Accredited Biofilm Test Methods: What It Means for the Wound Care Industry

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Introduction

Chronic wounds represent worldwide health issues and biofilms have been shown to colonise the vast majority of chronic wounds. Studies have found that treating biofilms has a beneficial impact on chronic wound healing. Consequently, wound care technologies have become increasingly targeted towards anti-biofilm claims. However, the absence of accredited biofilm test methods makes it difficult to support anti-biofilm claims. The recent addition of accredited biofilm test methods for MBEC, CDC reactor and Drip Flow Reactor (DFR) models for *S. aureus* and *P. aeruginosa*, 24 and 72 hour biofilms is the first step towards standardising biofilm testing methodology. Standardised biofilm test methods allows benchmarking testing of new and existing wound care technology, which has huge importance for the wound care sector as it will help inform medical professionals on the selection of the most appropriate treatment technology, which could reduce the health and financial costs of biofilm infected chronic wounds.

Aim

To validate and gain UKAS accreditation for MBEC, CDC reactor and DFR biofilm test methods.

Method

Perfectus Biomed Ltd. have UKAS accreditation for the MBEC, CDC reactor and DFR test methods under ISO 17025 for 24 hour (early) and 72 hour (mature biofilms) for both *S. aureus* and *P. aeruginosa.* Biofilms assays have all been designed to highlight any differentiation in product efficacy across key biofilm lifecycle stages.

Product treatment times of either 5 minutes at room temperature or 24 hours at 37°C were chosen to reflect environmentally relevant time periods and temperatures for the use of wound care products e.g. a 5 minute contact time could reflect the use of a wound care solution and a 24 hour contact time could reflect the use of a wound care dressing. All accredited biofilm methods involve a series of wash steps prior to treatment with the selected wound care technology. These wash steps ensure that planktonic organisms are removed and that biofilm cells alone are being tested. All assays have been designed such that appropriate negative and positive controls are included and quality checks are performed at each stage of the assay from the preparation of bacterial inoculum, quantification of established biofilm and quantification of expected Log reductions against known antimicrobial agents, with any deviations outside of the expected range of results resulting in the assay being rejected and repeated. All assays are also sampled with 3 biological replicates and 2 technical replicates to produce statistically testable data. All assays have been designed such that data is reported as colony forming units per ml^{-1}, rather than using estimations of biomass from absorbance measurements, which can be affected by the binding of test products.

Figure 1. Photographs of an MBEC device (left), CDC reactor (centre) and Drip Flow reactor (right).

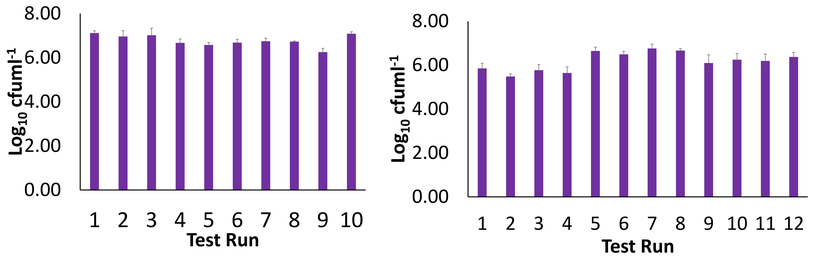

Figure 2. Data spread of *Pseudomonas aeruginosa* biofilms grown using the MBEC reactor model for 24 hours (left) and 72 hours (right).

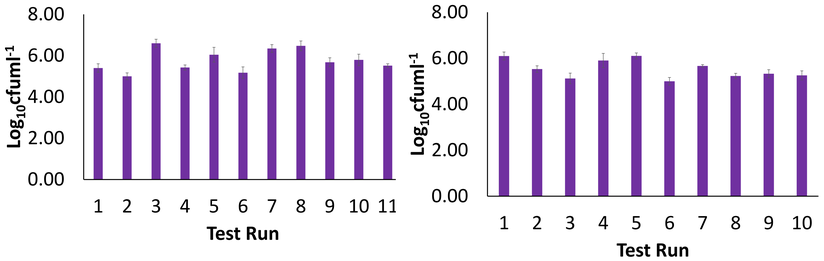

Figure 3. Data spread of *Staphylococcus aureus* biofilms grown using the CDC method for 24 hours (left) and 72 hours (right).

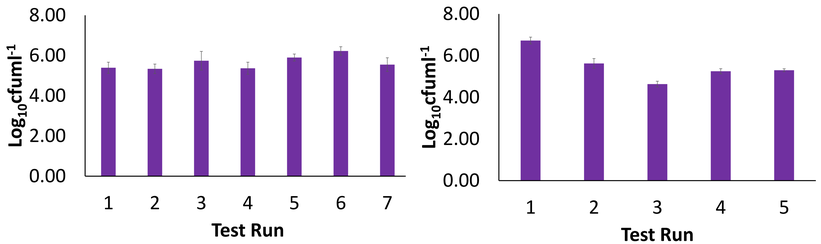

Figure 4. Data spread of *Staphylococcus aureus* biofilms grown using a drip flow device for 24 hours (A) and 72 hours (B).

Results

Each assay resulted in between 5 log and 7 log viable organisms being recovered from *S. aureus* and *P. aeruginosa* biofilms. The ability of a number of antimicrobial agents to breakdown of an established biofilm was determined. The products tested demonstrated a range in their abilities; from no log reduction to the recovery of no viable organisms. It was also found that following repetition of the study by the same operator and different operators all models used were able to grow reproducible biofilms under defined conditions which are suitable for efficacy testing.

Discussion

The importance of developing methods to test new and existing wound care products against biofilms has been made evident by studies analysing biofilm prevalence in diabetic foot ulcers, venous leg ulcers and pressure ulcers, which are health issues worldwide (James et al. 2008). It has been established that biofilms are both widespread in chronic wounds and less susceptible to antibiotic treatment than planktonic organisms. To test whether the removal of biofilms improved wound healing, Wolcott (2015) compared the effectiveness of standard of care, a wound gel that directly interfered with biofilm EPS, and standard of care plus wound gel biofilm and showed that a 50% reduction in wound volume was observed in 53%, 80% and 93% of cases, respectively. This showed that by directly treating biofilm there is an improvement in wound healing, which raises the importance of developing and accrediting assays to test new and existing wound care products against key biofilm forming bacteria.

Conclusions

It is important to have biofilm methods accredited as it gives; (i) an assurance that the test has been carried out to a high standard and the assays produce highly repeatable data between tests, between scientists and between laboratories; (ii) an assurance that study measurements are taken with instruments that have been maintained and calibrated to traceable international standards, and (iii) a quality seal that is recognised internationally.

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