Sporocides and How To Test For Them
Prof. Jean-Yves Maillard, Cardiff University
Broadcast from the 2019 Healthcare Infection Society Conference

Sporicides and how to test them

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Cardiff School of Pharmacy and Pharmaceutical Sciences
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ENDOSPORES AND SUSCEPTIBILITY

LOW

• Prions
• Bacterial spores
• Protozoal cysts
• Mycobacteria
• Non-enveloped viruses
• Gram-negative bacteria (vegetative)
• Fungi
• Protozoa
• Gram-positive bacteria (vegetative)
• Enveloped viruses

HIGH

SPORICIDES
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ENDOSPORES AND SUSCEPTIBILITY

EXOSPORIUM
Degradation?
Persistence on surfaces

SPORE CORE
Small Acid Soluble Proteins (SASPs)
Protection of nucleic acid
Low water content

SPORE COATS
Barrier to biocides

INNER MEMBRANE
Highly compressed
Barrier to biocide
Barrier to rehydration

CORTEX
Barrier to biocides
Physical pressure to inner membrane


ENDOSPORES AND SUSCEPTIBILITY

SPORULATION

SPORICIDAL ACTIVITY

OUTGROWTH

“SPORISTATIC” ACTIVITY

GERMINATION

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ENDOSPORES AND SUSCEPTIBILITY

SPORICIDAL ACTIVITY

**Highly reactive compounds**

- Ethylene oxide
- Glutaraldehyde
- Formaldehyde
- ortho-phthalaldehyde
- Sodium hypochlorite
- Sodium dichloroisocyanurate
- Chloramine-T
- Calcium hypochlorite
- Iodine and iodophors

“SPORISTATIC” ACTIVITY

- Phenols and cresols
- Quaternary ammonium compounds
- Biguanides (chlorhexidine)
- Organic acids and esters
- Alcohols

FORMULATIONS


ENDOSPORES AND SUSCEPTIBILITY

- Once germinated, a “spore” becomes significantly more susceptible to biocides


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ENDOSPORES AND SUSCEPTIBILITY

Sporicidal activity: difference between the number of viable spores added to the test vessel and surviving spores following exposure... as measured following germination and outgrowth, and multiplication of vegetative bacteria (to form a visible colony)

STANDARD TESTS FOR SPORICIDAL ACTIVITY

PURPOSE OF EFFICACY TEST PROTOCOLS

• End users can select a product that is appropriate for their use
  - provide reliable usage information on the efficacy of an antimicrobial product
• Sustainable and accurate product claims on label

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“Because the dormant spore form found in the health care environment causes concern for the infection control process, the EPA requires that all disinfectant products registered for use against *C. difficile* must be effective against the spore form of the organism, not the vegetative form.

However, testing is difficult because these strains don’t readily sporulate to high populations (>10⁸ spores/mL) using standard propagation methods and growth media.”

“Product X achieved a 100% kill of vegetative cells of *Clostridium difficile* ATCC 9689 (1.1 x 10⁸) dried out on a 12 inch square stainless steel test surface. (Wipe time: 30 seconds)”
STANDARD TESTS FOR SPORICIDAL ACTIVITY

Most common European efficacy protocols used for determining sporicidal activity against *C. difficile* (from label claim and web information on specific sporicidal product)

**BASIC TESTS**

Ability of a product to demonstrate bactericidal, fungicidal or sporicidal activity, without regard to specific conditions of intended use, is tested (Phase 1, step 1 tests)

**EN14347**: Basic sporicidal activity - *Bacillus subtilis*
- Temperature: 20ºC; contact time one of the following 30, 60, 120 min; no soiling *(no C. difficile)*

**ADVANCED’ SUSPENSION TESTS**

- **EN13704**: Sporicidal suspension test - *Bacillus subtilis, Clostridium sporogenes*
  - Temperature: 20ºC(4-75ºC); contact time: 60 min; low soiling (clean) *(no C. difficile)*

- **HIS (UK) sporicidal test** – Quantitative suspension test – *Clostridium difficile*
  - Clospore, Temperature: variable, contact time: 5 min; soiling

- **prEN 17126**: Quantitative suspension - *Bacillus subtilis and Clostridium difficile*
  - Temperature: 20ºC(4-30ºC for surface); contact time: 15 min 60 min; soiling

- **EN1276**: Bactericidal suspension test *(NO SPORES)*
  - Temperature: 20ºC(4-40ºC); contact time: 5 min (1-60 min)
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STANDARD TESTS FOR SPORICIDAL ACTIVITY

PRODUCT A - TRIGGER SPRAY €89.60

“Sporicidal, kill Clostridium difficile (C. diff) spores (EN 1276 & EN 14347), started with 15,300,000 c. diff spores and were reduced in one minute contact time to less than 10 C. diff spores in both clean & dirty conditions”

EN1276
Bactericidal NOT sporicidal

EN14347
BASIC sporicidal test
NO soiling NO C. difficile

STANDARD TESTS FOR SPORICIDAL ACTIVITY

Laboratory test report

ITEMS: XXX Sporicidal Wipe Liquid

TESTS: Disinfectant Test

METHOD: Bacteria – BS EN 13704:2002
Concentration: Neat
Temperature: 20 C
Contact Time: 15 and 30 mins
Interfering substance: Bovine Albumin 0.3 g/L
Recovery: Dilution neutralisation
Incubation media: Clostridial Agar

CLEAN CONDITION
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STANDARD TESTS FOR SPORICIDAL ACTIVITY

Efficacy carrier test protocols used for determining sporicidal activity

SURFACE TESTS

- ASTM E2197-11 (carrier stainless steel disks) *Bacillus subtilis*, *Clostridium sporogenes*
  - Temperature: variable; contact time: variable; soiling

- AOAC966.04: (carrier porcelain disks); *Bacillus subtilis*
  - Temperature: variable; contact time: variable; no soiling

PRODUCT TESTS

- ASTM2967-15: (carrier stainless steel disks) Quantitative test method for the evaluation of both the efficacy of wipes to remove microbial contamination from a non-porous surface and the transfer of microbial contaminant to a clean surface
  - Wiping: weight: 150-500g, contact time: 5 sec – 5 min rest; soiling
  - Viability (kill/removal) and transfer post-wiping

Possible scenarios for decontaminating high-touch environmental surfaces by wiping

Sattar & Maillard AJIC 2013;41:S97-S104.
**STANDARD TESTS FOR SPORICIDAL ACTIVITY**

**“SPORICIDAL” WIPES—efficacy testing against C. difficile NCTC12727**

<table>
<thead>
<tr>
<th>Wipes†</th>
<th>Bacterial Removal (log₁₀ cfu/disk ± SD)</th>
<th>Bacterial transfer following 10 s wiping time at 500 g surface pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>1.13 (± 0.36)</td>
<td>5 consecutive transfers. TNTC</td>
</tr>
<tr>
<td>NaOCl soaked wipe</td>
<td>2.02 (± 0.21)</td>
<td>5 consecutive transfers. TNTC</td>
</tr>
<tr>
<td>Cline® sporicidal wipe</td>
<td>4.09 (± 0.79)</td>
<td>No spore transferred</td>
</tr>
<tr>
<td>TriGene Advance</td>
<td>0.22 (± 0.07)</td>
<td>5 consecutive transfers. From 0 to TNTC</td>
</tr>
<tr>
<td>AzoMaxActive™</td>
<td>1.30 (± 0.33)</td>
<td>5 consecutive transfers. From 0 to TNTC</td>
</tr>
<tr>
<td>Sani-Cloth® Rapid</td>
<td>0.57 (± 0.07)</td>
<td>5 consecutive transfers. From 1 to TNTC</td>
</tr>
<tr>
<td>ActiB™</td>
<td>+0.08 (± 0.08)</td>
<td>5 consecutive transfers. TNTC</td>
</tr>
<tr>
<td>SuperNova®</td>
<td>1.14 (± 0.65)</td>
<td>5 consecutive transfers. From 83 to TNTC</td>
</tr>
<tr>
<td>Tuffix</td>
<td>0.67 (± 0.11)</td>
<td>5 consecutive transfers of ≤43 bacteria</td>
</tr>
<tr>
<td>Enduro Patient wipes</td>
<td>0.88 (± 0.13)</td>
<td>5 consecutive transfers. From 2 to TNTC</td>
</tr>
<tr>
<td>NewGenn®</td>
<td>0.84 (± 0.66)</td>
<td>5 consecutive transfers. From 40 to TNTC</td>
</tr>
</tbody>
</table>

1. At the time of testing i.e. 2010-2011; † no sporicidal claim

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### STANDARD TESTS FOR SPORICIDAL ACTIVITY


<table>
<thead>
<tr>
<th>BIOCIDES/PRODUCTS</th>
<th>Clean condition</th>
<th>60 min contact time</th>
<th>Log&lt;sub&gt;10&lt;/sub&gt; Reduction (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde - 2%</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>Ortho-phthalaldehyde-0.55%</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>Ortho-phthalaldehyde-0.65%</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>Didecyldimethyl ammonium chloride -1%</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>Bis(amino)propylylamine -1%</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>Amine -1% + quaternary ammonium-1%</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>Anoxy-Twin 1200 ppm</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>Aniosept Activ – 2%</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
<tr>
<td>NaOCl 5000 ppm</td>
<td>C. diff</td>
<td>B. sub</td>
<td>BS EN 14347</td>
</tr>
</tbody>
</table>

#### REALITY CHECK

Complex formulations

Exciplents altering the activity of XX (-%) against spores of *B. subtilis* PS333 (WT)

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**REALITY CHECK**

Ensuring sporicidal efficacy

**Factors inherent to the product**
- concentration
- formulation
- pH

**Factors inherent to the application**
- surface
- organic load (soiling)
- temperature
- contact time
- Relative humidity (fumigants)

**Factors inherent to the microorganism**
- *B. subtilis* spores
- *C. difficile* spores
- Sporulation
- Clinical vs. culture collection

**BIOAVAILABILITY**

**POTENTIATION/SYNERGY/ANTAGONISM**

**DELIVERY**

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**REALITY CHECK**

Sporicidal claims


... efficacy tests rely on an efficient and demonstrable neutralization protocol, and failure to quench the active(s) can lead to misinterpretation of 'sporicidal' activity...

... misinterpretation of the neutralization validation test results when following a standard protocol can also lead to erroneous sporicidal claims...

...Complex formulations, notably where several amine-based biocides are used, can be difficult to neutralize...

...Our current understanding of sporicides, in the sense that a sporicide should kill $10^3$-$10^5$ spores, has not really changed over the years, and only a few biocides have been shown to have sporicidal activity ... Amine-based products are, to date, not among these biocides...

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Complex QAC formulations, alcohol formulations, biguanide formulations are NOT SPORICIDAL

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THANK YOU

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www.webbertraining.com/schedulep1.php

December 6, 2018
INFECTION DISEASE HIGHLIGHTS AND LOWLIGHTS IN 2018, AND WHAT TO EXPECT IN 2019
Speaker: Dr. Larry Madoff, ProMED Editor, Director, Division of Epidemiology and Immunization, Massachusetts Dept. of Public Health

(South Pacific Teleclass)
CONTROL OF CARBAPENEMASE-PRODUCING ENTEROBACTERIACEA IN AN ENDEMIC SETTING: DO CLASSICAL IPC METHODS WORK FOR NEW AGE BUGS?
Speaker: Dr. Kalsivar Marimuthu, Tan Tock Seng Hospital, Singapore

(FREE Teleclass)
THE BEST WAYS TO GET YOUR HOSPITAL TO TALK ABOUT INFECTION CONTROL
Speaker: Prof. Andreas Voss, Radboud University, The Netherlands

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