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 = $\geq 6$ 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

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Antimicrobial Products</td>
<td>Dilutable liquids, powders, RTU formulations, spray products, towelettes</td>
<td>Dilutable liquids, powders, RTU formulations, and towelettes</td>
</tr>
<tr>
<td>Test Strain</td>
<td>ATCC 43598, ATCC 700792, ATCC 43599</td>
<td>ATCC 43598 (Toxin B only)</td>
</tr>
<tr>
<td>Spore Production</td>
<td>N/A</td>
<td>ASTM E2839-11&lt;br&gt;ASTM E2895-13</td>
</tr>
<tr>
<td>Spore Titer/purity</td>
<td>N/A</td>
<td>&gt;10^8 spores/ml; &gt;95%</td>
</tr>
<tr>
<td>Qualification of spore suspensions</td>
<td>N/A</td>
<td>Acid resistant (LR &lt;2 following exposure to 2.5 M HCl)</td>
</tr>
</tbody>
</table>
### EPA Guidance for *C. difficile* Claims (continued)

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