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### **Residual activity of three chemicals supplied by Quadralene**

The products supplied were Biocleanse concentrate (Benzalkonium chloride based product), a proprietary peracetic acid based formulation, and, Bleach (Sodium hypochlorite based product).

#### Preparation of inoculums

A methicillin resistant strain of *Staphylococcus aureus* (MRSA), NCTC 11940, was used for the test strain. An overnight culture of NCTC 11940 was grown up in nutrient broth at 37°C and the final count ascertained by the surface drop (Miles and Misra) technique.

#### Test for residual activity

A 1ml volume of Biocleanse concentrate at 5%v/v dilution was pipetted and spread over a defined area of 5 x 5 cm (25 cm<sup>2</sup>) and allowed to dry in a laminar flow cabinet. On the tile there were eight 5 x 5 cm<sup>2</sup> squares, each receiving 1 ml of the Biocleanse concentrate. The squares were labelled neat 0 hrs, neat 4 hrs, neat day 7, neat day 28, -2 dilution 0 hrs, -2 dilution 4 hrs, -2 dilution day 7 and -2 dilution day 28. The same was carried out with the peracetic acid based formulation at 5%v/v dilution and the Bleach at 10%v/v dilution.

A volume of 0.1 ml of a neat MRSA suspension containing a known number of colony forming units was spread over the neat 0 hrs square on the biocleanse, peracetic acid and bleach tile. 0.1 ml of a -2 dilution from the neat MRSA was spread on the -2 dilution 0 hr square on the biocleanse, peracetic acid and bleach tile. For each chemical, the tile was swabbed 5 minutes after inoculation to determine the level of surviving organisms. Each swab was then placed in 10 ml of TLTR neutralising solution (containing Tween 80, lecithin, sodium thiosulphate, ringers and maximum recovery diluent), pulverised vigorously, then plated onto blood agar plates using 0.5 ml, 0.1 ml and 10µl volumes. The TLTR neutralising solution is the standard neutralising solution used in the HPA-WEMS laboratory for challenge and residual activity testing.

Once the inoculum had been absorbed, the plates were incubated aerobically at 37°C for 18-24 hours, and the number of colonies counted and recorded.

Four hours after the initial inoculation of the tiles, 0.1 ml of the same neat and -2 MRSA overnight suspension was spread over a new defined 5 x 5 cm (25 cm<sup>2</sup>) area, neat 4 hrs for the neat MRSA and -2 dilution 4 hrs for the -2 MRSA dilution, on each tile, and the same method as above was followed.

One week later, and again at day 28, each tile was re-inoculated in the same way using a fresh neat and -2 dilution MRSA suspension and the procedure previously described repeated.

A control was carried out by pipetting 0.1 ml of a neat MRSA suspension onto a defined 5 x 5 cm (25 cm<sup>2</sup>) area on a tile with no chemicals added. After 5 minutes, the tile was swabbed, placed in 10 ml of TLTR neutralising solution, pulverised vigorously, then plated onto blood agar plates and incubated aerobically with the test plates.

## Results

### Recovery of MRSA from coated tiles

Number of hours/days following coating	Inoculum level of MRSA (cfu/0.1ml)	Chemical used on tile	Recovery after 5 min	
			Colony forming units/swab	log reduction*
0 hours	2.3x10 <sup>7</sup>	Biocleanse	<20	6.0
	2.3x10 <sup>7</sup>	Peracetic acid	5.0x10 <sup>5</sup>	1.6
	2.3x10 <sup>7</sup>	Bleach	<20	6.0
4 hours	2.3x10 <sup>7</sup>	Biocleanse	<20	6.0
	2.3x10 <sup>7</sup>	Peracetic acid	9.0x10 <sup>6</sup>	0.4
	2.3x10 <sup>7</sup>	Bleach	4.0x10 <sup>5</sup>	1.7
Day 7	2.0x10 <sup>7</sup>	Biocleanse	6.0x10 <sup>3</sup>	3.5
	2.0x10 <sup>7</sup>	Peracetic acid	9.9x10 <sup>6</sup>	0.3
	2.0x10 <sup>7</sup>	Bleach	1.5x10 <sup>6</sup>	1.1
Day 28	2.5x10 <sup>7</sup>	Biocleanse	5.3x10 <sup>3</sup>	3.7
	2.5x10 <sup>7</sup>	Peracetic acid	1.1x10 <sup>7</sup>	0.3
	2.5x10 <sup>7</sup>	Bleach	5.7x10 <sup>6</sup>	0.6
Control	2.6x10 <sup>7</sup>	N/A	9.8x10 <sup>6</sup>	0.4

- 1 log reduction is equivalent to a 10 fold drop in numbers

For the zero hour time period for the tiles inoculated with the MRSA organism, the Biocleanse and bleach covered tiles returned a 6 log reduction in actual counts. The peracetic acid covered tile returned a 1.6 log reduction in actual counts. The actual counts for each plate can be seen in appendix 1.

After the tiles had been coated with the three chemicals for 4 hours, the actual counts (MRSA) returned for the Biocleanse remained a 6 log reduction, but it had fallen for the bleach (a 1.7 log reduction in actual counts) and for the peracetic acid (a 0.4 log reduction in actual counts).

When the tiles were re-inoculated with MRSA one week later the Biocleanse tile log reduction was 3.5 log. The bleach tile count had fallen to a 1.1 log reduction in actual counts, while the peracetic acid tile had a 0.3 log reduction in microbial counts.

After 28 days, the log reduction for MRSA, for the Biocleanse tile was 3.7 log in microbial counts. The bleach overall log reduction had fallen to 0.6 log in microbial counts while the peracetic acid overall log reduction was 0.3 log.

The control result shows that there is a 0.4 log recovery reduction using the above method when no disinfectant is added to the tile.

On the tile dosed with bleach, it was also noticed that on the 4 hour, day 7 and day 28 testing times, the bleach had dried and left behind a grainy white crusty residue.

### Conclusion

Results on day 0 after the first challenge indicate that Biocleanse coated onto a tile is effective in killing very high levels ( $10^7$  colony forming units) of MRSA within 5 minutes. The same can be said for the bleach, but not the peracetic acid based formulation. In fact after 4 hours the peracetic acid based formulation log reduction was the same as the control log reduction, indicating no residual activity. Following re-inoculation of MRSA onto the tiles after various time periods, some residual activity could still be demonstrated 28 days after the Biocleanse coating had been applied to the tile. At this time (28 days) we could not detect residual activity for bleach or acetic acid.

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## Appendix 1

Result from colony counts on plates.

Chemical	Time Period	Dilution factor	Count on plate		
			0.5ml spread	0.1ml spread	0.01ml spread
Biocleanse	0 hr	Neat	0	0	0
Biocleanse	0 hr	-2	0	0	0
Biocleanse	4 hr	Neat	0	0	0
Biocleanse	4 hr	-2	0	0	0
Biocleanse	7 day	Neat	TNTC	60	3
Biocleanse	7 day	-2	19	4	0
Biocleanse	28 day	Neat	TNTC	53	5
Biocleanse	28 day	-2	0	0	0
Peracetic acid	0 hr	Neat	TNTC	TNTC	500
Peracetic acid	0 hr	-2	0	0	0
Peracetic acid	4 hr	Neat	TNTC	TNTC	TNTC
Peracetic acid	4 hr	-2	TNTC	TNTC	90
Peracetic acid	7 day	Neat	TNTC	TNTC	TNTC
Peracetic acid	7 day	-2	TNTC	TNTC	99
Peracetic acid	28 day	Neat	TNTC	TNTC	TNTC
Peracetic acid	28 day	-2	TNTC	TNTC	114
Bleach	0 hr	Neat	0	0	0
Bleach	0 hr	-2	0	0	0
Bleach	4 hr	Neat	TNTC	TNTC	TNTC
Bleach	4 hr	-2	TNTC	40	10

Chemical	Time Period	Dilution factor	Count on plate		
			0.5ml spread	0.1ml spread	0.01ml spread
Bleach	7 day	Neat	TNTC	TNTC	TNTC
Bleach	7 day	-2	TNTC	153	13
Bleach	28 day	Neat	TNTC	TNTC	TNTC
Bleach	28 day	-2	TNTC	TNTC	57

TNTC = Too numerous to count.