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

Live broadcast from

The 11th Healthcare Infection Society International Conference
ACC, Liverpool, UK, 26-28 November 2018
Healthcare Infection Society

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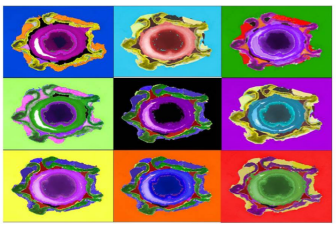
PRIFYSGOL CAERDYDD

Sporicides and how to test them



Jean-Yves Maillard
Cardiff School of Pharmacy and Pharmaceutical Sciences
Cardiff University

ENDOSPORES AND SUSCEPTIBILITY

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LOW

SUSCEPTIBILITY LEVEL

HIGH

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

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ENDOSPORES AND SUSCEPTIBILITY

Leggett *et al. J Appl Microbiol* 2012; 113: 485-98.

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

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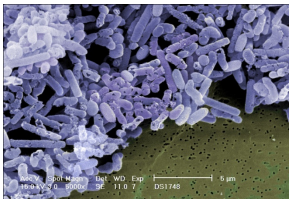
ENDOSPORES AND SUSCEPTIBILITY

SPORULATION

SPORICIDAL ACTIVITY



OUTGROWTH

"SPORISTATIC" ACTIVITY

GERMINATION

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ENDOSPORES AND SUSCEPTIBILITY

SPORICIDAL ACTIVITY

Highly reactive compounds

Ethylene oxide	Hydrogen peroxide
Glutaraldehyde	Peracetic acid
Formaldehyde	Chlorine dioxide
ortho-phthalaldehyde	Ozone

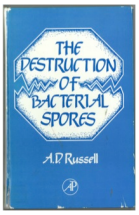
Sodium hypochlorite
Sodium dichlororisocyanurate
Chloramine-T
Calcium hypochlorite

Iodine and iodophors

FORMULATIONS

"SPORISTATIC" ACTIVITY

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



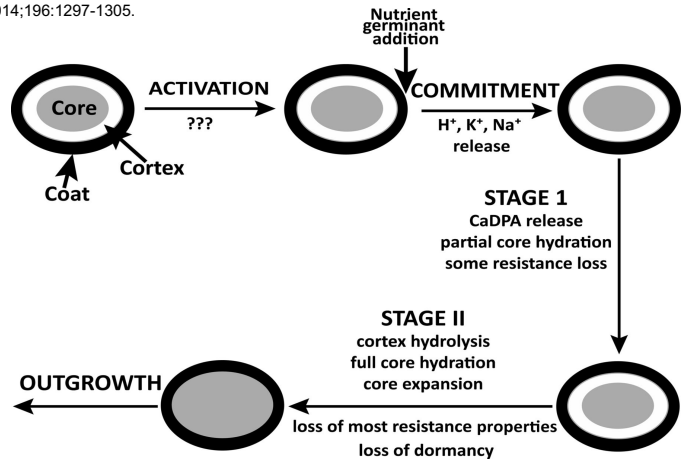
Professor Allan Denver Russell
(1936-2004)

Russell, A.D. (1990). Bacterial spores and chemical sporicidal agents. *Clinical Microbiology Reviews* 3, 99-119.

ENDOSPORES AND SUSCEPTIBILITY

➢ Once germinated, a "spore" becomes significantly more susceptible to biocides

Setlow *J Bacteriol* 2014;196:1297-1305.



Journal of Bacteriology

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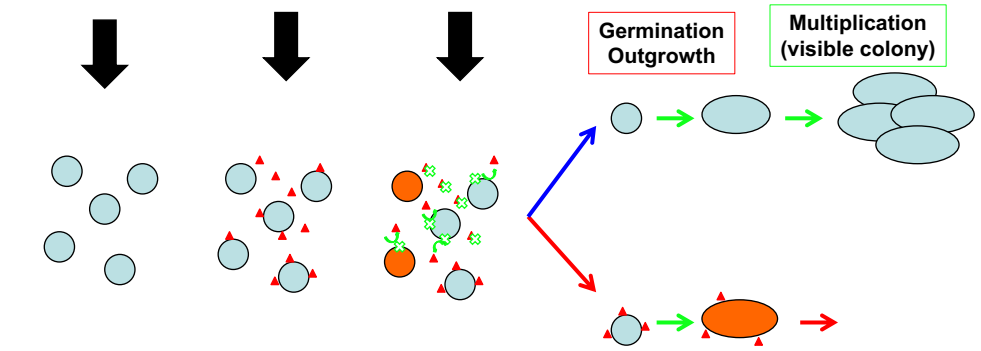
ENDOSPORES AND SUSCEPTIBILITY

Leggett *et al. J Appl Microbiol* 2016;120:1174-1180.



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)

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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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STANDARD TESTS FOR SPORICIDAL ACTIVITY
Clostridium difficile testing

By ATS Labs on March 9, 2011

"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 (>10^8 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 10^7) dried out on a 12 inch square stainless steel test surface. (Wipe time: 30 seconds)"

STANDARD TESTS FOR SPORICIDAL ACTIVITY
Clostridium difficile testing

- Anaerobic
- Optimisation of sporulation
- Spore purification
- Spore susceptibility
- Germination (dormant & superdormant)

MICROBIOLOGICAL METHODS

Clospore: A Liquid Medium for Producing High Titers of Semi-purified Spores of *Clostridium difficile*

JUSTO PEREZ, V. SUSAN SPRINGTHORPE, and SYED A. SATTAR[1]
University of Ottawa, Centre for Research on Environmental Microbiology (CREM), Faculty of Medicine, 451 Smyth Rd, ON, Canada K1H 8M5

prEN 17126: Quantitative suspension - ***Bacillus subtilis* and *Clostridium difficile***
BHIYT-L agar; Columbia broth + enzymatic digestion (purification)

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

Journal of Hospital Infection 89 (2015) 2–15
Available online at www.sciencedirect.com
Journal of Hospital Infection
Journal homepage: www.elsevierhealth.com/journals/jhin

Development of a sporicidal test method for *Clostridium difficile*

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ARTICLE INFO

Article history:
Received 15 September 2014
Accepted 22 September 2014
Available online 24 October 2014

Keywords:
Clostridium difficile
Disinfectant
Testing

SUMMARY

Background: Disinfectants with claimed activity against *Clostridium difficile* must be evaluated to ensure efficacy against the spores that comprise an environmental source of patient infection. Unfortunately there is, at present, no generally accepted method for evaluating these disinfectants. In the absence of such a method, laboratories have to adapt protocols that were not designed for products used in medical environments and consequently may use inappropriate test organisms, exposure times, and pass criteria.
Aim: To develop and evaluate a method for testing the activity of disinfectants against *C. difficile* spores using exposure times and pass criteria which are relevant to clinical practice.
Methods: A Joint Working Party of the Healthcare Infection Society (HIS) and the Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infections (ARHAI) of the Department of Health in England was assembled. The Working Party adapted a previously described enzyme-based method for spore purification (the Clospore method) using an exposure time of 5 min and a $5\log_{10}$ kill as a pass criterion.
Findings: Evaluation of the method by three laboratories demonstrated that the method is simple to follow and that the results are repeatable and reproducible.
Conclusion: The method described by the Working Party produces a clean suspension with a high titre of spores. It is recommended that, for a disinfectant used in the environment, the product should demonstrate a $5\log_{10}$ reduction in 5 min under clean or dirty conditions to fulfil the requirements of the test.

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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*)

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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)

'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*

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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

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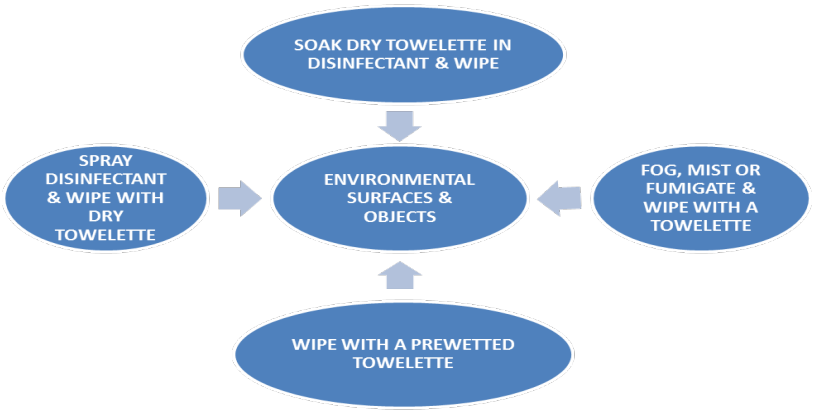
STANDARD TESTS FOR SPORICIDAL ACTIVITY

Possible scenarios for decontaminating high-touch environmental surfaces by wiping

Sattar & Maillard *AJIC* 2013;41:S97-S104.



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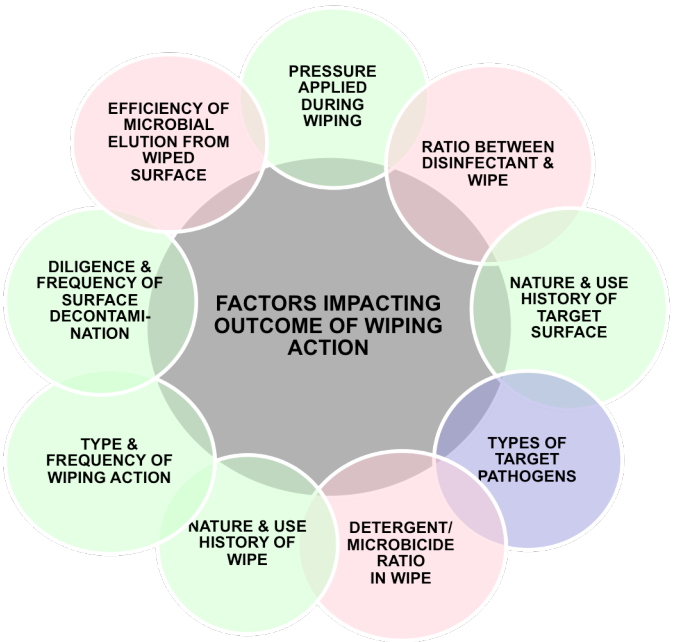

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Sattar & Maillard *Am J Infect Control* 2013 41;S97-S104

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STANDARD TESTS FOR SPORICIDAL ACTIVITY

"SPORICIDAL" WIPES– efficacy testing against *C. difficile* NCTC12727

Siani *et al. AJIC* 2011; 39(3), 212-218

Wipes[1]	Bacterial Removal (log_{10} cfu/disk ± SD) 500 g surface pressure	Bacterial transfer following 10 s wiping time at 500 g surface pressure
Negative control	**1.13 (± 0.36)**	5 consecutive transfers. TNTC
NaOCl soaked wipe	**2.02 (± 0.21)**	5 consecutive transfers. TNTC
Clinell® sporicidal wipe	**4.09 (± 0.79)**	No spore transferred
TriGene Advance	**0.22 (± 0.07)**	**5 consecutive transfers. From 0 to TNTC**
AzoMaxActive™	**1.30 (± 0.33)**	**5 consecutive transfers. From 0 to TNTC**
Sani-Cloth® Rapid	**0.57 (± 0.07)**	**5 consecutive transfers. From 1 to TNTC**
Activ8™	**+0.08 (± 0.08)**	5 consecutive transfers. TNTC
SuperNova®	**1.14 (± 0.65)**	5 consecutive transfers. From 83 to TNTC
Tuffie	**0.67 (± 0.11)**	5 consecutive transfers of ≤43 bacteria
Enduro Patient wipes	**0.88 (± 0.13)**	5 consecutive transfers. From 2 to TNTC
NewGenn[2]	**0.84 (± 0.66)**	5 consecutive transfers. From 40 to TNTC

[1] At the time of testing i.e. 2010-2011 ; [2] no sporicidal claim

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STANDARD TESTS FOR SPORICIDAL ACTIVITY

Wesgate *et al. J Hosp Infect* 2016;93:256-262.

60 min contact time	Log_{10} Reduction (± SD)							
BIOCIDES/PRODUCTS	**BS EN 14347**		**BS EN 13704**		**AOAC 966.04**		**ASTM E2197**	
Clean condition	*C. diff*	*B. sub*	*C. diff*	*B. sub*	*C. diff*	*B. sub*	*C. diff*	*B. sub*
Glutaraldehyde - 2%	1.87 (0.50)	0.56 (0.03)	1.57 (0.12)	0.44 (0.47)	1.60 (0.22)	0.12 (0.00)	1.17 (0.08)	0.66 (0.06)
Ortho-phthalaldehyde- 0.55%	>6.11 (0.33)	0.62 (0.11)	>5.96 (0.17)	0.48 (0.63)	5.05 (0.08)	0.19 (0.00)	5.83 (0.02)	0.29 (0.06)
Ortho-phthalaldehyde- 0.65%	>6.11 (0.33)	0.56 (0.07)	>5.96 (0.17)	0.77 (0.39)	5.05 (0.08)	0.12 (0.00)	>5.83 (0.00)	0.20 (0.09)
Didecyldimehtyl ammonium chloride -1%	0.69 (0.40)	0.01 (0.18)	0.23 (0.09)	0.07 (0.72)	0.44 (0.20)	0.54 (0.00)	0.39 (0.30)	0.41 (0.05)
Bis(aminopropyl)laurylamine – 1%	1.22 (0.90)	0.66 (0.01)	0.25 (0.09)	0.60 (0.54)	-0.08 (0.17)	-0.07 (0.00)	1.22 (020)	0.40 (0.10)
Amine -1% + quaternary ammonium- 1%	0.29 (0.60)	0.88 (0.03)	0.03 (0.03)	0.68 (0.01)	0.44 (0.04)	0.11 (0.00)	0.23 (0.17)	0.48 (0.10)
Anoxy-Twin-1200 ppm	>6.11 (0.33)	>6.27 (0.00)	>5.96 (0.17)	>4.88 (0.10)	5.05 (0.08)	>5.81 (0.00)	>5.83 (0.00)	>5.31 (0.02)
Aniosept Activ – 2%	6.09 (0.54)	6.81 (0.06)	>6.44 (0.03)	>6.18 (0.07)	5.05 (0.08)	>5.81 (0.00)	>5.83 (0.00)	*5.06 (0.24)*
NaOCl 5000 ppm	>6.11 (0.33)	>6.27 (0.00)	5.96 (0.17)	>4.88 (0.10)	5.05 (0.08)	>5.81 (0.00)	>5.83 (0.00)	4.28 (0.07)

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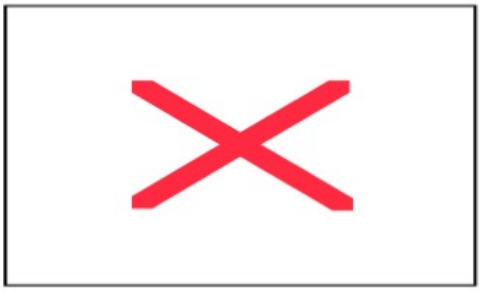
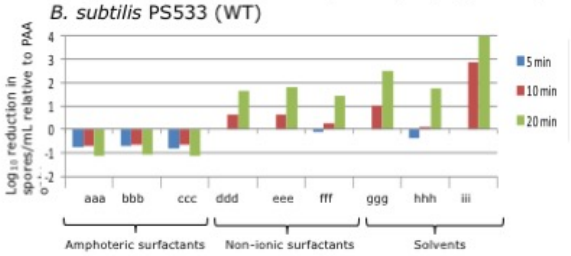
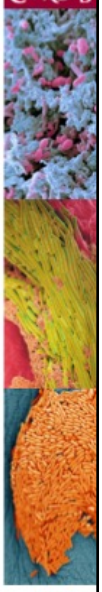
REALITY CHECK

Complex formulations



Excipients altering the activity of XX (-%) against spores of *B. subtilis* PS533 (WT)





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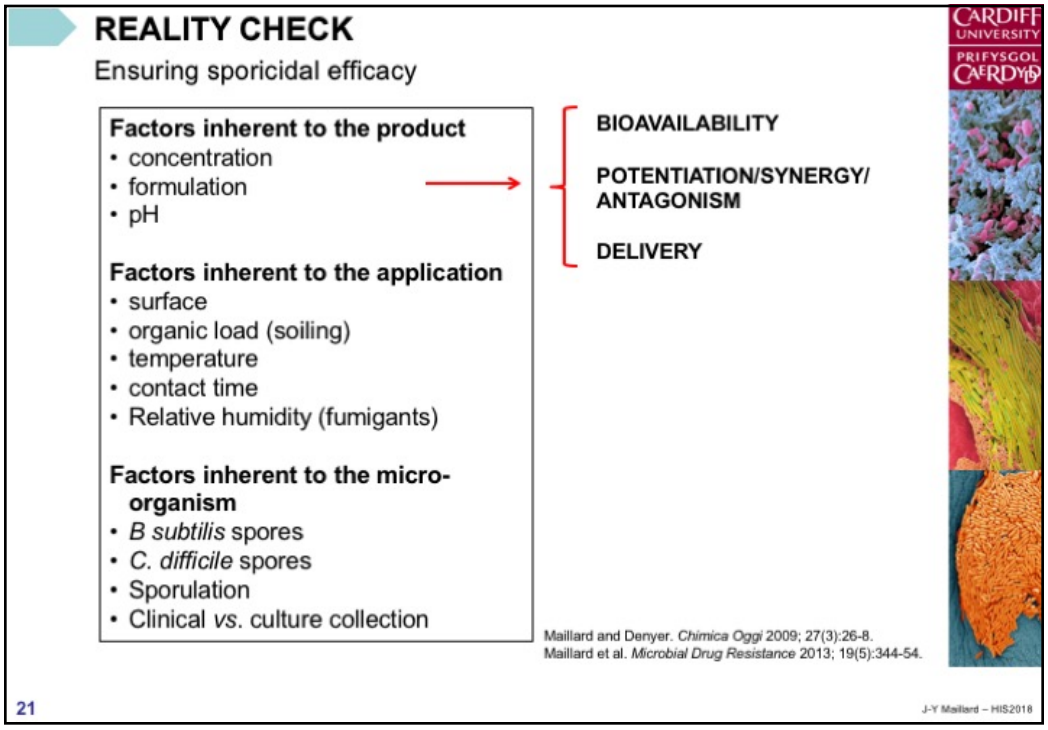

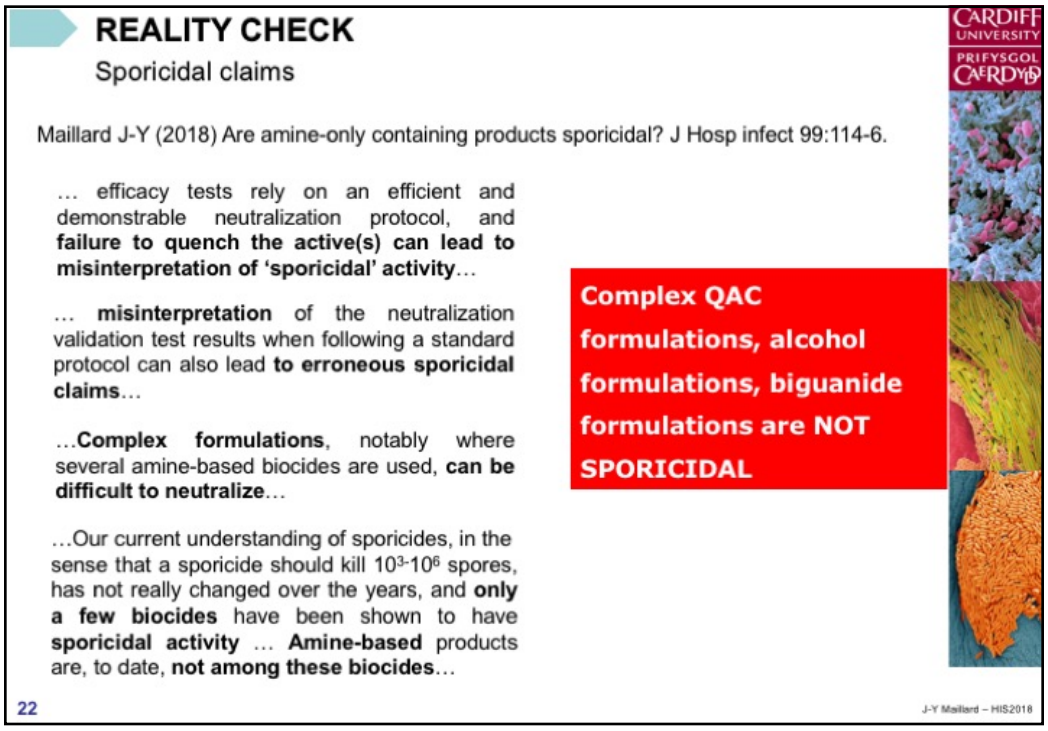

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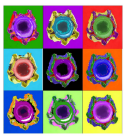
THANK YOU

Acknowledgements
R Wesgate
M Leggett
H Siani

Collaborators
P Setlow (University of Connecticut)
S Sattar (University of Ottawa)



Lovleen Joshi

www.webbertraining.com/schedulep1.php

December 6, 2018	INFECTIOUS 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
December 12, 2018	*(South Pacific Teleclass)* CONTROL OF CARBAPENEMASE-PRODUCING ENTEROBACTERIACEA IN AN ENDEMIC SETTING: DO CLASSICAL IPC METHODS WORK FOR NEW AGE BUGS? Speaker: **Dr. Kalisvar Marimuthu**, Tan Tock Seng Hospital, Singapore
December 13, 2018	*(FREE Teleclass)* THE BEST WAYS TO GET YOUR HOSPITAL TO TALK ABOUT INFECTION CONTROL Speaker: **Prof. Andreas Voss**, Radboud University, The Netherlands **Sponsored by Lonza (www.lonza.com) *****

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