ASTM Quantitative Petri plate Method (ASTM E 2896-12): Status, Research and Plans for Collaborative Study

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Topics

- Collaborative Study using QPM (ASTM E2896-12)
  - Status of QPM at ASTM
  - Proposed Modifications to QPM (focus on those that impact *C. difficile*)
  - Method Performance Evaluation
  - Collaborative Study Plan for *C. difficile*
  - Performance Indicators
- New Research Initiatives
- Acknowledgements

Status of QPM at ASTM

- 2011, April: Presented at the ASTM Spring Meeting/Assigned Work Item #32908
  -Formed Work Group (WG)~24 members
- 2011, May: Sent out proposed method to WG
  -Updated the method after incorporating comments/suggestions
- 2011, Nov: Method was 1st demonstrated at an EPA workshop
- 2012, Jan: 1st round balloting through ASTM Subcommittee E35.15
- 2012, Sep: 2nd round balloting through ASTM Main Committee E35
- 2012, Dec: ASTM published the Standard Test Method, E2896-12
- 2013, Oct: Proposed modifications to E2896-12 at the Fall Meeting
  -Formed Work Group~14 members
- 2013, Dec: Sent out revised method to WG
- 2014, Feb: After addressing comments/suggestions, method being updated along with 1-lab based data to depict repeatability.
- 2014, April: Launch E35.15 Subcommittee balloting

Collaborative Study - QPM

- QPM
  - Quantitative method
  - Product efficacy is considered a combination of mechanical removal and chemical inactivation.
  - With the proposed revisions, the method may be used to evaluate the sporicidal efficacy of antimicrobial towelettes in treating hard non-porous inanimate surfaces contaminated with spores of *C. difficile*.
  - Modifications to the method will be included in the collaborative study – see next section for proposed revisions

Proposed Modifications

Current Methodology	Proposed Modifications
1. Scope: Test Organisms: Pa, Sa, and Se	**1. Scope: Test Organisms:** Pa, Sa, Se and spores of *C. difficile* (ATCC 43598)
2. Referenced Documents:	**2. Referenced Documents:** Added 3 ASTM Methods
5. Significance and Use Soil load optional	**5. Significance and Use** A 3 part soil load to be added to inoculum (as per ASTM E2197-11)
6. Apparatus	**6. Apparatus** Added anaerobic chamber/jar and phase contrast microscope

Proposed Modifications (cont.)

Current Methodology	Proposed Modifications
7. Media and Reagents **Subculture media for daily transfer/Test Culture:** 1. Use of Synthetic broth (SB) for both daily and test cultures.	**7. Media and Reagents** **Subculture media for Test Cultue:** 1. Use of Tryptic Soy Broth (TSB) for both test cultures. -Will be used for all vegetative microbes.
Amount of culture added to subculture media: 1. 100 µL added from cryovial into 10 mL SB for daily cultures. 2. 100 µL added from daily cultures into 10 mL synthetic broth for test cultures.	**Amount of culture added to subculture media:** 1. 100 µL added from cryovial into 10 mL TSB for test cultures, and incubated for 18-24 h.
Recovery media TSA	**Recovery media** TSA and BHIY-HT (for C. diff)

Proposed Modifications (cont.)

Current Methodology	Proposed Modifications
8. Generation of Frozen Stock Culture See Text for vegetative bacteria	**8. Generation of Frozen Stock Culture** See Text for vegetative bacteria See ASTM E2839-11 or ASTM E2895-13 for *C. difficile* spore preparation
9. Organic soil: 1. No specific indication of what soil to use (FBS, HS, Heat-inactivated FBS used as examples).	**9. Soil load: See ASTM E2197-11** 1. All test cultures will be prepared using the three part soil from the QCT-2 Method. a) The ratio is: i. Mucin = 100 µL ii. BSA = 25 µL iii. Yeast = 35 µL iv. Test culture = 340 µL

Proposed Modifications (cont.)

Current Methodology	Proposed Modifications
11. Carrier Inoculation **Carrier drying:** 1. Carriers are dried at 36 ± 1°C in an incubator for 30-40 minutes or until visibly dry. a) The lids are placed on the Petri dishes during the drying process.	**11. Carrier Inoculation** **Carrier drying:** 1. Carriers are dried at 36 ± 1°C in an incubator for **30 ± 2 min.** **a) The lids of the Petri dishes are left ajar.**
12. Carrier load enumeration (control carrier counts): 1. Prepare serial dilutions and enumerate inoculum using membrane filtration. a) One mL of the sample dilution is filtered.	**12. Carrier load enumeration (control carrier counts):** 1. Prepare serial dilutions and enumerate inoculum using membrane filtration. **a) Entire sample from dilution tube is filtered.**

Proposed Modifications (cont.)

Current Methodology	Proposed Modifications
13. Wiping Procedure: 1. Three revolutions from outer margin of plate inward toward inoculated area. **Lift the towelette form plate and invert the last fold to expose an unused area (fold) of the wipe. Position the inverted towelette at the center of plate and** perform three circular revolutions form center to outer margin of the plate. without lifting the towelette or inverting the towelette, three additional revolutions are conducted from center to outer margin of the plate.	**13. Wiping Procedure:** 1. Three revolutions from outer margin of plate inward toward inoculated area in the center, and **without lifting the towelette**, continue three additional revolutions from center to outer margin of the plate.

Proposed Modifications (cont.)

Current Methodology	Proposed Modifications
14. Calculations: Current Formula:	**14. Calculations: Proposed Formula:**
$$\frac{(\text{CFU for } 10^{-1}) + (\text{CFU for } 10^{-2}) + (\text{CFU for } 10^{-3})}{10^{-1} + 10^{-2} + 10^{-3}}$$	$$\frac{\text{CFU for } 10^{-x}) + (\text{CFU for } 10^{-Y}) + (\text{CFU for } 10^{-Z})}{(a \times 10^{-x}) + (b \times 10^{-Y}) + (c \times 10^{-Z})} \times D$$ **where 10^{-x}, 10^{-y}, and 10^{-z} are the dilutions filtered, "a" "b" and "c" are the volumes filtered at each dilution (typically 9 or 10 mL), and "D" is the volume of medium originally in the vial with the carrier (39 or 40 mL). (An example is provided in response to a recommendation)**

Proposed Modifications (cont.)

Current Methodology	Proposed Modifications
Appendix: Neutralization Verification Assay: The LD counts in the *Neutralizer Effectiveness Control* and *Neutralizer Toxicity Control* should be within 1.0 log as compared to the LD in the *Organism Titer Control*.	**Appendix: Neutralization Verification Assay:** The LD counts in the *Neutralizer Effectiveness Control* and *Neutralizer Toxicity Control* should be within 0.5 log as compared to the LD in the *Organism Titer Control*.

Collaborative Study

- Goals
  - A collaborative study will be conducted on the revised QPM to address ASTM precision and bias requirements (follow MLB SOP MB-32)
  - Several method performance attributes will be assessed
- Tentative launch date: June/July 2014
- Familiarization of QPM for *C. difficile* is a component of this workshop
- Anticipate that data collection and reporting will be completed within 8 weeks of the launch date
- Data will be statistically analyzed
- Outcome will be reported back to ASTM E35.15 Subcommittee

Study Plan

- EPA (lead lab) plus 3 laboratories
- Test microbe: *C. difficile* (ATCC 43598)
  - see MLB SOP-MB-28 for the spore suspension preparation of *C. difficile* (see also ASTM E2839-11 or ASTM E2895-13)
  - Proposed control carrier counts: $\geq 10^6$ spores/carrier (desired 6.5 to 7.5 log/carrier)
- Randomized order of treatments (4 products)
- Three independent replications per treatment
- Test conditions (contact time, neutralizers) must be finalized
- Documentation consistent with EPA GLPs

Study Plan (cont.)

- A range of product actives will be included.
  - Presumed levels of efficacy ranging from high [sporicidal] to low [non-sporicidal]
  - Products with known sporicidal efficacy will be included
- Control plates
- Test conditions – various contact times, neutralizers, etc.
- Each participating lab will test all towelette products
- Preparatory steps necessary (e.g., spore suspension meeting all criteria) prior to product testing

Study Plan (cont.)

- Step 1: Pre-collaborative
  - Training/practice
  - Prepare spore prep
  - Achieve proposed control counts
- Step 2: Neutralizer Verification Assay (as per ASTM E2896)
  - Each lab will perform the assay on one of the products tested
- Step 3: Responsiveness Measurement Control
  - Two concentrations (high and low efficacy) of selected reference standard/towelette tested in a side-by-side fashion
- Step 4: Product Testing (for method performance evaluation)
  - Selected towelette products
  - One production lot per product
  - Three replications for each product

Study Plan (cont.)

- Five treated carriers, three control carriers
  - Example: Test four towelettes (products) per day (20 total treated carriers) against spores of *C. difficile*
  - One set of untreated control carriers per test day (3 control carriers)
  - 23 total carriers will be processed per test day

Method Performance Indicators

- Repeatability Standard Deviation (S_r) = Within Lab Variability
- Reproducibility Standard Deviation (S_R) = Between Lab Variability
- Responsiveness Measurement

Example of randomized test matrix (evaluating 4 test towelettes per day)

REPLICATION	TEST DAY	TOWELETTES
1	1	B,C,A,D
2	2	A,D,C,B
3	3	D,B,C,A

New research initiatives

- Determining shelf-life of spores
- Considering reference standard/proficiency tests
- Utilizing QPM platform for spray
- Considering use of a single quantitative method for liquid

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