# Advanced Micelle Technology Reduces Biofilms by 99.99%

Robert G Frykberg, DPM, MPH; Russell K. Griggs, M.S., CCRC

## PURPOSE

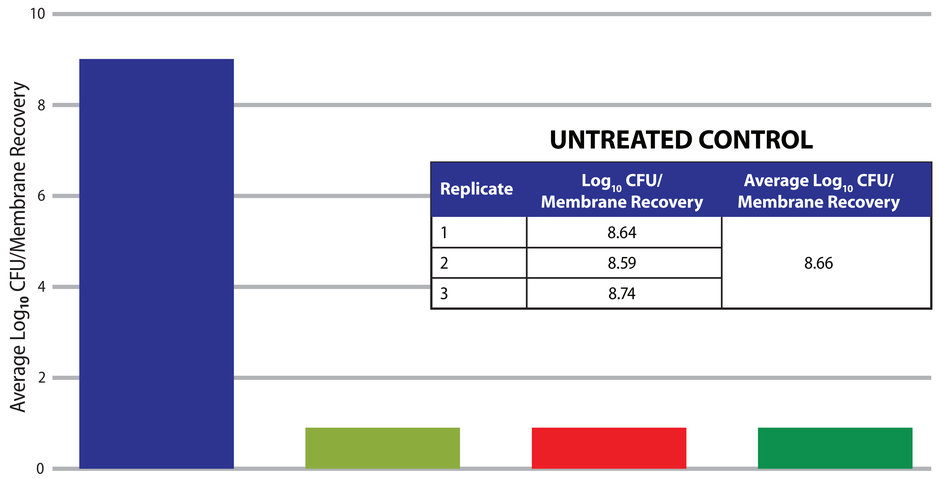
The purpose of this study is to evaluate the ability of three test articles to prevent the formation of *Pseudomonas aeruginosa* biofilm using the Colony/Drip Flow Biofilm Reactor (C/DFBR) methodology. Testing will be performed based upon published modifications [Lipp et al, 2010; DOI 10.12968/jowc.2010.19.6.48468 and Stoffel et al, 2020; DOI: 10.1111.wrr.12806] of ASTM E2647-20, *Standard Test Method for Quantification of a Pseudomonas aeruginosa Biofilm Grown Using Drip Flow Biofilm Reactor with Low Shear and Continuous Flow.*

Testing will be performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58. The characterization of the identity, strength, purity, composition, stability, and solubility of the test articles remains the responsibility of the Sponsor and will not be performed by the Testing Facility (21 CFR Part 58.105).

## SCOPE

Absorbent pads will be mounted onto glass slides, the prepared slides will be placed into a DFBR, and the DFBR sterilized. Sterilized polycarbonate membranes will be placed on top of the absorbent pads and then the top surface of the membranes inoculated with *Pseudomonas aeruginosa*. After a 30-minute air dry, a sterile rubber ring will be placed over the membrane and 1 mL of the test articles dispensed inside of the ring before starting a continuous 5 mL/hour flow of dilute growth media into the DFBR at room temperature. An additional 1 mL of two of the test articles will be dispensed inside of the ring again after 24 and 48 hours of continuous flow. After 72 hours of continuous flow conditions, membranes will be removed, rinsed, and transferred to containers of neutralizing eluent. Biofilm will be extracted by vortexing/sonicating and extracted biofilm samples plated onto agar. Three replicates of each test article will be evaluated with paired untreated control replicates. Mean log10and mean percent reductions attributable to each test article will be calculated relative to paired untreated control replicates.

## CHALLENGE SUSPENSION USED FOR BIOFILM DEVELOPMENT




### UNTREATED CONTROL

| Replicate | $Log_{10}$ CFU/ Membrane Recovery | Average $Log_{10}$ CFU/ Membrane Recovery |
|---|---|---|
| 1 | 8.64 | 8.66 |
| 2 | 8.59 | |
| 3 | 8.74 | |

## STUDY PRELIMINARY RESULTS

### ANTI-BIOFILM HYDROGEL

| Replicate | $Log_{10}$ CFU/ Membrane Recovery | Average $Log_{10}$ CFU/ Membrane Recovery | $Log_{10}$ CFU/Membrane Reduction Relative to Untreated Control | Average $Log_{10}$ CFU/ Membrane Reduction Relative to Untreated Control |
|---|---|---|---|---|
| 1 | 1.60 | 1.60 | 7.05 | 7.05 |
| 2 | 1.60 | | 7.05 | |
| 3 | 1.60 | | 7.05 | |

### ANTI-BIOFILM HYDROGEL & FENTONITE SUSPENSION

| Replicate | $Log_{10}$ CFU/ Membrane Recovery | Average $Log_{10}$ CFU/ Membrane Recovery | $Log_{10}$ CFU/Membrane Reduction Relative to Untreated Control | Average $Log_{10}$ CFU/ Membrane Reduction Relative to Untreated Control |
|---|---|---|---|---|
| 1 | 1.60 | 1.60 | 7.05 | 7.05 |
| 2 | 1.60 | | 7.05 | |
| 3 | 1.60 | | 7.05 | |

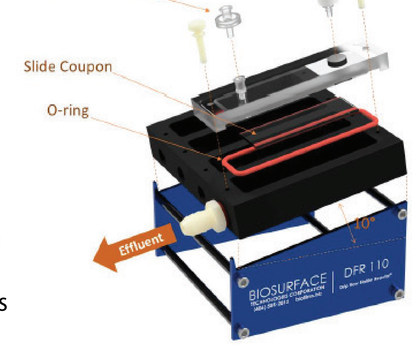
### ANTI-BIOFILM WOUND WASH

| Replicate | $Log_{10}$ CFU/ Membrane Recovery | Average $Log_{10}$ CFU/ Membrane Recovery | $Log_{10}$ CFU/Membrane Reduction Relative to Untreated Control | Average $Log_{10}$ CFU/ Membrane Reduction Relative to Untreated Control |
|---|---|---|---|---|
| 1 | 1.60 | 1.60 | 7.05 | 7.05 |
| 2 | 1.60 | | 7.05 | |
| 3 | 1.60 | | 7.05 | |

## DRIP FLOW BIOFILM REACTOR® TEST METHOD

In this method, a laboratory biofilm is established in batch mode for six hours and is then grown under low shear in continuous flow conditions for 48 hours. Biofilm accumulation is quantified by harvesting the biofilm from coupons of a known surface area, disaggregating the cell clumps and performing viable plate counts.

The DFR consists of a rectangular base (various materials available) held at a 10o angle by an anodized aluminum stand, Figure 1. Four or six separate channels are bored into the base resulting in independent sampling opportunities for each run performed. Each channel has two small pegs to hold the 18.75cm2 (25 x 75 x 1mm) glass coupon in place, a shallow trough that mitigates blockage of the effluent port during sloughing events and aids in coupon removal, and an effluent port which allows the continuous flow media to exit. Each channel also has an alternant influent port that can be used for catheter studies. The covers contain rubber O-rings to form an airtight seal, bacterial air vent gas exchange ports, and a Mininert Valve used for the inlet. The Mininert Valve consists of a rubber septum, into which a needle is placed to deliver the media, and a ported bottom to allow for larger drops of media to form than is possible with the needle alone. The flow of media is the only acting shear force on the biofilm.




## TESTING FACILITY

Nelson Labs® Bozeman
A Sotera Health company

NELSON LABORATORIES BOZEMAN, LLC
1755 South 19th Avenue Bozeman, Montana 59718
STUDY DIRECTOR – Russell K. Griggs, M.S., CCRC