Importance of Proper Methodology for Evaluating the Efficacy of Chemical-Based Antimicrobial Air Treatment Products Against Microbial Pathogens

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Abstract

Accurately assessing the efficacy of products claiming to inactivate or control airborne microorganisms of public health concern is of paramount importance. This poster focuses on key methodological aspects for evaluating the efficacy of chemical-based antimicrobial air treatment products. By developing and applying testing procedures that align with the intended product application pattern, the test methods will provide a more accurate assessment of a product's effectiveness for killing microbial pathogens, including bacteria, viruses, and fungi.

At the heart of the rationale for the proposed and discussed testing approach lies an understanding of the factors governing the interaction between an antimicrobial agent and target organisms. The efficacy of antimicrobial agents hinges on many variables, including the physical states of both the target microorganism and the antimicrobial agent, their concentrations, and ambient environmental conditions. How a chemical-based air treatment product contacts airborne pathogens and its mode of action are key considerations when designing efficacy studies.

The poster discusses demonstrates why a study that aerosolizes both the test organisms and the air treatment product will provide a better estimate of the antimicrobial efficacy of such products than the traditional surface-testing approach – by exposing coupons plated with test microbes to the test substance. We also report the significantly different results seen in side-by-side testing of several compounds against the same microorganisms on coupons vs. their use in the air against airborne organisms.

Goal

To demonstrate why appropriate methodology is critical when assessing efficacy of inactivating products with different modes of action: airborne vs. surface-borne organisms

Methodology

Test Chamber

Fully enclosed, 2.7m (H) x 2.7m (W) x 2.1(D) 304 stainless steel sealed (Figure 1.0). Test conditions: humidity range of 30% – 40% and a temperature range of 23°C – 26°C.

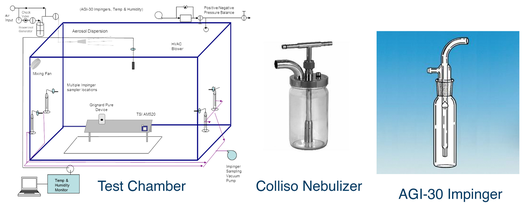

Figure 1. Experimental setup and devices.

Test organisms

Gram-negative bacteria species; Pseudomonas fluorescens (ATCC 13525), Salmonella typhimurium (ATCC 53648), and Klebsiella aerogenes (ATCC 51697); the gram-positive bacteria: methicillin-resistant Staphylococcus epidermidis (ATCC 12228) and Listeria innocua (ATCC 33090); viruses: non-enveloped bacteriophage MS2 (ATCC 15597-B1), enveloped Pseudomonas phage Phi 6 (ATCC 21781-B1); also, Mycobacterium smegmatis (607) and mold Aspergillus brasiliensis (ATCC 16404).

Air Treatment Experiments

Aerosolization of inactivating agent: Grignard Pure and benzalkonium chloride

Utilized in its undiluted form and aerosolized through proprietary vaporization or nebulization devices. Both GP and benzalkonium chloride released at preset intervals to maintain steady concentration.

Test procedure

The procedure is shown in the figure below. Briefly,

- Inactivating agent was released into the air first and continued to be periodically released to maintain its steady concentration.
- 20 min later, microorganism aerosolized for 20 minutes
- Microorganism samples collected using two AGI-30 impingers (Ace Glass Incorporated, Vineland, NJ), each filled with 20mL of sterile phosphate-buffered saline (PBS) enhanced with 0.005% Tween 80 at times t*= 15, 30, 60, and 90 min.
- The chamber was evacuated and decontaminated.
- Same experiments repeated without air treatment
- Efficacy of a particular treatment was determined by comparing microorganism recovery with and without air treatment.

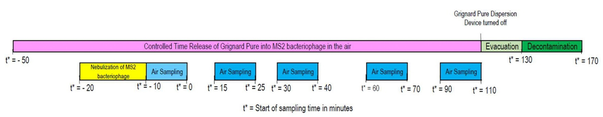

Figure 2. Controlled time release of GP to maintain a set GP concentration.

Surface Treatment Experiments

The methodology for surface testing followed well-established guideline by ASTM E1153: Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces as shown in the diagram below

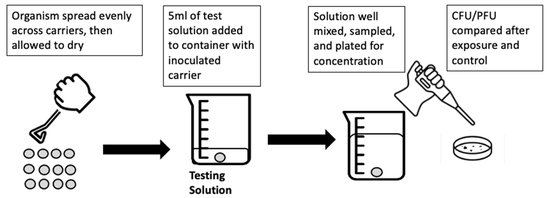

Figure 3. Hard Surface Test method

Settling slide experiments

- Inactivation of airborne microorganisms using Grignard Pure or benzalkonium chloride performed as described above.
- Blank glass slides were placed into the test chamber during air treatment experiment.
- The slides were removed after the experiment and placed in 50ml conical tubes filled with 20ml of PBS.
- Tubes with slides were well shaken and the solution was plated to determine the quantity of viable organisms that settled during the air treatment experiment.
- The same experiment performed without air treatment, and the recovery of settled microorganisms with and without treatment was compared.

Results

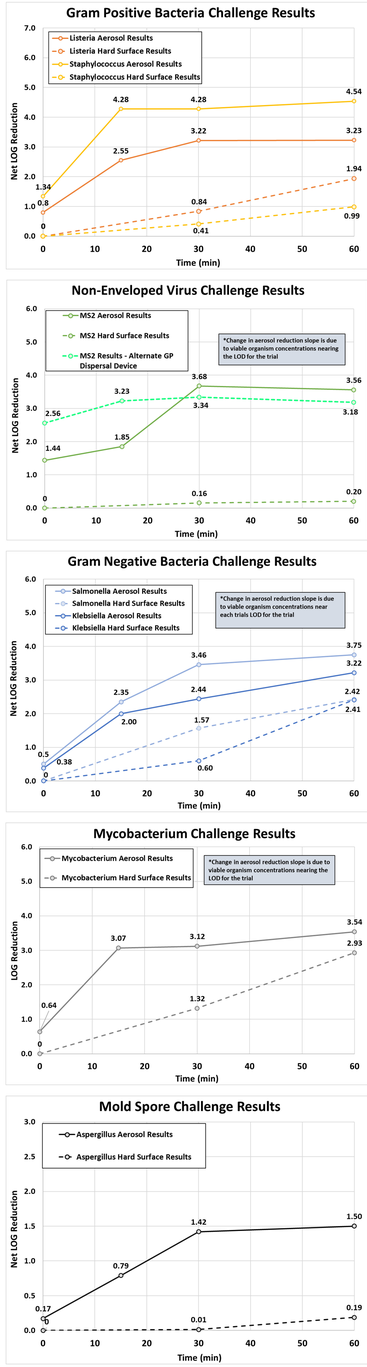

Figure 4. Inactivation of airborne and surface-borne antimicrobial agents by Grignard Pure at different exposure durations

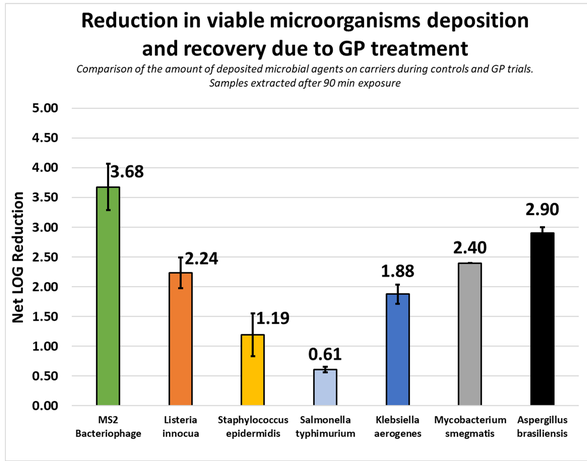

Figure 5. Reduction in viable microorganism deposition and recovery due to GP treatment

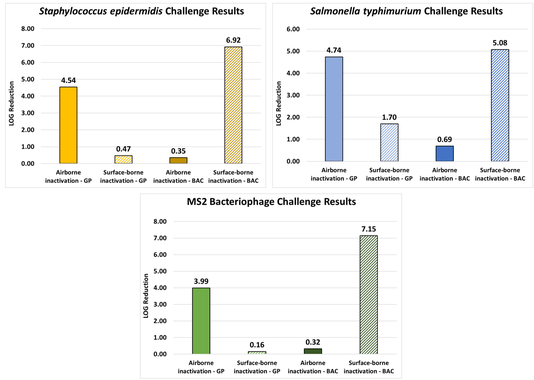

Figure 6. Comparison of airborne and surface-borne inactivation of microbial agents by Grignard Pure and benzalkonium chloride

Data for Grignard Pure are based on 60 min challenge. For benzalkonium chloride, the airborne test was 60 min, but surface test was limit to 5 min because of strong inactivation; however, control test result for benzalkonium chloride is based on 60 min test.

Summary and Conclusion

- The results from the aerosol studies indicated high inactivation efficacy of GP (1.0 – 3.5 Net Logs) in the first 30 minutes across all microbial species.
- For hard surface vs aerosol testing, the results indicated consistent and high efficacy of GP (4.0 – 5.0 gross logs) in all aerosol tests across the three microbial strains, while its performance on hard surfaces was notably poor (0.03 – 0.11 gross logs).
- In contrast, benzalkonium chloride demonstrated excellent performance in the hard surface tests, with substantial log reduction and this difference in performance of these three antimicrobial agents on surfaces versus in the air can be attributed to the distinctly different mechanisms of action associated with their respective active ingredients, as discussed in the previous section.