Residual Activity Test
- Clinell Universal Sanitising Wipes

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Project Report Prepared for GAMA Healthcare Ltd

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Summary

The ability of Clinell Universal Sanitising Wipes to disinfect contaminated steel surfaces has been assessed previously (HMS 2006). This report describes follow on studies investigating the residual biocidal activity present on a surface following the use of these Wipes. Residual activity was assessed using hospital grade stainless steel discs (7cm dia) which were wiped, left for a period of time (24, 48 & 72 hours) and then contaminated with 200µl of *Staphylococcus aureus* broth for 5 minutes. After 5 minutes the bacteria remaining on the surface were swabbed off and resuspended in neutraliser. Residual activity was assessed by comparing the numbers of bacteria recoverable from a wiped disc with the numbers recoverable from an unwiped control. The investigation was carried out in triplicate, in a dry atmosphere at room temperature (20-25^{o}C).

After 24 and 48 hours the disc retained sufficient residual activity to produce greater than a 3 $\log_{10}$ reduction in numbers of recoverable bacteria. After 72 hours the activity dropped slightly generating a $\log_{10}$ reduction in recoverable bacteria just below 3. In percentage terms the Wipes were able to generate sufficient residual biocidal activity to maintain a 99% reduction in bacterial load over a period of 72 hours. In reality residual activity after 24 hours is likely to have been greater than reported here since values of zero recoverable bacteria have been recorded as <15 cfu[1]. This gives the 24 hour results an elevated count and a reduced estimate of residual activity.

These results indicate that the Clinell Universal Sanitising Wipes impart significant residual activity to steel surfaces. This residual activity is maintained for up to 72 hours at least, when the surfaces wiped are kept in a dry atmosphere at room temperature (20-25^{o}C). The test surfaces used in this study were challenged with a high bacterial loading (>1x10^{6} cfu cm^{-2}), much higher than you would expect from hand/finger contamination. Consequently it is likely that Clinell Universal Sanitising Wipes impart sufficient residual biocidal activity to steel surfaces to protect against casual contact contamination.

[1] Cfu = Colony forming Units

Biocidal Surface Test – Clinell Universal Sanitising Wipes.

1 Introduction

The ability of Clinell Universal Sanitising Wipes to disinfect contaminated steel surfaces has been assessed previously (HMS 2006). This report describes follow on studies investigating the residual biocidal activity present on a surface following the use of these Wipes. Residual activity was assessed using hospital grade stainless steel discs (7cm dia) which were wiped, left for a period of time (24, 48 & 72 hours) and then contaminated with 200µl of *Staphylococcus aureus* broth for 5 minutes. After 5 minutes the bacteria remaining on the surface were swabbed off and counted. Residual activity was assessed by comparing the numbers of bacteria recoverable from a wiped disc with the numbers recoverable from an unwiped control. The investigation was carried out in triplicate, in a dry atmosphere, at room temperature (20-25^{o}C).

2 Experimental Procedure

2.1 Preparation of Test Discs

All discs used in this study were made of Hospital grade stainless steel. The discs were cleaned by soaking for >12 hours in 5% Decon 90 solution. They were then rinsed in distilled water and sterilized by soaking in 70% isopropyl alcohol for 12 hours. Excess isopropyl alcohol was poured off and the discs dried by evaporation in a Class II safety cabinet.

2.2 Preparation of the contamination fluid

The contamination fluid consisted of a 24 hour culture of *Staphylococcus aureus* (ATCC ????) grown at 37°C in Tryptone Soya broth (TSB). Prior to use the numbers of cfu/ml in the culture was determined via serial dilution in MRD[2] and duplicate pour plates using Tryptone Soya Agar (TSA). Plates were incubated at 37^{o}C for 24 to 48 hours.

2.3 Residual Activity Testing

Eighteen clean, sterile, steel discs were placed in sterile Petri dishes. Nine of these discs were each wiped with a single Clinell Universal Sanitising wipe. The discs were wiped for between 2 and 5 seconds and care was taken to ensure the entire disc was covered. The other nine discs were left unwiped to act as controls. At the start of the experiment all eighteen discs were exposed to the atmosphere by removing the Petri dish lids.

After each desired time period (24, 48 or 72 hours) 3 wiped discs and 3 unwiped discs were inoculated with 200µl of contaminating fluid, which was spread across the discs with a sterile plastic spreader. The contaminating fluid was left in contact with the discs for 5 minutes. After 5 minutes the discs were swabbed with two dry, sterile swabs and the swabs placed in 10ml of an

[2] Maximum Recovery diluent supplied by LabM

appropriate neutraliser. After neutralisation the neutralising broth was serially diluted in MRD and the numbers of cfu recovered determined by duplicate pour plates using TSA. Plates were incubated at 37^{o}C for 24 to 48 hours.

2.4 Data Analysis

Residual activity was determined by comparing the numbers of cfu recovered from the control discs with the numbers recovered from the wiped discs at each time period. The impact of residual activity being expressed as log_{10} reductions and percentage reductions in cfu recovered.

3 Results[3]

Expressed as Log_{10} reductions (Figure 1) in recoverable cfu the Wipes generated sufficient residual activity after 24 and 48 hours to produce a 3.4 log_{10} reduction and a corresponding 99.95% reduction (Figure 2). After 72 hours the residual activity dropped slightly producing a Log_{10} reduction of 2.8 and a percentage reduction of 99.7%. Beyond 48 hours the reproducibility of the results lessened as can be seen by the standard error about the mean (Figure 1).

The actual residual activity present after 24 hours is greater that that calculated since zero plate counts were recorded as <15 and included in the calculations as 15. This suggests that the actual Log_{10} reduction is somewhere between 4.6 and 3.4 and the percentage reduction between 99.997% and 9.95%.

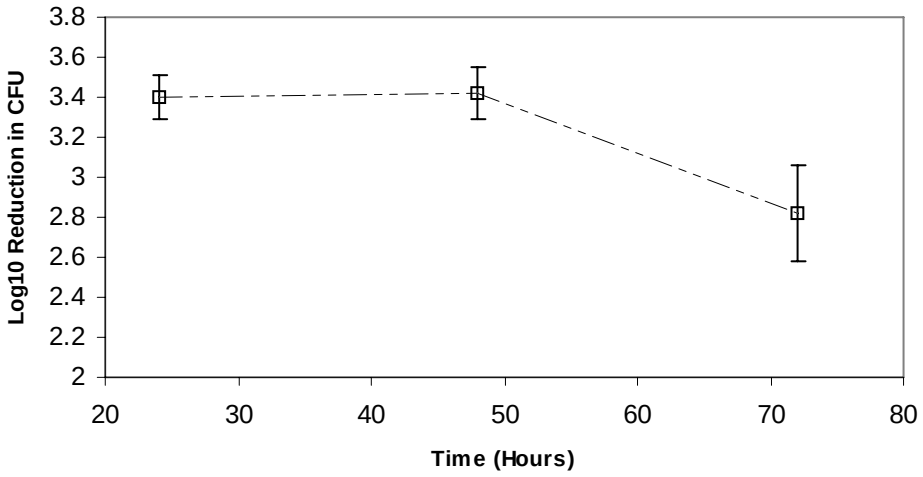

Figure 1. Impact of Residual Biocidal Activity on Recoverable CFU.

[3] Raw data can be found in the appendix

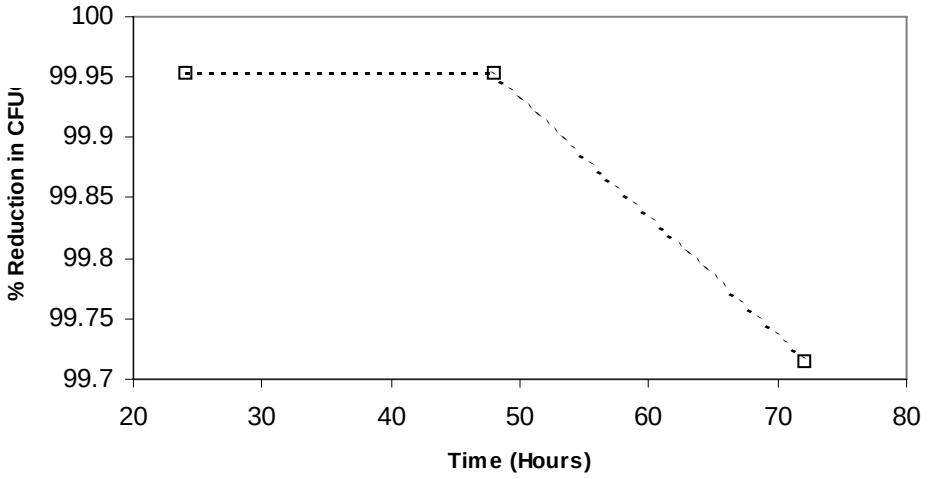

Figure 1. Impact of Residual Biocidal Activity on the Percentage of CFU recoverable.

4 Summary and Conclusions

On the basis of this investigation it is apparent that Clinell Universal Biocidal Wipes provide significant residual biocidal protection to steel surfaces. This protection is significant for up to 72 hours but is most active after 24 hours.

5 References

Huddersfield Microbiology Services (2006)

Appendix: Experimental Data

24 Hour Residual Test Results

Contaminating Broth			
Plate	-7	-8	-9
1	65	8	1
2	89	3	2

Control Discs			
Plate	-4	-5	-6
a1	40	5	0
a2	35	0	0
b1	80	7	0
b2	75	7	1
c1	17	1	0
c2	20	2	0

Test Discs			
Plate	-1	-2	-3
a1	0	0	0
a2	0	0	0
b1	0	0	0
b2	0	0	0
c1	0	0	0
c2	0	0	0

Disks In Agar	
Wipe 1	0
Wipe 2	0
Wipe 3	0
Control 1	tmc
Control 2	tmc
Control 3	tmc

48 Hour Residual Test Results

Broth			
Plate	-7	-8	-9
1	114	7	0
2	89	2	0

Controls			
Plate	-4	-5	-6
a1	Tmc	71	7
a2	Tmc	76	9
b1	Tmc	80	6
b2	Tmc	87	10
c1	Tmc	105	14
c2	Tmc	102	13

Wipes				
Plate	-1	-2	-3	-4
a1	Tmc	33	2	0
a2	Tmc	35	3	0
b1	Tmc	68	4	1
b2	Tmc	62	11	0
c1	169	17	6	0
c2	164	15	4	0

72 Hour Residual Test Results

Broth			
Plate	-7	-8	-9
1	116	10	0
2	105	12	2

Controls			
Plate	-4	-5	-6
a1	tmc	136	11
a2	tmc	154	7
b1	tmc	114	13
b2	tmc	145	18
c1	tmc	149	12
c2	tmc	144	10

Wipes				
Plate	-1	-2	-3	-4
a1	tmc	256	14	0
a2	tmc	261	20	2
b1	tmc	tmc	86	6
b2	tmc	tmc	77	4
c1	tmc	42	2	0
c2	tmc	50	9	1