MEMORANDUM

SUBJECT: Demonstration Studies on the Use of a Quantitative Test Method for Evaluating the Performance of Disinfectants on Porous Materials

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Purpose

The Microbiology Laboratory Branch conducted a series of demonstration studies in 2019 – 2020 to determine the feasibility of using quantitative methods to evaluate the effectiveness of antimicrobial agents on selected porous materials. MLB is providing this summary to the Efficacy Branch for consideration to support claims for measuring antimicrobial pesticide efficacy on porous materials.

Study Overview

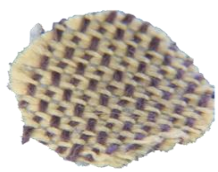
Two methods were developed to account for the differences in growth and recovery of bacteria versus viruses. The two methods use the basic platform of the "Quantitative Test Method for Evaluating Bactericidal and Mycobactericidal Activity of Microbicides Used on Hard, Non-porous Surfaces," modified for porous materials. Both methods use a 1-centimeter diameter disk to represent the porous surface. The microbial inoculum (with a soil load) is applied to the carrier, dried, and the disinfectant is overlaid on top of the dried inoculum. Following a contact time, viable microbes are quantified via recovery using membrane filtration or other recovery processes deemed appropriate for the microbe.

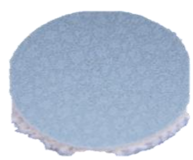
Recovery of the bacteria or virus from the porous material was the key point of the initial investigations. Once it was demonstrated the microorganisms could be recovered at an acceptable level, the laboratory applied citric acid, hydrogen peroxide, quaternary ammonium compound, and/or varying concentrations of sodium hypochlorite to the porous materials to measure the log reduction (LR) in viable microbe. For human coronavirus, only control count data were generated.

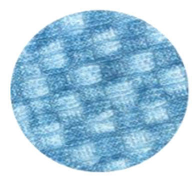
Selection of Representative Porous Materials

The porous materials noted in this summary are representative of materials used in clinical settings – materials typically not suitable for laundering. While there are a variety of materials in the marketplace, these materials were down-selected based on their suitability to be cut into 1-centimeter disks, ease of sterilization, ability to withstand vortexing and sonication, and capability of absorbing the microorganism and antimicrobial treatment. Samples of the materials were obtained directly from the material supplier and were cut into 1-centimeter disks by hand using a standard hole punch.

- Clinical Materials Evaluated:
  - Privacy Curtain Fabric (PCF): 54% Polyester, 46% Fire Resistant (FR) Polyester
  - Non-PVC Fabric (NVF): Polyurethane Face made with Polycarbonate and Polyether Resins, Polyester Backing.
  - Vinyl Seating Fabric (VF): Vinyl Face, Polyester Backing.
  - Duck Canvas (DCF): 100% woven cotton fabric.


Privacy Curtain


Non-PVC


Vinyl


Duck Canvas

Results - Controls

The test microbes were:

- *Pseudomonas aeruginosa* (ATCC # 15442)
- *Staphylococcus aureus* (ATCC # 6538)
- Human coronavirus 229E (ATCC VR-740)

Refer to Table 1 for a summary of the recovery of the three microbes from the four porous materials. Stainless steel carriers were included to represent a hard, non-porous surface.

Table 1. Mean Control Carrier Log Densities from Inoculated Porous Materials (and stainless steel)

Soft/Porous Carrier Type[1]	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	Human coronavirus
PCF	5.23±0.16	4.77±0.18	4.48±0.37
NVF	5.69±0.09	4.67±0.21	4.99±0.20
VF	5.59±0.16	5.69±0.24	5.00±0.14
DCF	4.42±0.28	4.71±0.22	4.01±0.28

[1] PCF = privacy curtain fabric, NVF = non-PVC fabric, VF = vinyl seating fabric, DCF = duck canvas fabric.

Stainless Steel	5.75±0.15	5.63±0.21	4.99±0.23

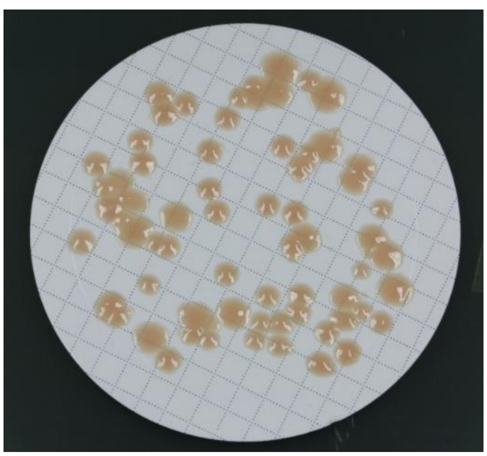
<u>Conclusions</u>. Based on the data in Table 1, adequate control carrier count levels were achieved for the microbe and surface combinations tested. In some instances, the recovery level from the duck canvas (DCF) fell below the desired target of 4.00 logs of viral particles per carrier specified in the study.

<u>Results – Antimicrobial Treatments</u>

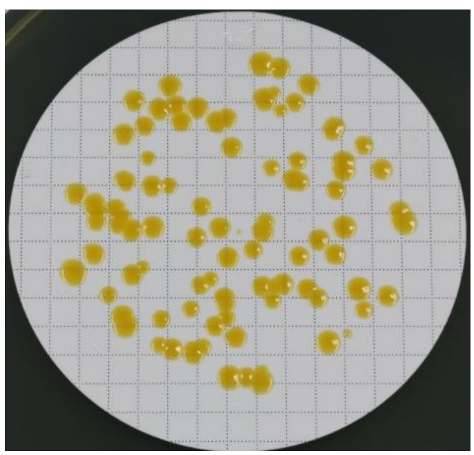
Once it was demonstrated the microbes could be recovered from the porous materials in sufficient numbers to conduct efficacy testing, the lab tested concentrations of sodium hypochlorite and ready-to-use (RTU) treatments against *Pseudomonas aeruginosa* and *Staphylococcus aureus* using the draft method. Two to three replications (independent test days) were conducted for each treatment-by-microbe combination to gain an understanding of variability. Sodium hypochlorite concentrations were selected for both *Pseudomonas aeruginosa* and *Staphylococcus aureus* to generate two distinct levels of log reduction.

The test conditions were: 1) addition of three-part soil load to the test suspension prior to carrier inoculation; 2) 5-minute contact time; and 3) 375 ppm hard water as the treatment diluent. The data summaries are presented by test microbe and treatment type. Stainless steel carriers were used as a test control for comparison purposes. The results are summarized in Figures 1-4.

<u>*Pseudomonas aeruginosa* and *Staphylococcus aureus*</u>



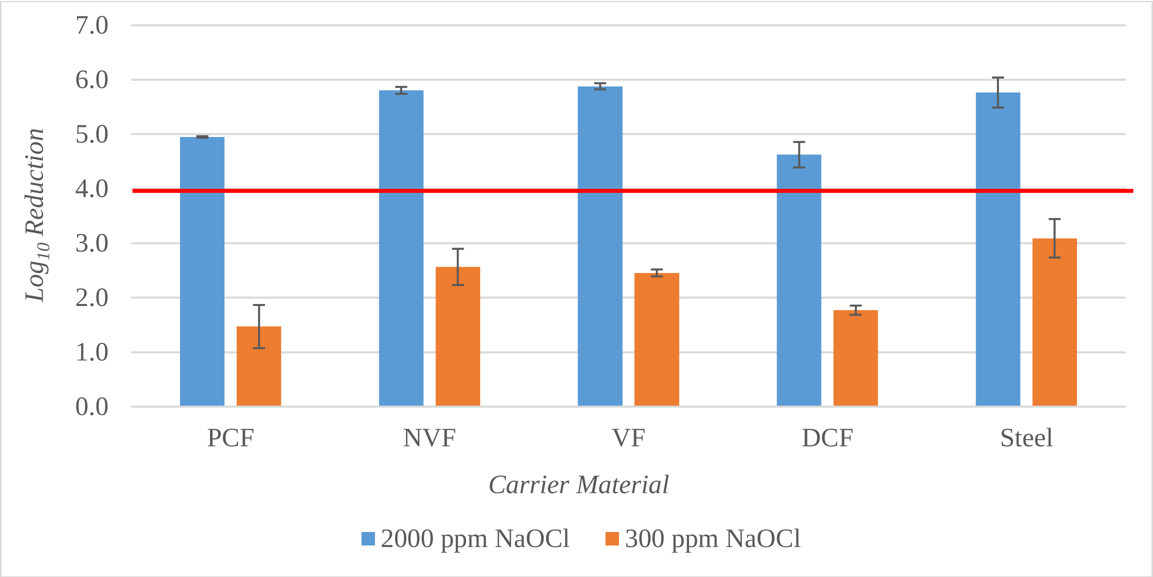
Pseudomonas aeruginosa colonies



Staphylococcus aureus colonies

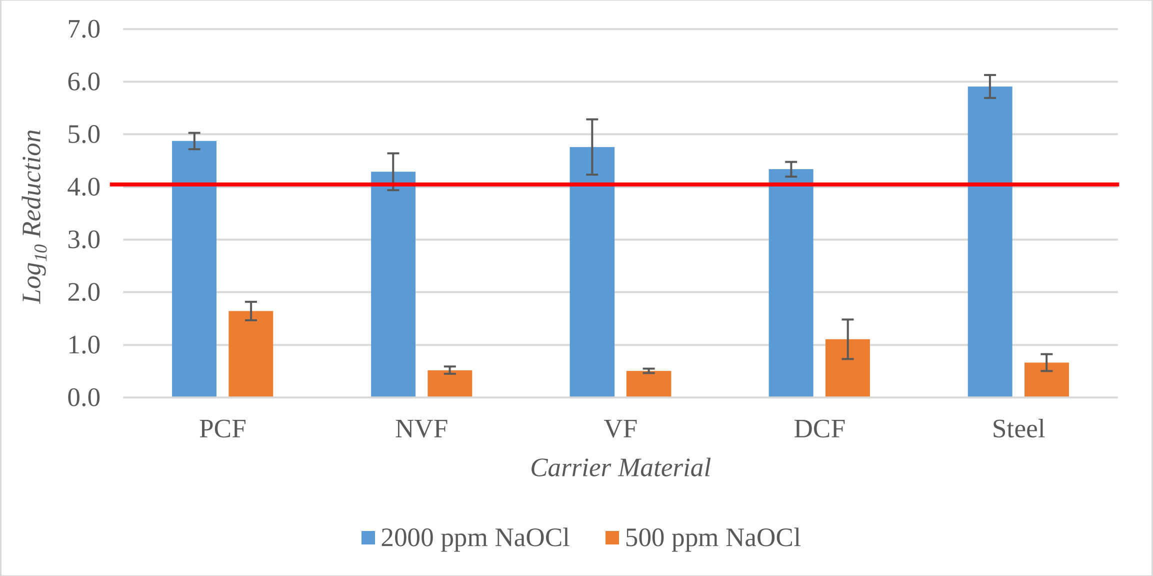
Data for Sodium Hypochlorite:

Figure 1. Summary of the log reduction (with error bars) of *Pseudomonas aeruginosa* when tested against two concentrations of sodium hypochlorite on four porous materials and stainless steel



Red line = proposed performance standard

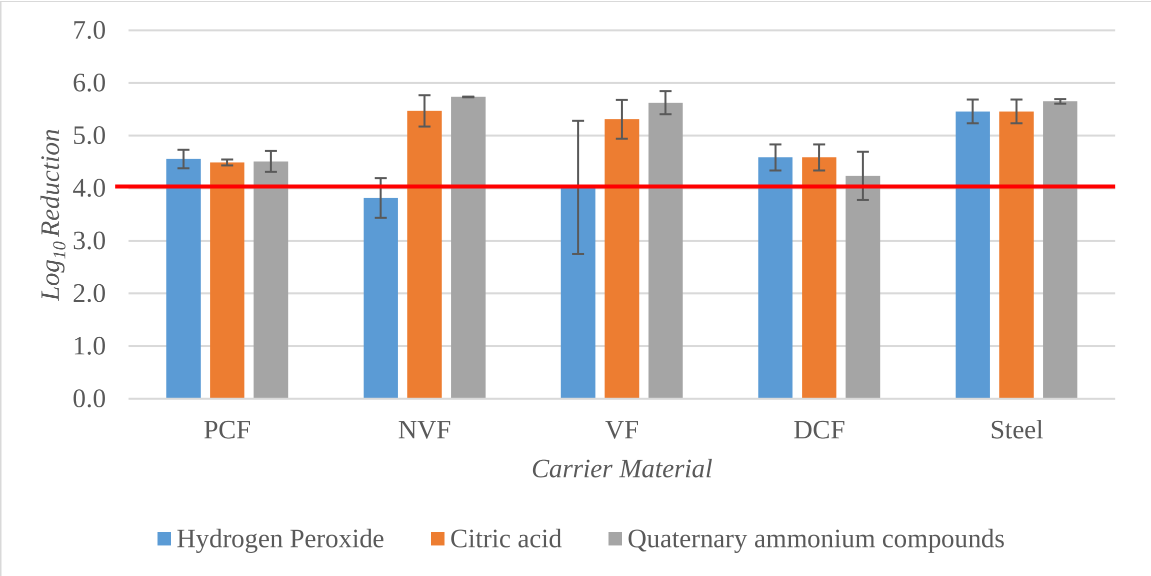
Figure 2. Summary of the log reduction (with error bars) of *Staphylococcus aureus* when tested against two concentrations of sodium hypochlorite on four porous materials and stainless steel



Red line = proposed performance standard

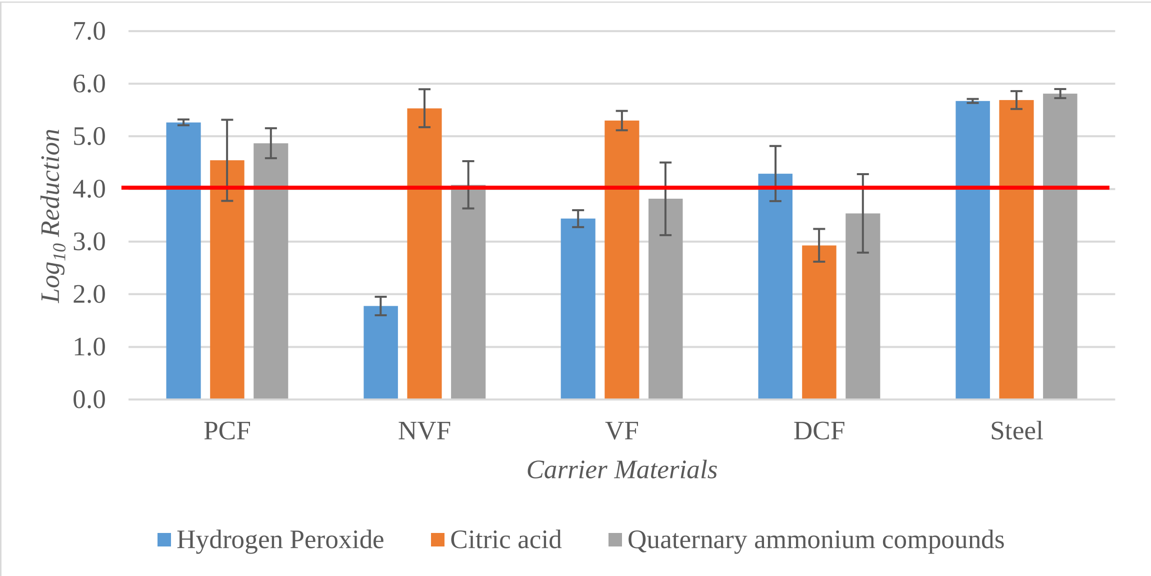
Data for Ready-to Use Treatments:

Figure 3. Summary of the log reduction (with error bars) of *Pseudomonas aeruginosa* when tested against three RTU treatments on four porous materials and stainless steel



Red line = proposed performance standard

Figure 4. Summary of the log reduction (with error bars) of *Staphylococcus aureus* when tested against three RTU treatments on four porous materials and stainless steel



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Conclusions and Recommendations

Based on the data generated from the demonstration studies presented here, the laboratory believes the proposed methods for testing porous materials are feasible and technically sound. The recovery data were deemed fully acceptable, based on the control counts and associated low variability for each of the three porous materials (PCF, NVF, and VF), to provide a strong basis for testing porous materials. The duck canvas fabric (DCF) is not deemed an ideal test material given that recovery sometimes fell below the desired target range and the usage of this material is less common in clinical settings.

The two methods are attached for your consideration. If you have any questions or need additional information, please feel free to contact me.