Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm

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Introduction

Biofilms are aggregated, structured communities of bacteria encased in an extracellular matrix which is composed of protein, DNA and polysaccharides. Microorganisms within a biofilm are challenging to treat compared to their planktonic counterparts. The Single Tube Method utilises the Centers for Disease Control (CDC) Biofilm Reactor (Figure 1). It is used to determine the efficacy of biocides against pre-formed biofilms. Data generated using this method has been used to gain biofilm removal claims in the USA. This method was designed to assess water soluble powder or liquid formulations against biofilm that has formed on hard or non-porous surfaces.

Aim

To determine the biofilm removal capabilities of a range of disinfectants using the Single Tube Method.

Method

Mature *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms were grown using a CDC reactor. The reactors were incubated under shear force for 48 hours in a batch system. At 48 hours, sterile media was introduced for 24 hours. Prior to treatment, the coupons were washed to remove planktonic organisms and placed into a 50 mL tube containing a splash guard. The biofilms were exposed to each disinfectant for 10 minutes. Following treatment, coupons were placed into neutraliser and remaining viable organisms were quantified per sample (Figure 2).

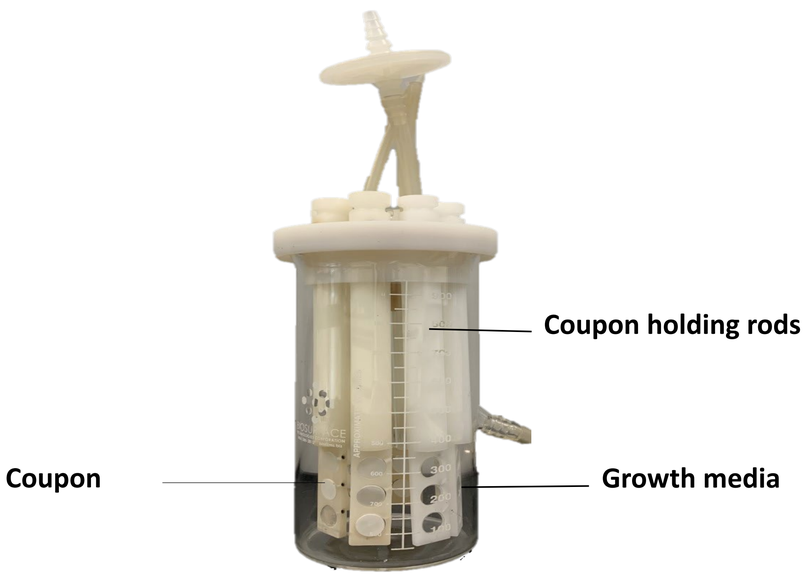

Figure 1. Photograph of CDC reactor.

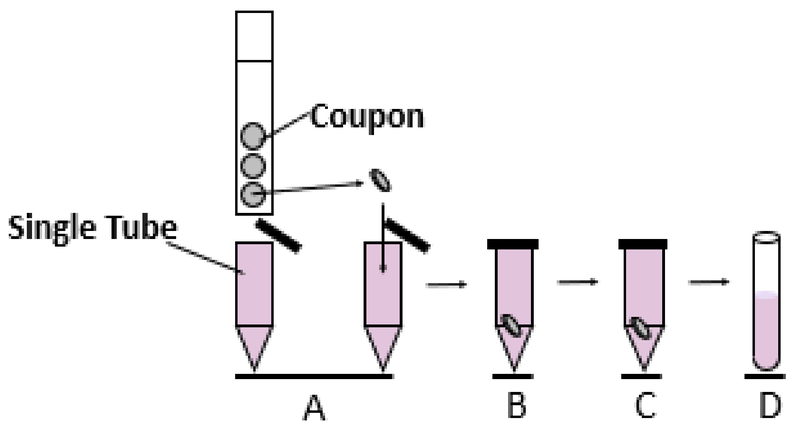

Figure 2. A. Removal/Rinsing B. Treatment. C. Neutralisation. D. Recovery

Results

An average of 7.97 Log_{10} CFU per sample *S. aureus* were recovered from coupons treated with the negative control. The quantity of *S. aureus* recovered from coupons treated with products A and B was not significantly different from the negative control. Averages of 4.47, 3.64, 3.15 and 2.53 Log_{10} CFU per sample *S. aureus* were recovered from coupons treated with products C, D, E and F. No viable organisms were recovered from coupons treated with the positive control (Figure 3).

An average of 9.03 Log_{10} CFU per sample *P. aeruginosa* were recovered from coupons treated with the negative control. The quantity of *P. aeruginosa* recovered from coupons treated with products A, B and C was not significantly different from the negative control. Averages of 5.89, 5.74 and 4.42 Log_{10} CFU per sample *P. aeruginosa* were recovered from coupons treated with products D, E and F. No viable organisms were recovered from coupons treated with the positive control (Figure 4).

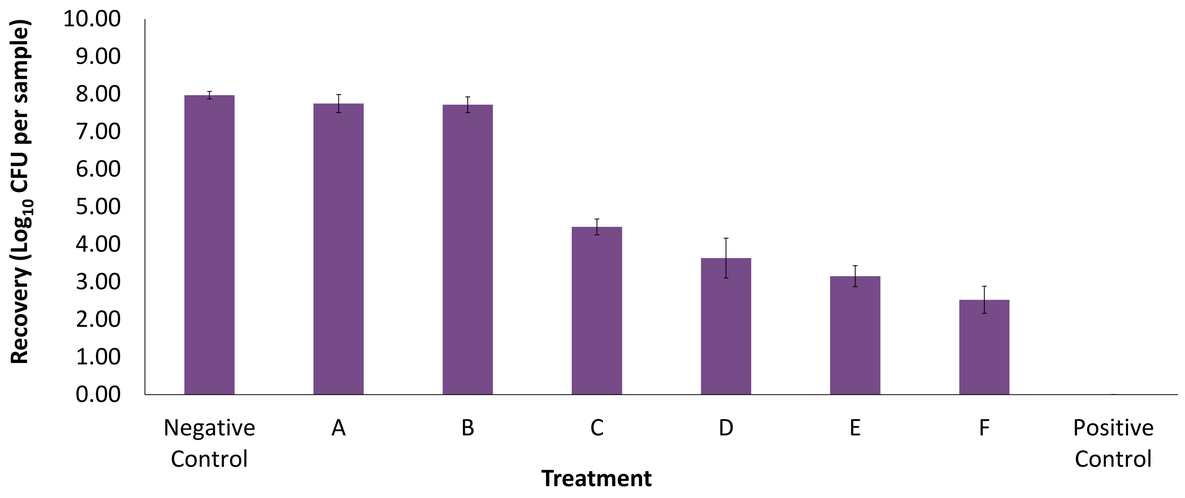

Figure 3. Average CFU per sample of *Staphylococcus aureus* recovered from coupons following 10 minutes treatment with a range of disinfectants.

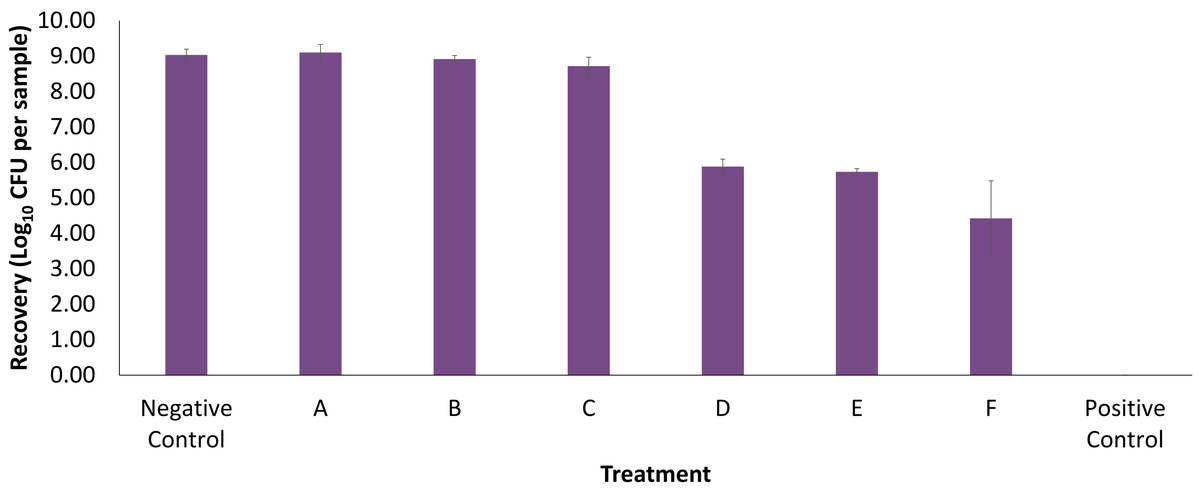

Figure 4. Average CFU per sample of *Pseudomonas aeruginosa* recovered from coupons following 10 minutes treatment with a range of disinfectants.

Discussion & Conclusions

Standard microbiological testing focuses on planktonic bacteria. Planktonic tests can underestimate the quantity of active required to effectively remove bacterial biofilm. The adoption of the Single Tube Method by the EPA is a positive step towards testing products in real-world environments, ultimately improving products on the market and positively impacting human health.

The test methodology differentiated between products that had little effect on *S. aureus* and *P. aeruginosa* biofilms, products that demonstrated an intermediate response and products that resulted in no viable organisms being recovered. With appropriate validation, the Single Tube Method can be used to assess additional bacteria and yeast species.

This project was carried out by Perfectus Biomed Ltd.
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