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Drip Flow Reactor Method Exhibits Excellent Reproducibility Based on a 10 Laboratory Collaborative Study

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ABSTRACT (50 word limit)

A standard method for growing *Pseudomonas aeruginosa* biofilm in the Drip Flow Biofilm Reactor was assessed in a 10-laboratory study. The mean log density was 9.29 $Log_{10}(CFU/cm^2)$. The repeatability and reproducibility SDs were equal to 0.22 and 0.24, respectively, providing statistical confidence in data generated by the method.

SHORT COMMUNICATION (1000 words)

Approved standard test methods are verified for the statistical attributes of repeatability, reproducibility, ruggedness and responsiveness. Most scientific publications report the repeatability standard deviation (SD_r) only, with small values indicating that experiments conducted in the same laboratory give a similar outcome (e.g., Log_{10}(CFU) of microorganisms). Some publications also report method responsiveness, an indication of whether the method can distinguish between variable test conditions. For instance, for an antimicrobial efficacy test, the method would give a different response for low versus high concentrations of an antimicrobial. Although knowledge of repeatability and responsiveness is important, reproducibility and ruggedness results are more informative of method performance across multiple laboratories. A method is rugged if small changes to the protocol do not significantly change the outcome. A ruggedness assessment is normally done before a method is proposed as a standard. A method is reproducible if nearly the same result is achieved when the method is implemented in multiple laboratories. A reproducibility standard deviation (SD_R) is calculated from an Interlaboratory Study (ILS), also known as a ring trial or collaborative study, with smaller values indicating better reproducibility.

A standard test method that describes how to grow a *Pseudomonas aeruginosa* ATCC 15442 biofilm under low fluid shear close to the air-liquid interface using the Drip Flow Biofilm Reactor was assessed for reproducibility in a 10-laboratory collaborative study. Preliminary assessments of the method's repeatability and ruggedness are described by Goeres et al (2009) which led to initial standardization of the method with ASTM International as Standard Test Method E2647.The ten participating laboratories were provided a training video, data sheets and study specific instructions that were explained during a training call prior to data collection. The names of the participating laboratories are list in the ASTM Research Report (ASTM International RR: E35-1015). Each laboratory conducted three separate experiments, completed on separate weeks. Four replicate biofilm samples were collected from each experiment. A scatterplot of the data is

shown in Figure 1. From these ILS results, the repeatability and reproducibility SDs of the control log densities were calculated.

A linear mixed effects model was fit to the control log densities (Pinheiro & Bates 2000, Bates et al. 2015) with random effects for lab and experiment. This model provided an estimate of the repeatability and reproducibility SDs. Results of the statistical analysis are in Table 1.

The mean log density was 9.29 $Log_{10}(CFU/cm^2)$ for all 30 experiments from 10 labs which is within 0.07 logs of the original viable biofilm log density of 9.36 reported in Goeres et al 2009. And the repeatability SD of 0.23, based on sampling one coupon, is strikingly similar to the repeatability SD of 0.28 reported in 2009. The reproducibly SD from the ILS was 0.27, with the largest contribution to the variance attributed to within experiment sources. This is explained by the natural heterogeneity of biofilm growth and the importance of using a properly a calibrated pump to ensure that each channel receives the same flow of nutrients.

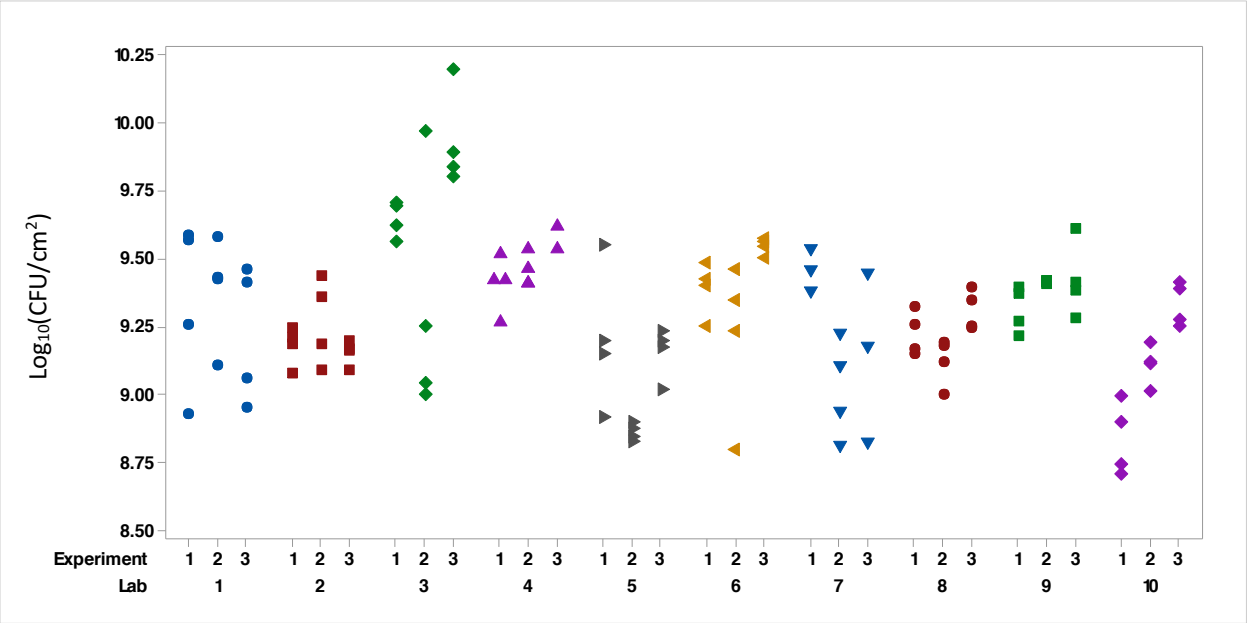

Figure 1. Each point is the log density ($Log_{10}(CFU/cm^2)$) for a biofilm sample harvested from an individual coupon housed in a single channel of the drip flow reactor from a single experiment at a single laboratory. Along the horizontal axis are listed the 10 labs and three experiments done in each laboratory.

Table 1. Summary of the repeatability and reproducibility assessment of biofilms grown in the DFR from a 10-laboratory study.

				Sources of Variability (%)				
No. Lab	**No. Exp.**	**No. Samples**	**Mean LD**	**Within Exp.**	**Among Exp.**	**Among Lab**	**Repeatability SD**	**Reproducibility SD**
10	30	116	9.29	41%	31%	28%	0.2275	0.2682

The 0.27 reproducibility SD demonstrates that if a new lab runs the protocol in a single experiment, then the log density of bacteria in a single resulting biofilm sample is predicted to be 0.27 away from the "true" mean log density. In other words, the Drip Flow Biofilm Reactor produces biofilms

with highly reproducible log densities. This provides confidence in the data reported for this test method and directly addresses the "reproducibility crisis" currently being experienced in other fields of science (Parker et al. 2018).

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