PROPOSAL

Interim Guidance for the Efficacy Evaluation of Products with Biofilm Claims

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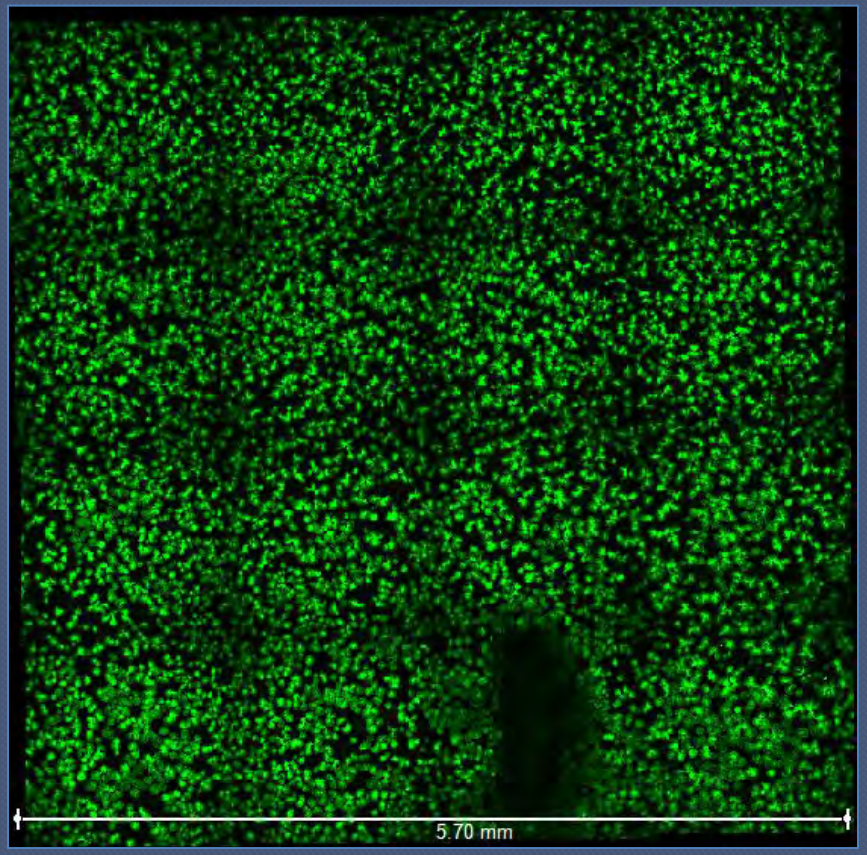
Background

- Biofilm is considered to be a “pest” by the EPA.
- Therefore, any label claim to prevent, destroy, repel or mitigate biofilm on an inanimate environmental surface is a pesticidal claim which requires registration under FIFRA – including product efficacy data.
- Biofilm express unique characteristics, and therefore require unique and relevant test methods for measuring product efficacy.
- The choice of method will dictate the type of label claim.



Scope

- Agency's proposed interim guidance for the efficacy evaluation of antimicrobial pesticides sold as water soluble powders or liquids that are labeled for treating hard non-porous surfaces contaminated with bacterial biofilm.
- Registrants and applicants may propose and submit alternative practices to the Agency for assessment and the Agency will evaluate them for appropriateness on a case-by-case basis.
- This guidance may be updated in the future.

Product Efficacy

- The following quantitative test method may be used to generate efficacy data:
  - ASTM E2871-12: Standard Test Method for Evaluating Disinfectant Efficacy against *Pseudomonas aeruginosa* Biofilm Grown in CDC Biofilm Reactor using Single Tube Method.
- A claim for controlling biofilm may be added to a registered antimicrobial product with an existing claim.

Example 1

- For a limited-spectrum disinfectant claim, the product must pass all of the following efficacy tests:
  - AOAC Use-Dilution Method for *Staphylococcus aureus* (AOAC 955.15) or *Salmonella enterica* (AOAC 955.14) and the
  - ASTM Single Tube Method (ASTM E2871-12) for *P. aeruginosa*

Example 2

- For a broad-spectrum disinfectant claim, the product must pass all of the following efficacy tests:
  - AOAC Use-Dilution Method for *S. aureus* (AOAC 955.15)
  - AOAC Use-Dilution Method for *S. enterica* (AOAC 955.14) and the
  - ASTM Single Tube Method (ASTM E2871-12) for *P. aeruginosa*

Example 3

- For a hospital or healthcare disinfectant claim, the product must pass the all of the following efficacy tests:
  - AOAC Use-Dilution Method for *P. aeruginosa* (AOAC 964.02)
  - AOAC Use-Dilution Method for *S. aureus* (AOAC 955.15) and the
  - ASTM Single Tube Method (ASTM E2871-12) for *P. aeruginosa*
  - ASTM Single Tube Method (ASTM E2871-12) modified for *S. aureus*

Options

- Applicants should consult with the Agency for other formulations prior to developing registration data. The Agency may require submissions of protocols for review.
- Modifications to the test method will be necessary to accommodate other biofilm forming microbes.

Test Strains/Test Cultures

- *Pseudomonas aeruginosa*
  - Produce stock cultures and a mature biofilm of *P. aeruginosa* according to the EPA's Microbiology Laboratory Branch's (MLB) standard operating procedure (SOP) MB-19-02.
    - Note: *P. aeruginosa* (ATCC 15442) may display three colony types.

Test Strains/Test Cultures

- *Staphylococcus aureus*
  - ASTM standards do not currently address the culture of *S. aureus*.
  - Produce a mature *S. aureus* biofilm per EPA MLB SOP MB-19-02 with the following exceptions:
    - *S. aureus* ATCC 6538 is utilized.
    - Biofilm cultures are initiated using a frozen stock culture of *S. aureus* (refer to Attachment 2 of EPA MLB SOP MB-05-11 for preparation of frozen stock cultures)
    - Use tryptic soy broth (30 g/L).
    - Incubate batch phase at 36 ± 1°C with a baffle speed of 60 rpm.
    - Use tryptic soy broth (300 mg/L) as the growth medium for continuous flow operation (CSTR) mode.

Test Parameters

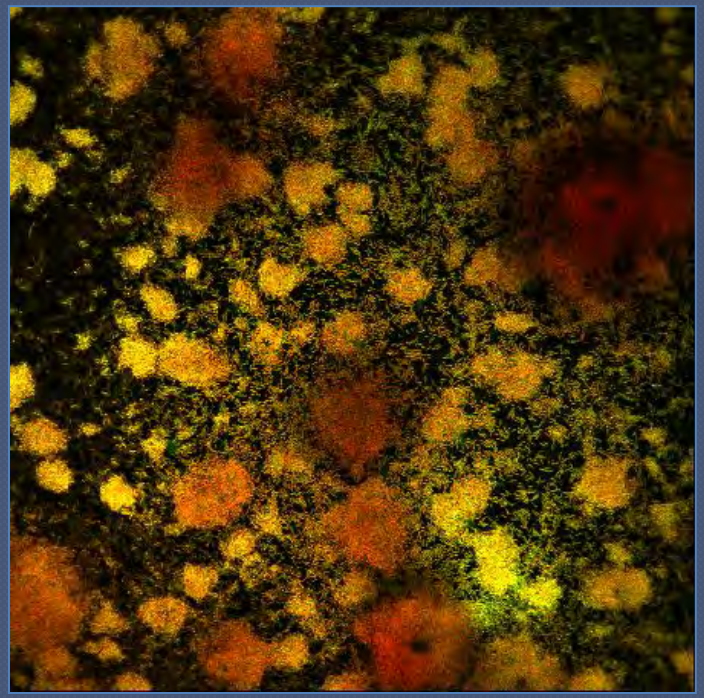
- Product Performance
  - A minimum 5 log reduction in viable bacteria in biofilm is required.
  - The mean log density for carriers inoculated with *P. aeruginosa* or *S. aureus* must be at least 8.0 (corresponding to a geometric mean density of 1.0×10^8).
- Contact Time
  - The contact time for testing should not exceed 10 minutes.

Other Specifics

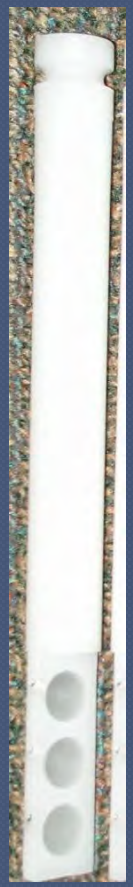
- Carrier Material
  - Borosilicate glass coupons
- Number of Batches
  - Test three batches at the lower certified limit(s) (LCL)
- Test Carriers per Batch
  - Evaluate a minimum of five carriers against the disinfectant and 3 carriers as controls.
- Neutralizer Confirmation
  - Conduct neutralization testing in advance of efficacy testing to determine the appropriate neutralizer for the product.
  - Use MLB SOP MB-20-01 Attachment 1.
  - Submit neutralizer confirmation data along with product efficacy data.

Label Claims

- Examples of Claims:
  - Kills 99.999% of bacteria in biofilm on a hard, non-porous surface
  - Kills a minimum of 99.999% of bacteria in biofilm
  - Reduces at least 99.999% of bacteria growing in biofilm
- Other related claims:
  - Kills biofilm bacteria
  - Controls slime-forming bacteria
  - Specifically designed and formulated to destroy bacteria in biofilm
  - Penetrates biofilm, killing the bacteria living there

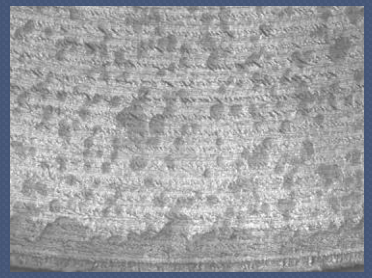


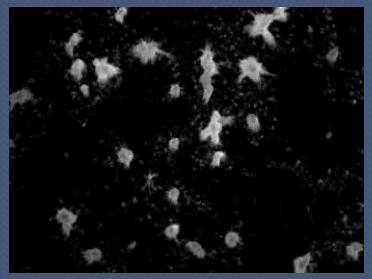
CDC Biofilm Reactor



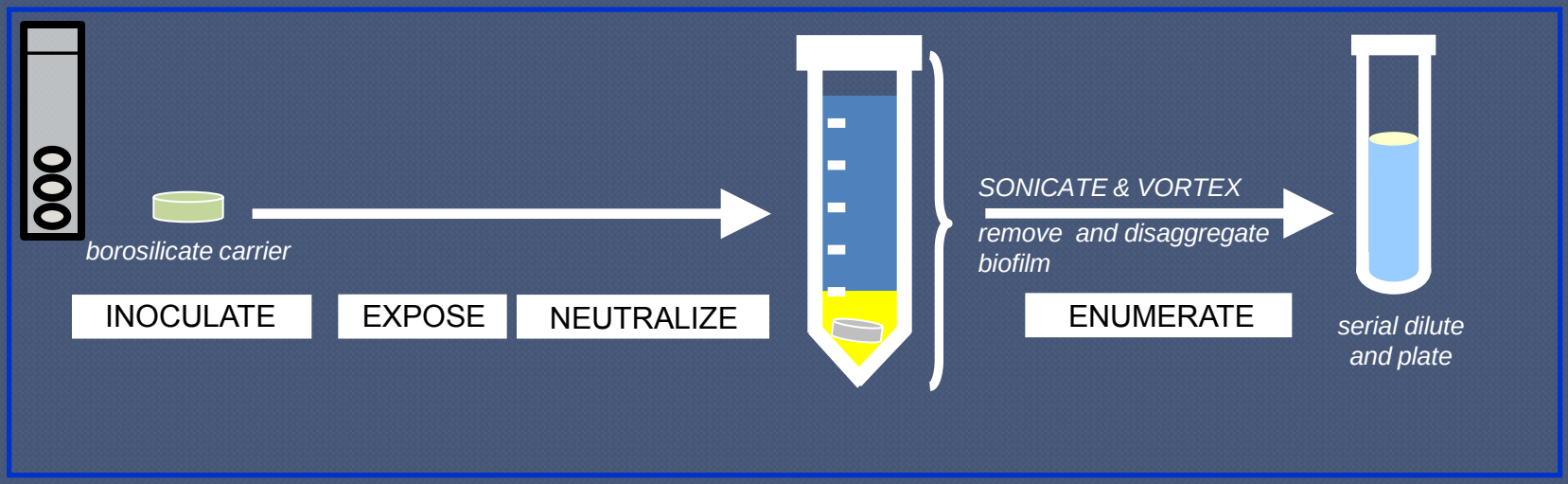
Eight Rods per reactor

Three coupons per rod





Single Tube Method

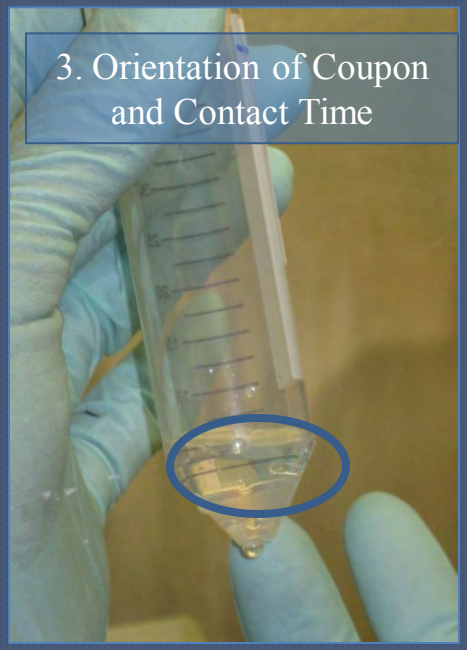


Key Aspects/Steps:

- 4 mL disinfectant
- 36 mL neutralizer
- Variable contact times & concentrations
- 20±1°C treatment temperature

- Vortex 30 s
- Sonicate 30 s
- Repeat twice
- Vortex 30 s

Single Tube Method



Next Steps

- Propose revisions to the ASTM methods; especially for testing of *S. aureus* biofilm or make revisions to MLB's SOPs
- To increase accuracy of the assay:
  - Filter the entire contents of reaction tube containing the carrier
  - Data generated from filtration can be used to assess LRs as high as the control counts in the event of complete kill.
- Pre-screening test chemicals may be necessary to determine the appropriate dilutions required to achieve countable plates
- Work with MSU on method performance criteria
- Finalize guidance and post by September 2014

Acknowledgements

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  - Rebecca Pines – EPA Microbiology Laboratory
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