solutions were left for 60 seconds at room temperature.

(3) Then diluted tenfold in neutralizing buffer (to halt antimicrobial action).

(4) Diluted and undiluted solutions were spread on Tryptic Soya Agar (TSA) and incubated at 37˚C.

(5) Colony forming units (CFUs) were recorded after 24 h growth, each CFU deemed representative of one viable cell surviving the treatment.

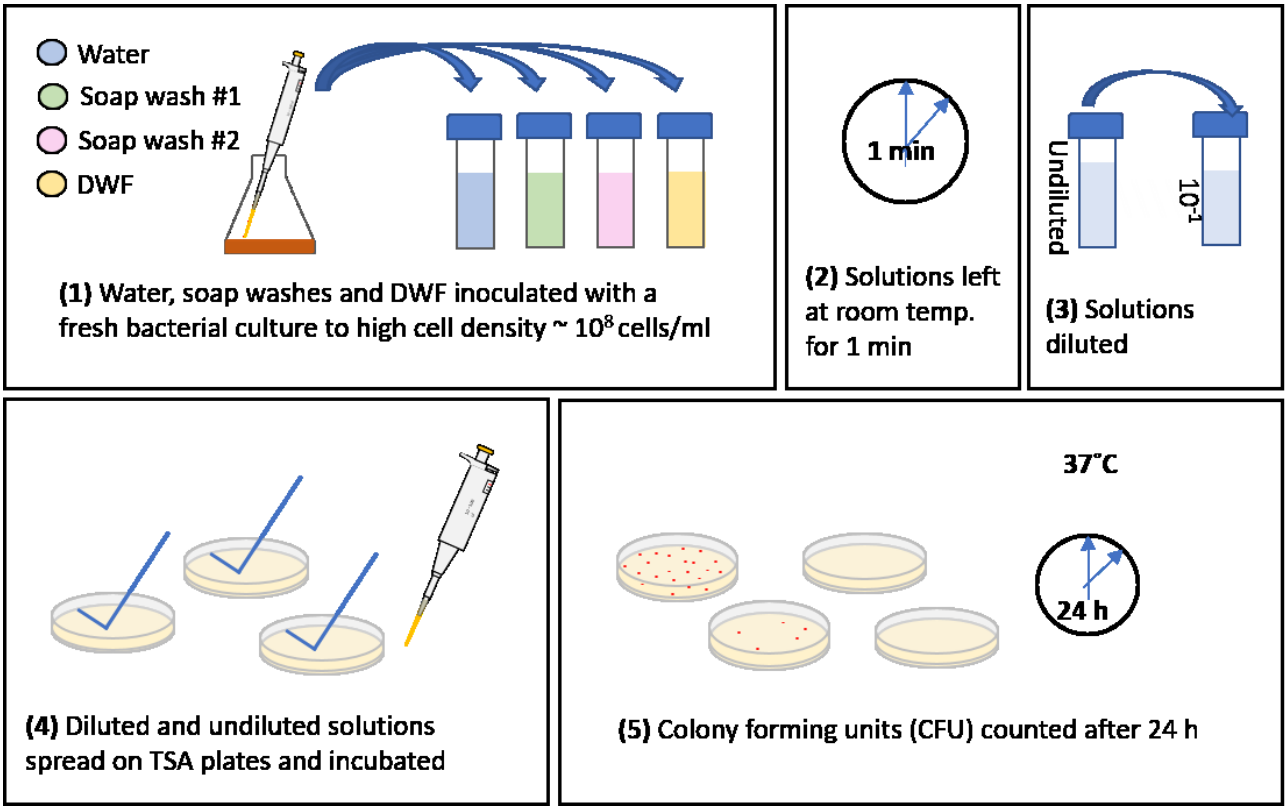


**Figure 1: Schematic overview of the protocol** employed to determine the bactericidal activity of DeWipe Formula (DWF) relative to other available products (water and soap washes). All steps were performed under standard sterile laboratory conditions.

### 2B. Bactericidal activity of DWF-soaked DeWipes

A schematic overview of the protocol for testing the efficacy of DWF-soaked DeWipes at removing and killing *E. coli* and *S. aureus* is illustrated in Figure 2. The protocol was modified from the BSI standardised BS EN16615:2015 testing method. The following steps, repeated twice for each microorganism, describe this procedure:

(1) Benchkote® was employed as the polyethylene test surface. Two test grids were marked out, each consisting of four square test areas (T1-T4), each with an area of 25 $cm^2$. The Benchkote® was sterilised with an ethanol-soaked DeWipe and air dried.

(2) The fresh bacterial culture was diluted to approximately 1 x $10^8$ CFU/ml. The test grid was spiked/inoculated with 50 µl of bacterial suspension adjacent to each T1 area.

(3) While the inocula were drying, DeWipes were soaked for 30 minutes in either sterile distilled water (W; control treatment) or DWF (DWF; active treatment).

(4) The soaked wipe was wrapped around a sterile plastic box and used to push the bacterial inoculum from the inoculation area, sequentially across test areas T1-T4 and back again, in one swift, fluid motion.

(5) Each test area was swabbed using an individual cotton swab moistened with sterile saline. Each test area was then swabbed a second time with a dry swab to ensure all moisture was collected.

(6) For each test area, both swabs were placed in the same sterile saline and vortexed for 1 minute. Used wipes were also vortexed separately in saline for 1 minute.

(7) Solutions from the previous step, i.e. homogenates, were diluted tenfold in sterile saline.

(8) Diluted and undiluted homogenates were spread on TSA agar plates and incubated at 37 ˚C.

**(9)** After 24 h growth, CFUs were recorded, each CFU deemed representative of one viable cell.

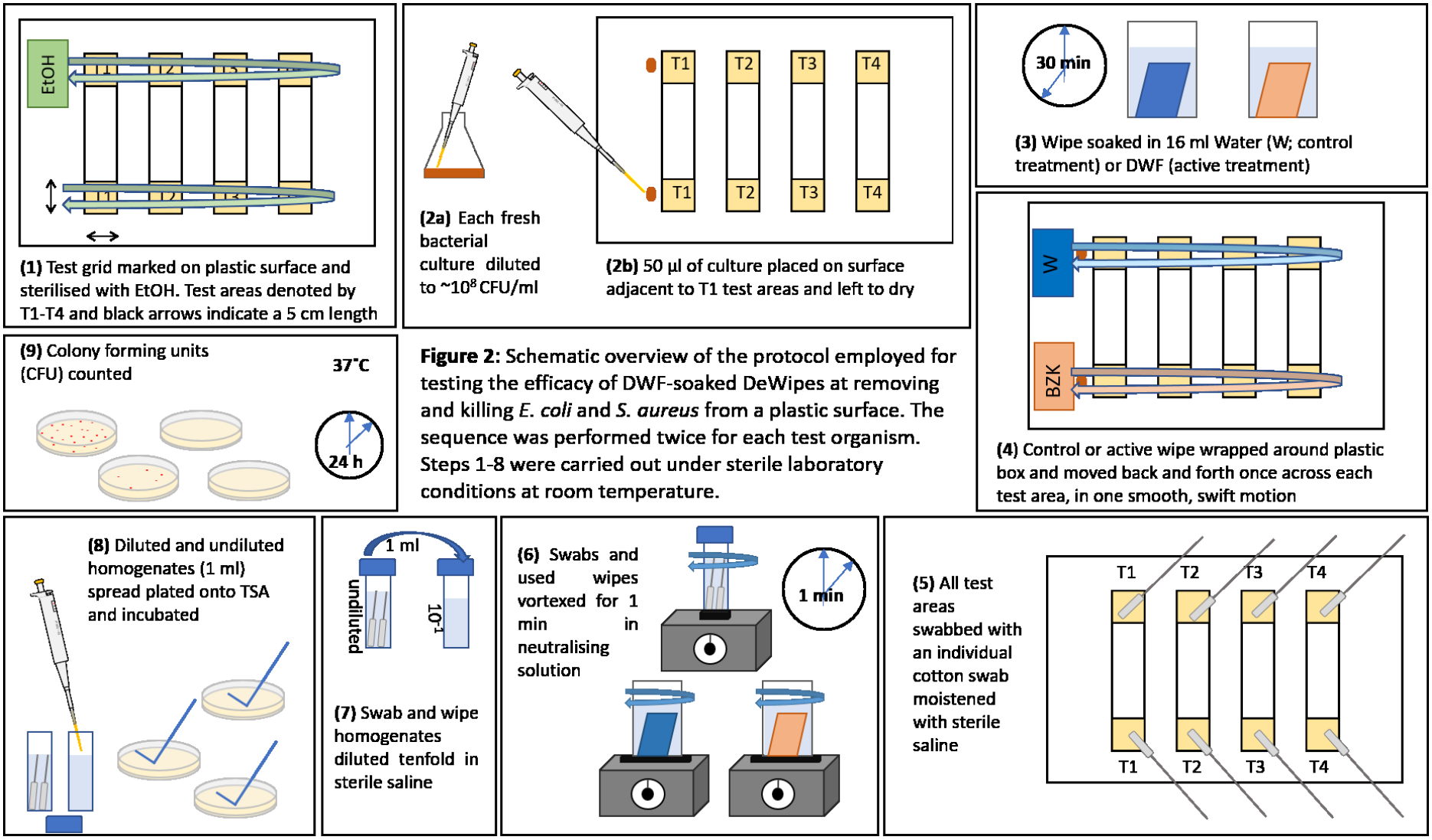


**Figure 2:** Schematic overview of the protocol employed for testing the efficacy of DWF-soaked DeWipes at removing and killing *E. coli* and *S. aureus* from a plastic surface. The sequence was performed twice for each test organism. Steps 1-8 were carried out under sterile laboratory conditions at room temperature.

**Figure 2: Schematic overview of the protocol** employed to determine the bactericidal activity of DWF-soaked DeWipes relative to other available products (water and soap washes). All steps were performed under standard sterile laboratory conditions.

## (3) Results

### 3A. Bactericidal activity of DWF in solution

The results of this investigation indicate that DWF solution exhibits significant bactericidal activity towards *E. coli* and *K. pneumoniae*, two important and widely distributed opportunistic pathogens. When exposed to DWF solution for 1 minute, more than 99.9% of *E. coli* and *K. pneumoniae* cells were killed (Figure 3). This reduction for DWF solution, to levels below detection (in this case an 8-log reduction), was significant compared to the other treatments, which exhibited little bactericidal activity towards these organisms. Whilst a reduction in the viability of *S. aureus* was evident in the presence of the DWF solution and soap wash treatments, the level of reduction did not meet the 3-log (i.e. 99.9%) reduction criteria required for success. The reduced effectiveness of the DWF solution towards *S. aureus* may reflect the different cell wall composition (Gram-positive organism) compared to *E. coli* and *K. pneumoniae* (Gram-negative), however further laboratory testing is required to fully understand the efficacy of the solution against all microorganisms, especially relating to the intended end-use.

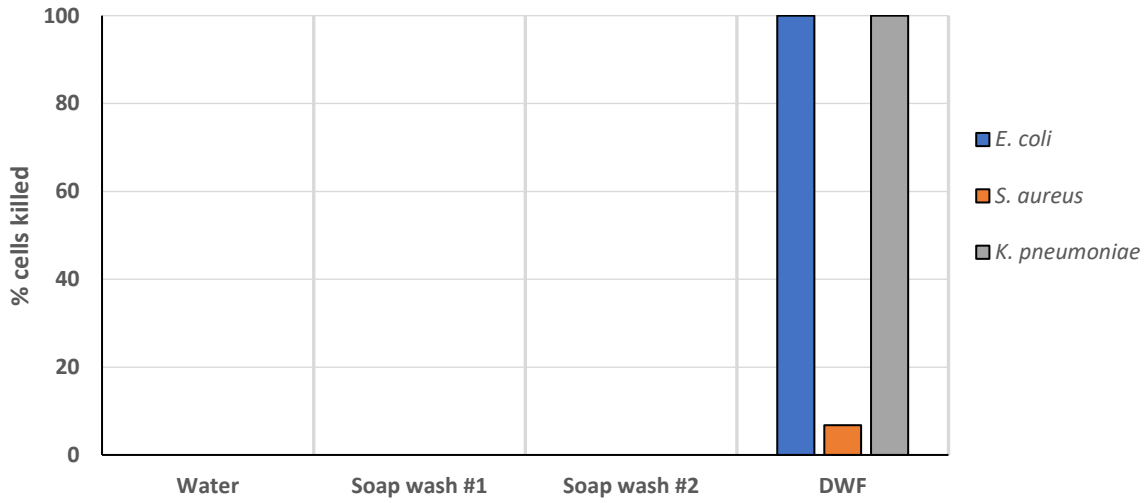


**Figure 3: Shows a large proportion of bacteria are killed by DeWipe formula**. The percentage reduction in intial viable (live) microbial numbers following treatment in solution with DeWipe Formula (DWF), water or soap washes. The three different bacteria were tested at initial cell concentrations of approx. $10^8$ cells/ml.

### 3B. Bactericidal activity of DWF-soaked DeWipes

The results of this investigation indicate that DWF-soaked DeWipes successfully removed both *E. coli* and *S. aureus* from a non-porous plastic surface. Following brief application of the DWF-soaked DeWipe to the inoculated polyethylene surface, both organisms were reduced to levels below detection (Figure 4). Conversely, inoculated surfaces treated with water-soaked DeWipes (controls) still harbored viable bacterial loads.

Given the need for safe DeWipe disposal after their use, we tested the viability of bacterial cells contained within the wipes post-use and therefore their potential to constitute a possible biohazard. Whilst the water-soaked DeWipes contained significant viable bacterial loads, the DWF-soaked DeWipes did not harbor detectable viable bacteria (Figure 5), suggesting that they had effectively killed any bacteria picked up during the wiping process.

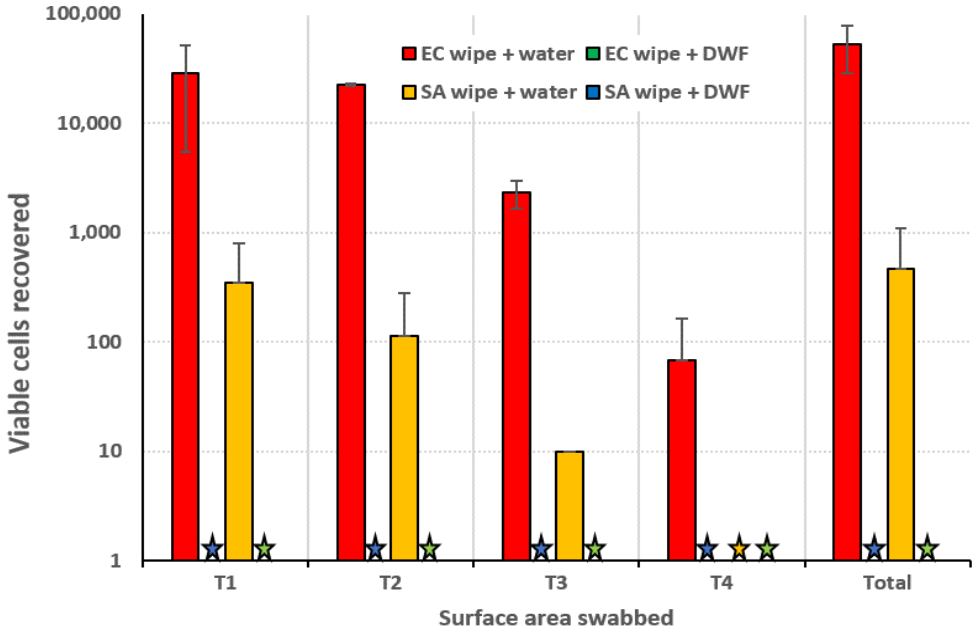


**Figure 4: Shows no detectable bacteria were present on surfaces treated with wipes containing the DeWipe formula**. The recovery of culturable bacteria (CFU) from a plastic surface following standardised wiping with a DeWipe soaked in water or DWF solution. Averages are shown for each treatment (bars), with whiskers indicating variation in repeat treatments. T1-T4 represent test areas, each of 25 $cm^2$ (Figure 2), while Total represents the cumultative number of culturable bacteria recovered per treatment (areas T1-T4). EC = *E. coli* and SA = *S. aureus* as test organisms. *Stars represent treatments where viable microbial numbers were below detection*.

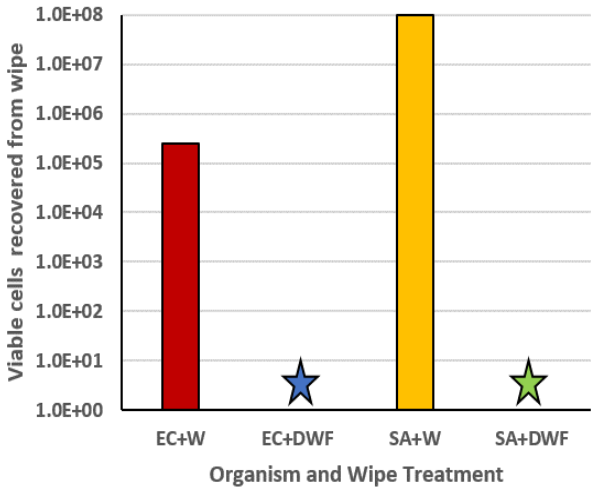


**Figure 5: Shows no detectable bacteria were present on the used wipes**. The recovery of culturable bacteria from water (W) or DWF-soaked DeWipes, following their use to remove a bacterial inoculum (*E. coli* (EC) or *S. aureus* (SA)) from a non-porous polyethylene surface. Stars represent treatments (exclusively DWF-soaked wipes) where viable microbial numbers were below detection, therefore killing >99.9% of microbial cells captured (relative to the control water-soaked wipes). *Stars represent treatments where viable microbial numbers were below detection.*

## (4) Conclusions and recommendations

These results are encouraging and indicate that the DeWipes containing DeWipe antimicrobial formula, are able to significantly reduce the viability of the important bacterial pathogens tested by >99.9%.

It is recommended that further investigation is undertaken to validate eficacy of the DeWipe Formula on other bacteria and viruses (particularly its use in a DeWipe format) to fully understand the capabilities and limitations of the product. This testing should include:

- Analysis of additional bacterial species under laboratory conditions, particularly those likely to be of concern to the potential product end-user.
- Analysis of additional surfaces, both porous and non-porous.
- Determination of the optimal concentration of DWF in a DeWipe format.
- Testing the product under ‘real-world’ applications.

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