

US EPA ARCHIVE DOCUMENT



OECD Quantitative Method

Ongoing Activities (focus on bactericidal)

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Disclaimer

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Areas of Interest

- ◉ Based on the outcome of the 2012 collaborative study, MLB has conducted a series of demonstration/feasibility studies to enhance the method's ease-of-use and overall performance:
 - Increasing the mean control carrier count range
 - Use of Tryptic Soy Broth for culturing *Pseudomonas aeruginosa*
 - Use of membrane filtration and direct plating

Mean Control Counts

- ◉ Adjust control count range 4.5-5.5 to 5.0-6.0 logs/carrier
 - Increasing the bacterial challenge to provide a 1 log difference between the minimum control counts and the anticipated performance standard.
 - This increase may further improve method performance.
- ◉ Two preparations of carriers (at 2 inoculation levels) evaluated side-by-side per test day using two non-efficacious treatments in a replicated study.
 - Based on MLB's study, the resulting LRs are not impacted by the higher carrier counts.

Synthetic Broth and Tryptic Soy Broth

- ⦿ There is only one available source of synthetic broth (HiMedia).
- ⦿ Tryptic soy broth is already used for *S. aureus* in the OECD method.
- ⦿ Two preparations of carriers (from different growth media) were evaluated side-by-side per test day using 2 non-efficacious treatments in a replicated study.
 - Based on MLB's study, *P. aeruginosa* grown in SB and TSB yielded consistent and comparable control log densities and treated log reductions.

Membrane Filtration and Direct Plating

- Direct plating saves time and resources
- MLB evaluated *P. aeruginosa* and *S. aureus* using two non-efficacious treatments in a replicated study
- Mean control carrier counts were the same for direct plating and filtration
- Direct plating resulted in *slightly* higher recovery for treated and control carriers; however, use of filtration for assessing the entire content of a vial (10^0) is beneficial.

Recommendations

- Evaluate four treated and three control carriers
- Consider use of a suspension-based test for neutralizer confirmation assay
- Increase control carrier count range to 5.0-6.0
- Use TSB (undiluted) to grow *P. aeruginosa*
- Direct plate control carrier counts
- Filter entire contents of vial associated with treated carrier
 - Direct plate from any dilutions prepared in duplicate

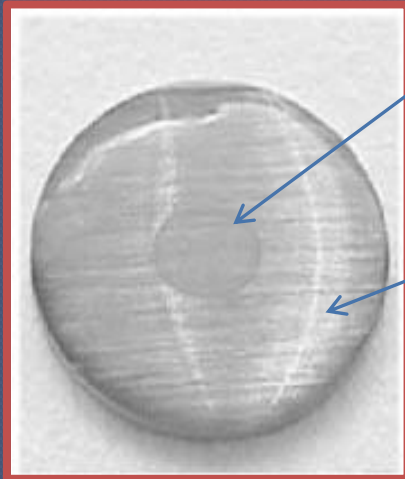
Next Steps

- ⦿ Consolidate the proposed revisions in MLB's SOP and conduct a small-scale study in 2014
 - Request volunteer laboratories (3)
 - EPA will lead the study
- ⦿ Study Aspects
 - Use data from the 2012 collaborative to prioritize treatments
 - *S. aureus* and *P. aeruginosa*
 - Control counts
 - LR for reference standard
 - LR for lower efficacy treatments
- ⦿ Based on outcome, update MLB SOP and ultimately the OECD Guidance Document

Next Steps

- ◉ Formation of the Stakeholder Working Group and the OECD Technical Committee
- ◉ Transition with less focus on revisions and more on implementation, including best practices for use of the method (testing criteria)
 - Limited revisions to the Test Guidelines – bactericidal first
- ◉ Resolve remaining issues with *M. terrae*
- ◉ Conduct virus collaborative
- ◉ Conduct demonstration studies on fungi

Questions/Comments



10 µL dried
inoculum

50 µL
disinfectant

1 cm brushed
stainless steel disk



Vial with
inoculated
carrier



Count colonies and
determine log density
for control and treated
carriers.