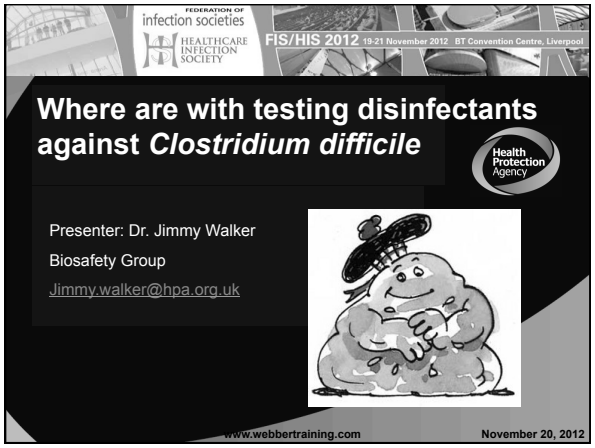

Learning Objectives

Health Protection Agency

Where are with *C. difficile* rates?

Understanding the limitations of disinfection

What standards are currently available?

How do you choose a new one?

Where are we with testing and validation?

Identifying the way forward

The A Team

Health Protection Agency

ARHAI/HIS TASK FORCE ON SPORICDAL DISINFECTANTS

Terms of Reference

1. To develop an accepted standard for laboratory testing of disinfectants which claim to have activity against *C. difficile* spores

2. To develop a network of Laboratories with capability to perform in vitro assays of sporicidal activity of biocides

3. To explore the creation of an accreditation scheme for laboratories which perform in vitro assays of sporicidal activity of biocides

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Guest Editorial

Sporicides for *Clostridium difficile*: the devil is in the detail

M.H. Wilcox [a,*], A.P. Fraise [b], C.R. Bradley [b], J. Walker [c], R.G. Finch [d]

[a] Microbiology, Leeds Teaching Hospitals & University of Leeds, Old Medical School, Leeds General Infirmary, Leeds, UK
[b] Hospital Infection Research Laboratory, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK
[c] Biosafety Unit, Research Department, Health Protection Agency, Microbiology Services, Porton Down, Salisbury, UK
[d] University of Nottingham & Nottingham University Hospitals NHS Trust, Clinical Sciences Building, City Hospital Campus, Nottingham, UK

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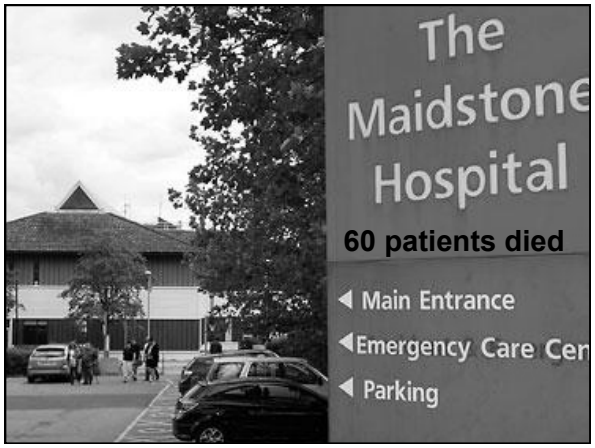
SUMMARY

A taskforce has now been formed with representatives from the Department of Health's Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI), the Hospital Infection Society (HIS), the Department of Health (England) and the Health Protection Agency. The aims of the ARHAI/HIS Taskforce on Sporicidal Disinfectants are: to develop an accepted standard for laboratory testing of disinfectants which claim to have activity against *C. difficile* spores; to develop a network of laboratories with capability to perform *in vitro* assays of sporicidal activity of disinfectants; and to explore the creation of a national quality assessment scheme for laboratories which perform *in vitro* assays of sporicidal activity of disinfectants.

Healthcare-associated infections

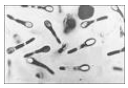
"Infections that patients acquire during the course of receiving treatment for other conditions within a healthcare setting"



Healthcare Acquired Infections

- Hospitals are busy places
- Long hours
- Tiring work
- Stressful activities
- Cluttered environment
- Life and death decisions

Clostridium difficile



Gram-positive, rod-shaped bacteria, forms endospores and have a strictly fermentative mode of metabolism

Most important cause of hospital-acquired antibiotic associated diarrhoea. Causes more serious intestinal conditions such as colitis and pseudo membranous colitis in humans (toxin mediated).

Present in the gut of up to 3% of healthy adults and 66% of infants. However, *Clostridium difficile* rarely causes problems in children or healthy adults, as it is kept in check by the normal bacterial population of the intestine.

Spreads via the faecal oral route characterised by 'explosive diarrhoea' 10^7 to 10^9 cfu per gram.

What surfaces do you touch?

About 5% of near-patient sites demonstrate presence of Gram-negative bacilli indistinguishable to those from the patient

Microorganisms recovered from linen and nightwear; bedside table, bed rail and chair; door handle; infusion pump and respirator; and expected bathroom sites

The perineum has been highlighted as an important source of environmental contamination for hands of both patients and staff

Lemem et al 2004 JHI

Transmission of *C. difficile*

Transient and persistent carriage of hospital organisms on the hands of healthcare workers. Dancer et al 2010 JHI

Spores of *Clostridium difficile* survived for five months and epidemic vancomycin-resistant enterococci (VRE) for up to four years. Fekety et al 1981 AJM

59% clinical staff caring for patients with *C.difficile*, had positive cultures for *C. difficile* from their hands. McFarland et a 1989 NEJM

Prior room occupancy has been shown to be a risk for acquisition of both Gram-negative and Gram-positive organisms.

This suggests that terminal cleaning and/or disinfection regimens for isolation rooms containing patients colonised and/or infected with MRSA, VRE, *C.difficile*, Acinetobacter and Pseudomonas fail to remove all microbial contamination, thus exposing a new admission to the remnants of a persistent environmental reservoir.

Carling et al 2010 AJIC

Figure 1: Trends in rates of CDI (FY 2008/09 to FY 2011/12)

Figure 1a: Total rates

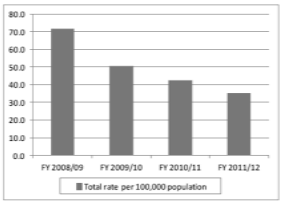
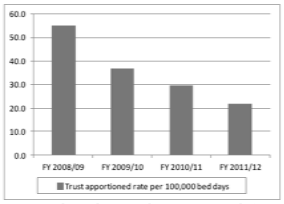
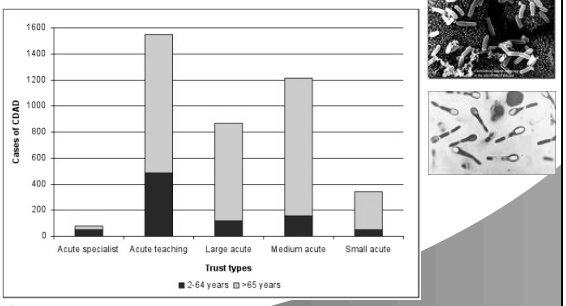


Figure 1b: Trust apportioned rates

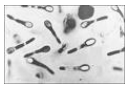


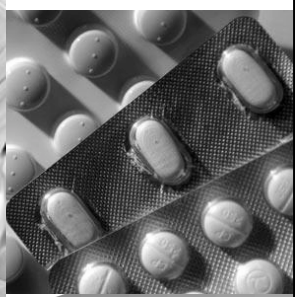
A total of 18,005 cases of CDI occurring in patients aged 2 years and over between April 2011 and March 2012 (FY 2011/12). 21,707 (2010/11 – 17% reduction. 36,095 (08/09 – 50%reduction)

***C. difficile* incidence in London by age group and Trust type: April – September 2007**



Source: www.hpa.org.uk: mandatory reporting since 2004 in patients 65 and over: since 2007 reported for aged 2 and over.





Kills 99% of ALL known germs stone dead!

What confidence do you have in your disinfectants?

Chemical Disinfection

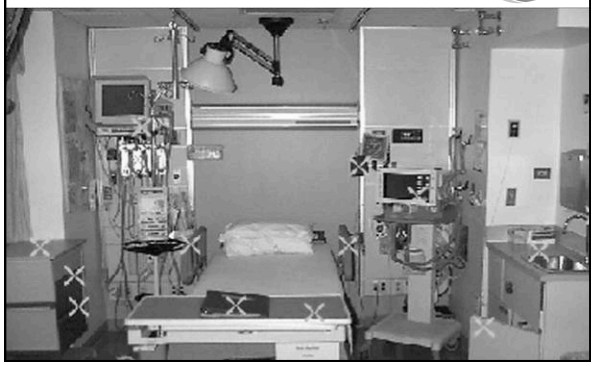
Typical definition: ***The destruction of micro-organisms, but usually not bacterial spores: disinfectants do not necessarily kill all micro-organisms but reduce them to safe levels which make the disinfected object safe to handle.***

"Disinfection does not sterilise a surface, object or medical device"

Increasing resistance to disinfection methods

Agent	Example
Prions	CJD, BSE
Spores	*Clostridium difficile*
Coccidia	*Cryptosporidium*
Mycobacteria	*Mycobacteria tuberculosis*
Cysts	Giardia
Fungi	*Aspergillus niger*
Non-enveloped viruses	Polio virus
Gram-negative bacteria	*Escherichia coli*
Gram-positive bacteria	*Staphylococcus aureus*
Lipid enveloped viruses	Human Immunodeficiency Virus

Role of cleaning?



Cleaning in *C. difficile* outbreaks

***C. difficile* rates fell by 48%, with a sustained and significant reduction on the rate of nosocomial CDI when all surfaces, floor to ceiling, were wiped with dilute bleach applied with towels to thoroughly wet the surfaces.** Hacek et al 2010 AJIC

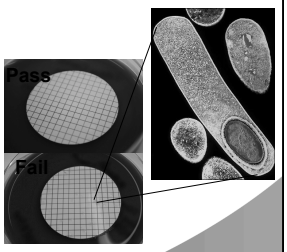
Another group implemented daily cleaning with 0.55% bleach wipes on two medical wards with a high incidence of *C. difficile*. Pre-intervention - 31 new cases of *C. difficile* on the wards. After cleaning - 4 cases on these wards over the following year, representing a 7-fold decrease. Orenstein et al 2011 ICHE.

No other interventions introduced other than targeted cleaning with bleach wipes.

Past experiences

Work for PASA

- EN13704
- Bactericidal
- Filter method



Commercial Sporocides

Nos	Product	Log_{10} reduction from 10^8 challenge			
		Clean (0.3% albumin)		Dirty (3% albumin)	
		1 MIN	60 MIN	1 MIN	60 MIN
1	Chlorine dioxide	>4	> 4	>4	> 4
2	Chlorine dioxide	>4	> 4	> 4	> 4
6	Chlorine dioxide	>4	> 4	3	> 4
7	Chlorine dioxide	>4	> 4	3	4
8	Chlorine dioxide	3	> 4	3	> 4
9	didecyldimethylammonium	3	> 4	<3	> 4
10	Chlorine dioxide	3	> 4	<3	3
11	Chlorine dioxide	3	> 4	<3	<3
14	Triamine	2	> 4	<3	> 4
15	Chlorine	2	> 4	<3	> 4
16	Chlorine	2	> 4	<3	> 4
17	Chlorine	<3	> 4	<3	> 4
18	Electrolysed Brine(H_2O_2, ClO_2, O)	<1	> 4	<3	3
19	Chlorine	<3	> 4	<3	3
20	Chlorine dioxide	2	> 4	<3	<3
26	Triamine (wipes)	<3	> 4	<3	<3
27	Peracetic acid (wipes)	<3	4	<3	<3
28	Triamine	<3	3	<3	<3
29	Triamine	<3	3	<3	<3
30	Chlorine dioxide	<3	<3	<3	<3
31	Benzalkonium chloride, Didecyldimonium chloride, Bronopol, Polyaminopropyl biguanide hydrochloride	<3	<3	<3	<3
32	Benzalkonium chloride, Didecyldimonium chloride, Bronopol, Polyaminopropyl biguanide hydrochloride	<3	<3	<3	<3

Speight et al 2011 JHI

Summary of current EN standards for assessing sporicides

Criteria	EN 14347	EN 13704
Phase	Phase 1	Phase 2 Step 1
Area of Application	All areas	Food, industrial, domestic and institutional
Test organism(s)	*Bacillus subtilis* *Bacillus cereus*	*Bacillus subtilis*
Additional test organisms	None	*Bacillus cereus* *Clostridium sporogenes*
Contact time (mins)	30, 60 or 120	60
Additional contact times	None	5, 15 or 30
Interfering substances	None	0.03% albumin
Log reduction required	≥4.0	≥3.0

Development of a national standard in parallel to the development of EU norm

EU norm	Preferred UK National Standard
Sporicidal activity evaluated using ribotype 027 Additional ATCC 9689	027 not considered appropriate ATCC is toxigenic; use a non toxigenic strain such as NCTC 11209
Use cooked meat broth	Prefer blood agar If they insist on using cooked meat – suggest they only use a commercially available standardised method
Contact time of 60 mins – looks at surface and instrument disinfection	Look at surfaces with shorter contact times
Glutaraldehyde as a reference agent	Prefer chlorine as reference agent
Repetitions of test	Clarification required on the number of repetitions of each test required
Take sample of the neutralised test method in duplicate	Clarification of how many duplicated test need to be done Recommend that a known disinfectant is used every time the test is carried out.
	Avoid mandating one neutralising agent
Method – shock at 80°C for 10 mins, store for 2 year	Keep HIRL 70°C for 30 mins, store for 1 year

Limitations of Filtration methods

Time (mins)	NaDCC			QAC		
	R1	R2	R3	R1	R2	R3
1	<2.52	<2.52	<2.52	<2.52	<2.52	<2.52
5	3.90	3.56	3.51	<2.52	<2.52	<2.52
60	>4.74	>4.74	>4.74	<2.52	<2.52	<2.52

Courtesy of M Wilkinson, HIRL

Sporicidal Testing Trial

Test disinfectants	500 ppm NaDCC (HazTab) QAC diluted to 1.5% with stdd hard water)
Contact times	1, 5 and 60 mins
Test organism	*Clostridium difficile* NCTC 11209 (new freeze dried culture to be used)
Organic load	3g/litre bovine serum albumin Final concentration in the test 0.03%
Neutralization method	Chemical/dilution neutralization LTSHS for NaDCC LTS for QAC
Test frequency	3 replicates on Day 1 3 replicates on another day

Preparation of spore test suspension

1. Inoculate 6 blood agar plates with a culture of *Clostridium difficile* (NCTC 11209). from the -20°C freezer
2. Incubate anaerobically at 37°C for 3 - 5 days.
3. Scrape the growth from the surface of the blood agar plates into 10ml sterile water. Vortex to break up any clumps. Store in the fridge at 4 - 8°C for 3 - 5 days.
4. Centrifuge at 3000 rpm for 10 mins
5. Remove supernatant, add 10 ml sterile water, mix well and repeat centrifugation as described above.
6. Repeat 2 times.
7. Heat-shock the suspension at 70°C for 30min.
8. Enumerate the number of spores in the suspension by carrying out ten-fold dilutions
9. Spore suspensions may be kept in the fridge for 1 year.

Time (mins)	NaDCC			QAC		
	R1	R2	R3	R1	R2	R3
1	0.08	0.06	0.00	0.02	0.00	0.02
5	0.97	0.57	0.62	0.00	0.00	0.00
60	6.08	6.08	>6.08	0.06	0.02	0.08

Courtesy of M Wilkinson, HIRL

Factors affecting testing

- One of the labs had no problems with the method.
- Two of the laboratories unable to grow sufficient quantities of spores from plates so unable to achieve sufficient spore reduction.
- Back to the drawing board
- Jean-Yves Maillard tried out the Clospore method

PEREZ ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 94, NO. 2, 2011 1

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

Clostridium difficile **continues to cause infections in healthcare and other settings. Its spores survive well indoors and require sporicidal chemicals for infection control. However, proper testing of disinfectants is impeded due to difficulties in obtaining viable spores of high enough quality and titers to meet current regulations for sporicidal claims. A new liquid medium (Clospore) has been developed, based on a systematic review of the compositions of 20 other available media.** ***C. difficile*** **spores grown in the new medium and treated with a mixture of lysozyme and trypsin yielded final suspensions with >10^9 CFU/mL of viable spores, with a purity of >91% as tested by spore-staining and phase-contrast microscopy. The spores showed a biological decay rate of**

surroundings (9). Until recently, the label claims of many environmental surface disinfectants against *C. difficile* were based mostly on tests using its vegetative form, which is easier to inactivate. Now, the U.S. Environmental Protection Agency (EPA) will accept such claims only when the testing is conducted using the spores (10).

Although high titers of *C. difficile* spores can be obtained using semi-solid media (11, 12), the process yields crops of variable quality for routine testing of sporicidal activity. Thus far, good sporulation in liquid media has been difficult and, again, yields titers not high enough to demonstrate acceptable levels of sporicidal activity (13). After a systematic comparison of the compositions of 20 available media, we describe here a liquid medium along with a semipurification process to produce high-titered suspensions of *C. difficile* spores for use in testing environmental surface disinfectants.

Preparation of spore test suspension

1. Inoculate Clospore broth medium* with a culture of *Clostridium difficile* (NCTC 11209). from the -20°C freezer
2. Incubate anaerobically at 37°C for 7 - 10 days.
3. Centrifuge at 10, 000 g for 10 mins
4. Add Enzymic solution
5. Remove supernatant, add 10 ml sterile water, mix well and repeat centrifugation as described above.
6. Repeat 2 times.
7. Heat-shock the suspension at 70°C for 30min.
8. Examine the spores microscopcially and enumerate the number of spores in the suspension by carrying out ten-fold dilutions
9. Spore suspensions may be kept in the fridge for 1 year.

* Liquid culture is preferred but Clospores plates can be used if centrifuge not available

Porton

	Initial inoculum	NaDCC Log_{10} reduction ± SD		
		1 min	5 mins	60 mins
Batch 1				
Day 1	7.45	0.49 ± 0.08	2.55 ± 0.12	7.45 ± 0.00
Day 2	7.28	0.61 ± 0.08	3.58 ± 0.20	5.91 ± 1.22
Batch 2				
Day 1	7.70	0.49 ± 0.02	2.11 ± 0.15	7.17 ± 0.93
Day 2	7.55	0.51 ± 0.08	2.02 ± 0.155	7.56 ± 0.00

Cardiff

	Initial inoculum	NaDCC Log_{10} reduction ± SD		
		1 min	5 mins	60 mins
Batch 1				
Day 1	7.10	0.45 ± 0.12	1.16 ± 0.13	0.38 ± 0.17
Day 2	7.92	1.44 ± 0.23	1.28 ± 0.07	1.47 ± 0.37

Ongoing work

Ring trial to continue till all laboratories complete initial study

Blu Test Scientific also joining the ring trial.

Once data set is completed results will be published.

Tender for administration and organisation of an external quality assurance scheme

- To prepare standard disinfectant test products to send out to participating laboratories within the UK
- To collate responses from all participating laboratories and carry out appropriate statistical analysis
- To distribute test products every 3 months for new laboratories and subsequently every six months
- To provide statistical information and feedback to participating laboratories
- To have procedures in place for notifying laboratories producing results which are statistical outliers
- To provide a biannual report on accredited laboratories to the HIS Sporicidal Task Group

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- ***Tina Bradley, Carole Fry, Adam Fraise, Chris Woodhall, Peter Hoffman, Roger Finch, Jean-Yves Maillard, Mark Wilcox,*** **John Coia**
- ***Susan Macken***
- **Rebecca Westgate**
- **Martyn Wilkinson**
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