

US EPA ARCHIVE DOCUMENT

Antimicrobial Efficacy Test Methods Workshop Highlights from Workshop Sessions DAY 2: February 19, 2014 – Antimicrobial Test Methods		
Method/Activity	Aspect	Comments/Recommendations
Opening Remarks	Laboratory SOPs	- MLB SOPs are posted at http://www.epa.gov/pesticides/methods/atmpa2z.htm. Action Item: Participants to review MLB SOPs and provide any feedback on SOPs.
AOAC Use Dilution Method Performance Standard		- Changes to performance standard are posted on http://www.epa.gov/oppad001/umd_performance_standard_revision_document.pdf. - Recommendation: Use the latest version of the AOAC methods to generate data for GLP submissions. EPA reiterated the need to update SOPs accordingly. Action Item: Participants to provide feedback on issues associated with changes to the performance standard. Action Item: CSPA to poll members regarding necessity of bioscreening stainless steel penicylinders. Action Item: CSPA to poll members for average rate of contamination per set of carriers. How would members define contaminated carriers (e.g., 1/60 positive carriers, growth due to contaminant?) - Comment: Will contamination in a test system be permitted for registrant submitted efficacy data? MLB expressed concerns about allowing contamination due to the impact on the number of valid carriers in a test (e.g., 59). There is a history of EPA allowing a single contaminated tube.
Antimicrobial Testing Program	Updates	- ATP website for user community was recently updated (2/10/14). - Contact for errors on the site or concerns over product representation: Tracy Lantz (Lantz.tracy@epa.gov; 703-308-6415)
Method/Activity	Aspect	Laboratory Sessions
OECD Method/virology (BEAD/MLB SOP MB-25-02)	Drying time and method	- EPA provided an overview of current method development on the use of the OECD method and TCID₅₀ for testing viruses; currently using the feline calicivirus as a surrogate. Action Item: Participants to provide feedback on best practices for drying inoculated carriers
	Freezing virus	Action Item: Participants to provide feedback on best practices for freezing/storing

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		virus stocks, including: ▪ Volume in vials ▪ % serum added to virus ▪ PEG extraction for purification
	Potential revisions	• Action Item: Participants to provide feedback on best practices, including: ▪ Is adsorption needed for FCV? ▪ Is scraping and filtering needed for virus collection?
	Calculation of virus titer	• Reed and Muench vs. Spearman-Kärber calculators; which approach is most accurate/useful? • Action Item: EPA will seek input on use of both calculation methods.
	Testing other viruses	• BEAD/MLB draft SOP is specific to FCV/CRFK. Industry is interested in testing other viruses using the OECD method and requests that MLB's SOP be more general or include more viruses. • EPA stated that the FCV/CRFK model will be used for the upcoming collaborative study due to the high order in the microbial hierarchy and current acceptance by EPA as a surrogate for Norwalk virus.
	Handouts	• Action Item: EPA to provide handouts available to participants. • Action Item: EPA announced plans on conducting a collaborative study on the OECD method for viruses in late 2014. Volunteer labs (3-4) will be required for the study.
Biofilm – ASTM E2562-12 (Standard Test Method for Quantification of <i>Pseudomonas aeruginosa</i> Biofilm Grown with High Shear)	Carriers	• EPA discussed and demonstrated the use of the ASTM Single Tube Method for the evaluation of products against bacterial biofilm. • Comment: How do you know if all biofilm is removed from coupon? ▪ Action Item – EPA will consider adding step to the SOP and the standard to stain coupons with crystal violet and view the surface microscopically. It is unclear how this information will be used.
	<i>Staphylococcus</i> work	• EPA explained that the primary focus has been on <i>P. aeruginosa</i>, although initial work has been completed with <i>S. aureus</i>. The results look promising for <i>S. aureus</i> control counts. <i>S. aureus</i> requires greater concentration of nutrients than <i>P.</i>

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and Continuous Flow Using CDC Biofilm Reactor) and ASTM E2871-12 (Single Tube Method)		<i>aeruginosa</i>, and must be incubated at 36°C, not room temperature. Action Item: EPA will revise the SOP MB-20 to include <i>S. aureus</i> and will initiate revisions to the standard(s) once the necessary data have been generated.
	<i>Pseudomonas aeruginosa</i>	Multiple (3) colony phenotypes exist, and each should be included in the test culture; do not select for colony type.
	Claims/ Decision on log reduction	- EPA proposed a 99.999% reduction in viable bacteria for the claim - Comments: How was the 5 log reduction selected? Is this too high?
	Limit of detection	EPA explained that a substitution rule was used in its demonstration tests (e.g., 0.5 substituted for 0 CFU recovered at the 10⁻¹ dilution and that this approach may potentially under-estimate log reduction, thus is proposing to filter contents of the primary vial.
	Units	CFU/carrier is the unit preferred by EPA, not CFU/cm².
	Filters	The method calls for PES membrane (0.45 µm). If the neutralizer has catalase, filtration may be problematic.
	Neutralizer	Neutralization is typically achieved by the use of 36 mL, but some products may require additional volume (adjust tube size to allow for neutralizer volume).
	MLB SOP MB-20	Action Item: Will reflect version 13 of ASTM Standard, precision/bias statement, in the next update of MB-20.
	Possible Modifications	- Filter contents of primary tube (10⁰ tube) to increase method sensitivity - Modify the culture prep procedure; eliminate shaking step (if possible) - Standardize incubation timeframe (24±2hrs) - Add <i>Staphylococcus</i> to MLB's SOPs
ASTM E2896-12 (Quantitative Petri Plate Method [QPM])	Handouts	Action Item: Make handouts available to participants.
	Modifications to ASTM method	EPA will submit revisions to the standard to ASTM for balloting.
	Wiping/ scraping glass	Important to use consistent pressure, from test to test, during wiping of carrier and scraping during neutralization step.

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	Petri dish	When scraping, avoid splashing.
	Time recording for contact	Action Item: EPA to provide a copy of a timing sheet for the method to demonstrate time recording for wiping/neutralization of carrier (e.g., end wipe at 6 sec, neutralize at 36 sec [timer time]; to demonstrate that time recorded on test sheets includes wiping time).
	Towelettes with two different types surfaces	- Some towelettes have a smooth surface and a rougher surface. - Which side is used for the efficacy evaluation? - Recommendation: Follow label directions (if label specifies side for disinfection use) or consider selecting smoother side for wiping. - Recommendation: Record on paperwork the side that was used for wiping.
	Pre-clean step?	- Revised QPM will include mandatory three-part soil load. Action Item: EPA will address if a pre-clean claim will be permitted even though products will be evaluated with soil load?
Method/Activity	Aspect	Comments/Recommendations
AOAC Germicidal Spray Test and Tuberculocidal Test Methods	Method revisions	- EPA presented the significant revisions to both methods over the 2012-2013 timeframe. - EPA indicated that several footnotes are present in the MLB SOP-07 (TB test) which point out procedures not found in the standard method – the steps were agreed to at the last workshop (2012), but the AOAC standard has not been revised. - EPA encouraged users to revise their SOPs to be current with the most recent version of the method, and continue to use/cite EPA SOPs.
Proposed Biofilm Guidance	Proposed guidance for new claims	EPA proposal on new biofilm claims was provided. The basic information is presented in the associated ppt presentation.
Collaborative Study on Agitated Versus Static TB Cultures	Study Overview	Background, study plan and timeline provided – see associated ppt presentation.
Bactericidal –		EPA provided a list of proposed revisions to the method and stressed the need to

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Ongoing Efforts		focus on method implementation. • Action Item: EPA will work with stakeholders for conducting a limited amount of verification testing.
Mycobactericidal-Collaborative Study Results	Protocol revision	• EPA reviewed the findings of the 2013 collaborative study involving the OECD method and <i>M. terrae</i>. • Method continues to look very promising; however, revisions are in order. Preparation of stock and test cultures deemed problematic. • Action Item: EPA to make first effort at revising protocol for growing <i>M. terrae</i> stock and test cultures. Will take into account recommendations made by stakeholders (see next category below). ▪ Plan to revise in May timeframe and report back to work group shortly thereafter. • Action Item: EPA will request feedback from participants on changes.
Industry Presentation: TB Collaborative: Challenges, Resolutions, and Recommendations by the EWG	Recommendations	• Stakeholder representatives (from Lonza and Stepan) provided feedback and options to consider for optimizing the growth and harvest of <i>M. terrae</i> as well as conducting the efficacy test. ▪ For example, using sterile bags to incubate plates individually to cut down on contamination, using forced circulation incubators for better temperature regulation, and covering tops of filter units with foil to reduce potential for contamination.
Viruses-Ongoing Efforts and Plans for a Collaborative Study	Collaborative study	• EPA explained the strategy for developing the OECD method with viruses. • Action Item: EPA to launch collaborative study in late 2014 timeframe. See associated ppt presentation. ▪ EPA seeks 3 to 4 volunteer labs.
Fungi – Future Efforts	Test guidelines	• Action Item: EPA will seek stakeholder feedback on the OECD test guidelines for generating the conidial suspension and other associated aspects of testing fungi. EPA requests 3-4 stakeholders to verify the procedures in the lab.
	Test organism	• Action Item: EPA will determine if <i>Aspergillus</i> is suitable for product claims.

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OECD Validation Management Group – Maintenance of OECD Guidance Documents and Harmonizing Test Conditions	New U.S. working group	- EPA and OECD Validation Management Group member (Diane Falbo) provided background on the path forward to fulfill goal of harmonization. Two workgroups will be formed; one to work through the technical revisions and the other will function as a steering committee under OECD. Action Item: Stakeholders to create new U.S. working group to facilitate the need for additional revisions to the method. - Need 8-10 participants. - If interested, contact Brigid Klein of CSPA at bklein@cspa.org.
Method/Activity	Aspect	Comments/Recommendations
ASTM 2896-12 QPM	<i>C. difficile</i> Collaborative study	- EPA announced plans to conduct collaborative study involving <i>C. difficile</i> in 2014. Action Item: EPA seeking feedback on product selection for low/high treatments in collaborative study. - Low/inefficacious treatment – Can industry provide towelettes products with no active ingredient (impregnated with all inactive ingredients but not the active)? - How to send/mail the low treatment/high treatment towelettes to collaborators and maintain a blinded study? Store in unmarked containers? Need to consider importance of dispenser orifice. - EPA will seek 5-8 volunteer labs to conduct the collaborative.
General	Upcoming collaborative studies	Action Item: EPA to develop and disseminate a table summarizing all planned collaborative studies. See table below.
		Action Item: EPA to post pdf versions of presentations. - Make link available to participants via CSPA.