NOTE TO THE EDITOR

Evaluation of alcohol wipes used during aseptic manufacturing

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Keywords
antimicrobial wipes, efficacy, production unit.

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2008/1872: received 30 October 2008, revised and accepted 28 November 2008

doi:10.1111/j.1472-765X.2009.02574.x

Abstract

Aim: During aseptic manufacturing and specifically during the transfer of items into an isolator, disinfection of surfaces is essential for reducing the risk of final product contamination. Surface disinfection can be carried out by a variety of methods, however the most accepted current practice is a combination of spraying with 70% alcohol and wiping. The aim of this study was to evaluate the effectiveness of two wipe systems by determining their ability to remove, kill and transfer bacterial contaminants from standardized surfaces.
Methods and Results: The protocol used to achieve these objectives was based on a newly published method specifically designed to test wipes. Alcohol impregnated wipes performed better at reducing microbial bioburden than the alcohol spray/dry wipe applications. Impregnated wipes drastically reduced (1–2 $\log_{10}$ reduction) a small bioburden (approx. 2 $\log_{10}$) of spores of *Bacillus subtilis* and methicillin-resistant *Staphylococcus aureus* from the surface, but failed to remove (<0·2 $\log_{10}$ reduction) *Staphylococcus epidermidis*. The alcohol spray/dry wipes did not manage to remove (<0·2 $\log_{10}$ reduction) spore or bacterial bioburden from surfaces and was able to transfer some viable microorganisms to other surfaces. Both wipe types showed poor antimicrobial efficacy (<1 $\log_{10}$ reduction) against the test bacteria and spores.
Conclusions: As far as the authors are aware this is the first time that such a practical study has been reported and our results suggest that the best wipes for surface disinfection in aseptic units are the alcohol (IPA) impregnated wipes when compared with the dry wipes sprayed with alcohol.
Significance and Impact of the Study: The impregnated wipes performed better than the dry wipes sprayed with alcohol and should be used for surface disinfection in aseptic units.

Note to the Editor

Aseptic manufacturing can be described as a multiple step process by which many materials, equipments, medicinal products and containers are transferred into (and manipulated in) an environmentally controlled workspace to produce a sterile product. Items used for product preparation usually start with some surface bioburden and as such need to be disinfected, usually by a combination of spraying with alcohol and wiping prior to and during transfer(s) into the isolator cabinet where the final product is manufactured (Hiom *et al.* 2004; Beaney 2006; Jackson and Wilson 2006). In hospital aseptic units, high quality assurance (QA) standards have been introduced to maximize patient safety [e.g. ISO 13408-1; ISO 14644: Beaney 2006; Medicines and Healthcare products Regulatory Agency (MHRA) 2007]. Examples of typical aseptic products would include intravenous (IV) cytotoxic drugs, trans-parenteral nutrition (TPN) and central intravenous additive (CIVA). Transfer disinfection is usually based on a combination of 70% alcohol sprays and sterile wipes (Hiom 2000; Cockcroft *et al.* 2001; Hiom *et al.* 2004;

Beaney 2006), where sterile wipes may be pre-alcohol impregnated, dry and/or alcohol wetted just before use. Current practice to validate transfer disinfection consists of contact plate enumeration of a flat surface before and after disinfection procedures. This is somewhat qualitative in nature, is exposed to the variabilities in efficacy of contact plate performance (Pinto *et al.* 2009) and is not accurate enough to accurately assess wipe efficacy. Indeed individual technique for trigger spraying and wiping differs between end users and there is no universal standard procedure for doing this. As alcohol is not a sporicidal agent there is also the postulation that to remove spores a wipe procedure is essential. Thus transfer disinfection remains a major QA issue when dealing with aseptic manufacturing. The aim of this study was to evaluate the most effective wipe systems used during aseptic manufacturing to (1) remove, (2) kill and (3) to prevent transfer between surfaces of surface bacterial contaminants.

Staphylococcus epidermidis (NCIMB 8853), *Bacillus subtilis* (ATCC 6051), and clinical isolates of methicillin resistance *Staphylococcus aureus* (MRSA; University Hospital of Wales, Cardiff, UK) were used in this study. Slope cultures of *Staph. epidermidis* and MRSA were prepared from freezer stocks onto Tryptone Soya Agar (TSA; Oxoid Ltd, Basingstoke, UK). Secondary slopes were prepared and incubated at 37°C overnight and washed with 5 ml of Tryptone Sodium Chloride [TSC; 8·5 g l^{-1} sodium chloride (Fisher Scientific, Loughborough, UK) and 1 g l^{-1} Tryptone (Oxoid)]. Working cultures were prepared by centrifuging the suspension at 4200 ***g*** for 10 min and resuspension in fresh TSC. Viable counts were performed on TSA using the pour plate method.

Spores of *Bacillus subtilis* (ATCC 6051) were prepared according to BSEN14347 (Anonymous 2005). Spore suspensions containing $1{\cdot}23 \times 10^9$ spore ml^{-1} were maintained in distilled water and stored at 4°C until used.

Sterile 70% denatured ethanol (IPA) wipes (Klerwipe^{TM} - 70/30), and sterile low particulate dry wipes without antimicrobial formulation (Klerwipe - 100^{TM}) both provided by Shield Medicare Ltd (Franham, UK) were used. Ethanol (70% v/v; Fisher Scientific) was used to spray (three squirts) the unmedicated dry wipes before use. The activity of the ethanol was quenched by dilution in TSC. To test the quenching ability of TSC, a neutralizer efficacy test based on the BSEN 13697 (Anonymous 2001) was performed. Two sterile stainless steel discs (2 cm diameter with a Grade 2B finish, Goodfellows Cambridge Ltd, Huntingdon, UK) were inoculated with 20 μl of the test suspension and left to dry in the incubator for 25 min at 37°C. Two sterile flat-bottomed glass bottles (100 ml, 4–5 cm diameter; Fisher) containing 10 ml of TSC and 5 g of glass beads (3 mm diameter; Sigma, Poole, UK) were used for testing the quenching efficacy of TSC. A small section of the test wipe was added to one bottle and left in contact for 5 min. The inoculated stainless steel discs were then transferred to each bottle ensuring that the inoculated side of the disc was facing the beads and shaken at 150 rev min^{-1} for 1 min. Surviving bacteria were enumerated after incubation at 37°C for MRSA and *Staph. epidermidis* and at 30°C for *B. subtilis*.

To measure the efficacy of wipes to remove bacterial bioburden dried onto a surface, the protocol described by Williams *et al.* (2007) was used. Bovine serum albumin (0·6% w/v; BSA; Sigma) was used to mimic 'dirty' conditions by adding 1 ml of BSA solution to 1 ml bacterial or spore suspension. Stainless steel disks were then inoculated with 20 μl of the bacterial or spore test suspension (approx. 2 $\log_{10}$ CFU) and left to dry for 25 min at 37°C. A section of the alcohol impregnated disinfectant wipe or dry wipes sprayed with alcohol was attached to a steel rod and then rotated against the disc at a speed of 60 rev min^{-1} for 10 s using a drill (IKA, Labortechnik, Staufen, Germany), exerting a weight between the disc and the wipe of 100 g (±5 g). Each disc was then transferred to 100 ml sterile flat-bottomed glass bottle, containing 5 g of glass beads and 10 ml neutralizer, and shaken for 1 min at 150 rev min^{-1}. After 5 min contact time, 1 ml sample of each media was plated out in triplicate on TSA and was incubated overnight at 37°C for *Staph. epidermidis* and MRSA and 30°C for *B. subtilis*. The actual number of cells removed from the disc was calculated by subtracting the number of cells recovered after wiping from the number of cells initially on disc.

The bactericidal efficacy of wipes was tested by direct inoculation of the wipes with the bacterial or spore suspension (Williams *et al.* 2007). A section of test wipe (alcohol impregnated wipe), control wipe (unmedicated) and wipe sprayed with ethanol were prepared and directly inoculated with 20 μl of test suspension (approx. 2 $\log_{10}$ CFU). After 100 s exposure, wipes were transferred into 100 ml sterile flat-bottomed glass bottle, containing 5 g of glass beads and 10 ml neutralizer, and shaken for 1 min at 150 rev min^{-1}. After 5 min contact, 1 ml sample of each inoculated wipe was transferred to TSA and plates incubated overnight at 37°C.

To evaluate the risk of wipes transferring bacterial bioburden between surfaces an adpression test was performed (Williams *et al.* 2007). After surface wiping as described above, eight TSA plates were successively inoculated by pressing the wipes on the surface exerting weight of 100 g (±5 g) and incubated overnight at 37°C for MRSA or *Staph. epidermidis*, and at 30°C for *B. subtilis*.

Each experiment was performed in triplicate. Statistical analysis was performed by using one-way analysis of variance (ANOVA) and Kruskal–Wallis Test (Minitab® software, PA, USA).

Table 1 Comparison of efficacy between the alcohol impregnated wipe Klerwipe™ - 70/30 and the nonimpregnated Klerwipe - 100™ sprayed with ethanol 70% v/v

	Impregnated Klerwipe™ - 70/30			Dry sterile Klerwipe - 100™ sprayed with ethanol 70% v/v		
	Spores of *Bacillus subtilis*	*Staphylococcus epidermidis*	Methicillin resistance *Staphylococcus aureus* (MRSA)	Spores of *B. subtilis*	*Staph. epidermidis*	MRSA
Efficacy to remove bioburden from surfaces						
Log_{10} ± SD CFU/spores inoculated	1·92 ± 0·01	1·96 ± 0·01	1·89 ± 0·01	2·17 ± 0·00	2·04 ± 0·00	1·90 ± 0·01
Log_{10} ± SD CFU/spores remaining on the surface following wipping	0·00*	1·77 ± 0·03	0·53 ± 0·12	2·02 ± 0·02	1·89 ± 0·01	1·71 ± 0·03
Efficacy of wipes to kill inoculum						
Log_{10} ± SD reduction in viable cell	1·05 ± 0·01	0·78 ± 0·10	0·90 ± 0·0	0·68 ± 0·03	0·55 ± 0·04	0·78 ± 0·0
Ability of wipes to transfer bioburden						
Number of consecutive transfer showing growth	0	2	0	4	3	4

*no recovery on any occasions

The diluent TSC was shown to quench effectively the antimicrobial effect of the impregnated, or alcohol sprayed wipes (data not shown). There was no significant difference between the number ($P > 0·05$) of *Staph. epidermidis* and MRSA used to inoculate disc surface for each experimental replicate. The efficacy of the wipes to remove microbial bioburden from the surface after 10 s wiping is shown in Table 1. Although, MRSA and the spores of *B. subtilis* were readily removed from the surfaces by impregnated wipes, such removal was not observed with *Staph. epidermidis*. The alcohol impregnated wipes removed a higher number of vegetative bacteria and spores dried on the surface than the dry wipes sprayed with alcohol ($P > 0·05$).

The ability of the wipes to transfer the bacterial bioburden to other surfaces was tested using multiple adpression to eight consecutive TSA plates. The number of cells inoculated and dried on the disks before wiping was between 83 and 148 spores (*B. subtilis*), and between 78 and 110 CFU (MRSA/*Staph. epidermidis*). When the impregnated wipes were used, no transfer was observed for the spores of *B. subtilis* or for MRSA. However, *Staph. epidermidis* was transferred to the first two consecutive plates. When the dry wipes sprayed with alcohol were used the spores of *B. subtilis*, and MRSA were transferred to the first four consecutive plates, and *Staph. epidermidis* to the first three plates. The number of viable cells transmitted to TSA plates was gradually reduced from approximately five to one with the successive adpression (data not shown). The number of cells transferred to consecutive plates was consistent and not different ($P > 0·05$) between the three replicate experiments (data not shown).

Direct inoculation method was used for measuring wipe bactericidal and sporicidal activity. The control consisted of dry unmedicated wipes. Approximately 2 log_{10} were inoculated onto the wipes. After 10 s contact the number of viable cells recovered from the control unmediated wipes were 1·94 ± 0·01, 2·02 ± 0·01 and 1·91 ± 0·01 for *B. subtilis*, *Staph. epidermidis* and MRSA, respectively. There was no significant difference ($P > 0·05$) between the numbers of spores or CFU inoculated onto the control wipes compared with the numbers recovered from these wipes. Both the alcohol impregnated wipes and the dry wipes sprayed with alcohol showed a poor sporicidal and bactericidal activity (Table 1).

Assurance of sterility during aseptic manufacturing of pharmaceutical products relies heavily on appropriate disinfection regimens. Presence of bioburden on materials is an important risk factor and steps taken to minimize microbial bioburden will decrease the risk of contamination to the final product (Beaney 2006). Disinfection procedures must be carried out with a suitable disinfectant and effective wiping regimen (Hiom *et al.* 2004; Beaney 2006; Medicines and Healthcare products Regulatory Agency (MHRA) 2007). However, until now, there have been no appropriate techniques that could provide an accurate, quantitative measure of wipe antimicrobial efficacy. The development of a quantitative three steps protocol by Williams *et al.* (2007) enabled a robust comparison between two wipe products for the transfer disinfection of materials during aseptic manufacturing. The results from this study clearly highlighted the difference in activity between the alcohol-impregnated wipe and the dry wipes sprayed with alcohol and raises concerns about the use of the later. The dry wipes sprayed with alcohol demonstrated minimal effect at reducing the bacterial bioburden on a surface and were able to transfer viable micro-organisms between surfaces. Transfer

disinfection ensures that surfaces have as low a bioburden as practically possible and is an essential part of QA during aseptic manufacturing (Beaney 2006). The inability of a wipe to kill micro-organisms is tentatively linked to the ability to transfer the micro-organisms to other surfaces (Williams *et al.* 2007).

In this short study the microbial bioburden was low to reflect conditions found in practice. Hence, some results, notably the adpression test could not be statistically analysed. However, the continuous transfer of bacteria following the use of the dry wipes sprayed with alcohol is of concern.

In conclusion, this study reflected conditions found in practice where two main wipe procedures for the transfer disinfection of materials used for aseptic manufacturing were observed: alcohol pre-impregnated wipes *vs* dry wipes sprayed with alcohol. Until now, no clear evidence existed to the superiority of either procedure. However, our results clearly demonstrated, in a quantitative manner, that alcohol pre-impregnated wipes are more effective at reducing surface bioburden then dry wipes sprayed with alcohol.

Acknowledgement

The authors wish to thank Paul Spark (Cardiff & Vale NHS Trust) for providing the wipes and recommendations on their use during aseptic manufacturing of pharmaceutical products.

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