# Assessment of the Ability of Stimulan® Beads to Release a Combination of Antibiotics to Effectively Eradicate Pre-Formed Biofilms *In Vitro*

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## Introduction

Over 65% of bacterial infections treated clinically in the developed world are known to be caused by organisms growing in biofilms. Biofilms are matrix-enclosed communities of bacteria which are significantly more resistant to host defences and to conventional therapies compared to their planktonic counterparts. Periprosthetic infections can lack a definitive diagnosis because matrix-enclosed sessile bacteria are less immunogenic and elicit a reduced inflammatory response compared to planktonic cells. Implantable devices are highly susceptible to infection and biofilm formation. Once a biofilm has been established in a periprosthetic joint can be difficult to diagnose and eradicate. Successful treatment of periprosthetic joint infection requires optimal surgical procedures combined with long-term antimicrobial therapy directed against surface-adhering microorganisms. Given the limited efficacy of traditional antibiotics in implant-associated infections, novel strategies such as releasing antibiotics directly to the site of infection are a promising future option for biofilm prevention and eradication.

## Aim

To assess the ability of Stimulan® beads containing a mixture of Vancomycin and Gentamicin or Vancomycin and Tobramycin to release a combination of antibiotics and effectively eradicate pre-formed biofilms *in vitro.*

## Method

- Single species *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms were established on polycarbonate coupons within a CDC biofilm reactor for 72 hours (Figure 1).
- Established biofilms were removed from the reactor and rinsed in phosphate buffered saline to remove planktonic organisms.
- Washed biofilms were exposed to a challenge plate containing suspended Stimulan® beads enclosing a mixture of Vancomycin and Gentamicin (VG) or Vancomycin and Tobramycin (VT) for 24 hours at 37°C ± 2°C.
- Positive and negative controls were tested concurrently.
- Following exposure, remaining attached organisms were recovered by sonication. Serial dilutions and plate counts were performed on the resultant suspensions. Log reductions compared to the negative control were reported.

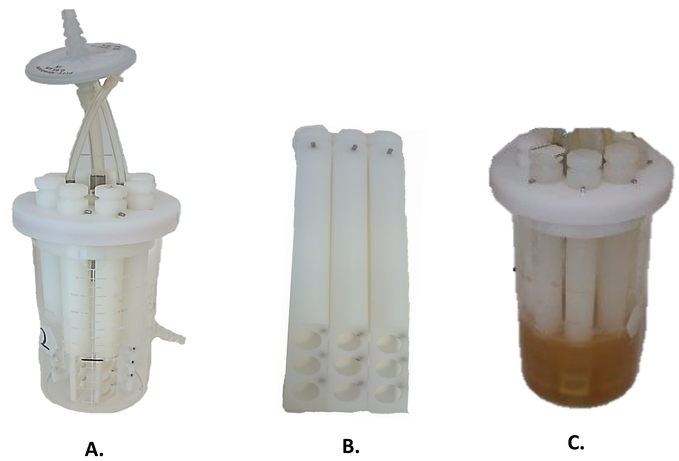


**Figure 1.** Photographs of (A) CDC reactor, (B) Coupon holding rods, (C) CDC reactor containing 72-hour pre-formed single species biofilms.

## Results

An average of 6.60 ± 0.23 $Log_{10}CFUmL^{-1}$ viable *S. aureus* and 6.78 ± 0.12 $Log_{10}CFUmL^{-1}$ viable *P. aeruginosa* were recovered from the negative controls (Figure 2). No viable *S. aureus* were recovered from biofilms treated with the positive control or Stimulan® beads containing a mixture of Vancomycin and Gentamicin or Vancomycin and Tobramycin. No viable *P. aeruginosa* were recovered from biofilms treated with the positive control or Stimulan® beads containing a mixture of Vancomycin and Gentamicin. An average of 0.33 ± 0.52 $Log_{10}CFUmL^{-1}$ (below 1 Log) viable *P. aeruginosa* were recovered from biofilms following treatment with Stimulan® beads containing a mixture of Vancomycin and Tobramycin.

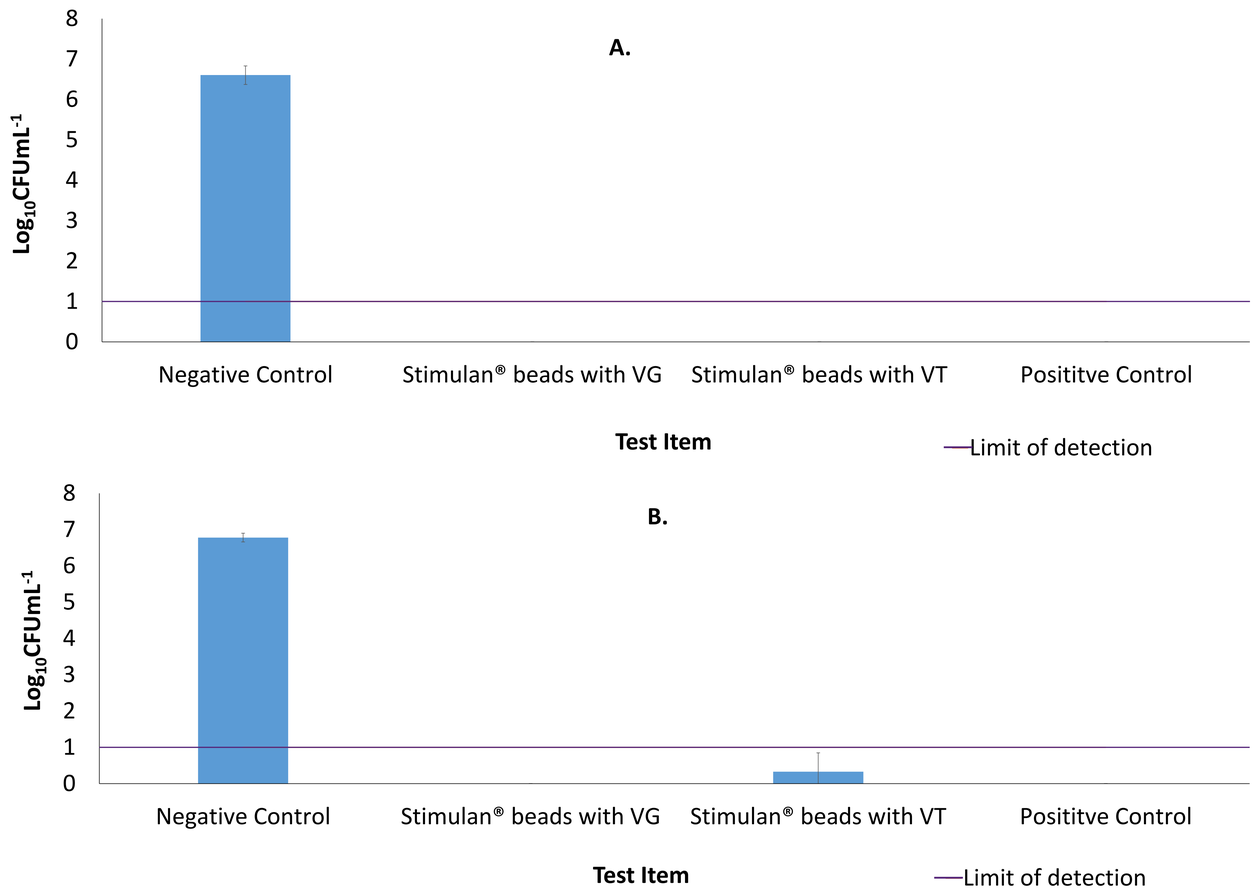


**Figure 2**. Quantity of total viable microorganisms recovered following 24-hour treatment of 72-hour pre-formed *Staphylococcus aureus* (A) and *Pseudomonas aeruginosa* (B) biofilms.

## Discussion

In *vitro* biofilm test methods are more representative of the real-world scenario compared to their planktonic equivalents. Biofilm microorganisms are attached to a surface, have developed under a dynamic environment and reside within extra polymeric substances. Exposure of the biofilm to Stimulan® beads containing a mixture of Vancomycin and Gentamicin or Vancomycin and Tobramycin resulted no viable organisms being recovered from pre-formed biofilms in the test method described. Further testing is required to confirm clinical performance. Eradication of biofilm infections may result in improved patient outcomes and a decrease in healthcare costs. Demonstration of biofilm eradication *in vitro* provides a strong basis for future work to show the potential for effective treatment clinically.

### References:

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