EPA Guidance for *Clostridium difficile* Testing

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EPA Interim Guidance for *C. difficile* Claims

- Interim Guidance Issued in 2009
  - Based on best available science at the time and studies submitted to support registration
- Scope: Antimicrobial products – dilutable liquids, powders and ready-to-use - to treat hard non-porous surfaces in health care settings
- Claim: Kills *C. difficile* spores on hard, non-porous surfaces
- Recommended Efficacy Test Methods
  - AOAC Sporicidal Activity of Disinfectants Test (SAT , AOAC 966.04)
  - Quantitative Three Step Method (TSM, AOAC 2008.05; ASTM E2414)
  - Quantitative Carrier Test Method (QCT-2, ASTM E2197)

EPA Interim Guidance for *C. difficile* Claims (continued)

- Test Strains: ATCC 43598, ATCC 700792, ATCC 43599
- Controls: Quantitative = $>10^6$ spores/carrier; Qualitative = between five and six logs spores/carrier
- Organic soil load: None; label must specify pre-clean step
- Number of Test Carriers:
  - For AOAC SAT
    - 30 carriers for sterilants –2 separate batches of the product
    - 60 carriers for hospital disinfectants - 3 batches of product, one at least 60 days old
  - Quantitative Tests – number of carriers tested as per method
- Performance Standard: Quantitative = ≥6 log reduction; Qualitative = zero positive carriers (out of 60)
- Special label instructions required

Enhancements to the Interim Guidance

- Five years since Interim Guidance Issued
- Knowledge gained through additional research and testing
- Focus on standardization of the spore suspensions and use of quantitative methodology
  - AOAC Sporicidal Activity of Disinfectants is not in use – requires large volume of spores
- Use of a single strain is preferable – ATCC 43598 is a relevant clinical strain
- Incubation timeframe for recovery of treated spores is >2 days
- Spore Recovery:
  - BHIY-HT provides optimal nutrition for recovery of spores
  - PES membranes used to optimize recovery
- Spore quality and storage conditions are critical

Enhancements to the Interim Guidance

- Standard sporulation methods have been collaboratively studied and accepted as ASTM methods
- Long term storage conditions for spores were studied by EPA
- Soil load should be an essential component to the inoculum per the ecology of *C. difficile*
- Testing of towelettes should be based on the actual formulation
  - Quantitative Petri Plate Method appears to be suitable for evaluating towelettes

EPA Guidance for *C. difficile* Claims

Attributes	Existing Interim Guidance (2009) Last Updated May, 2012	Proposed Changes
Antimicrobial Products	Dilutable liquids, powders, RTU formulations, spray products, towelettes)	Dilutable liquids, powders, RTU formulations, and towelettes
Product Efficacy Test	Qualitative: AOAC SAT 966.04 Quantitative: AOAC 2008.05; ASTM E2197-02	Quantitative: AOAC 2008.05; ASTM E2197-11, ASTM E2896-12.
Test Strain	ATCC 43598, ATCC 700792, ATCC 43599	ATCC 43598 (Toxin B only)
Spore Production	N/A	ASTM E2839-11 ASTM E2895-13
Spore Titer/ purity	N/A	$>10^8$ spores/ml; >95%
Qualification of spore suspensions	N/A	Acid resistant (LR <2 following exposure to 2.5 M HCl)

EPA Guidance for *C. difficile* Claims (continued)

Attributes	Existing Interim Guidance (2009)	Proposed Changes
Neutralizer confirmation	N/A	Must test/submit data with Product efficacy
Soil Load	N/A	3 parts soil load as OECD (prior to Inoculation)
Recovery Medium/Filter	N/A	BHIY-HT/Hydrophilic PES
Incubation Conditions	N/A	Anaerobically at 36°C for 3-5 days for treated carriers; 48 ±4 hrs for control
Carrier counts	$\geq 10^6$ spores/carrier	$\geq 10^6$ spores/carrier (desired target of 6.5 to 7.5 LD)
Product Performance	≥6 Log Reduction (LR)	Mean LR ≥6 logs