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MICROBIOLOGICAL TEST REPORT

DATE of REPORT: 26/11/14

CUSTOMER: CMC Hygea Ltd, Ballymaclode Woods, Dunmore Road, Co. Waterford
Attention: Mr Derrick Watson

SAMPLES:

- 3 x Unused ABLiS devices supplied by CMC as negative controls
- 3 x ABLiS devices (5 month usage Beacon Hospital, 90^0C post-clinical use wash, same manufacture batch as unused controls)
- 1 x standard polypropylene basin from regular usage in Beacon Hospital

SAMPLES RECEIVED: 23/10/2014

CONDITION: All sample materials received intact and at ambient temperature

ANALYSES REQUIRED: Comparative antibacterial activity testing

TEST METHOD: BS ISO 22196:2011 ***Measurement of antibacterial activity on plastics and other non-porous surfaces.***

Options used in testing:	Sample size:	5cm x 5cm
	Cover film size:	4cm x 4cm
	Inoculum volume:	0.3ml
	Recovery volume:	10ml
	Exposure times:	24 h and 72 h at RH >95%
	Incubation temperature:	35^0C
	Challenge controls:	0 time on standard polypropylene
	Challenge species:	*Staphylococcus aureus* ATCC 6538P
		Pseudomonas aeruginosa Clinical isolate
		Escherichia coli ATCC 8739
		VR *Enterococcus faecalis* Clinical isolate
		MR *Staphylococcus aureus* Clinical isolate

Neutralising solution:

A combination of 5% sodium thioglycolate, 1% sodium thiosulphate and 2.5% sodium bisulphite was added to SCDLP neutralizing broth for the neutralization of silver) (Liau, S.Y., Read, D.C., Pugh, W.J., Furr, J.R., Russell, A.D. "Interaction of silver nitrate with readily identifiable groups : relationship to the antibacterial action of silver ions." *Letters in Applied Microbiology*. 1997. Volume 25, issue 4. p. 279-283

DATE TESTING COMMENCED: 27/10/14 et seq

Introduction

CMC Hygea Ltd have developed a polypropylene utility basin for patient hygiene activities in a clinical setting. The basin is designed for use with a fitted disposable inner liner. Both basin and liner are treated with embedded antimicrobial agent designed to reduce infectious risk from cross-contamination, handling and repeated use.
The device is known by the acronym ABLiS (Antimicrobial Basin Liner System).

A number of these antimicrobial-treated ABLiS devices have been in daily use at the Beacon Hospital, Sandyford, Dublin 18 for 5 months alongside regular untreated polypropylene basins.

The aim of the current study was to compare samples taken from both used ABLiS devices and standard basins and compare their ability to inhibit growth or reduce challenge doses of a selection of bacteria applied to their surfaces for periods of 24 or 72 hours.

In addition, the same tests were applied to unused ABLiS devices, enabling a comparison between antimicrobial efficacy when new and after a period of use. This can reveal loss of efficacy due to leaching of antimicrobials or alterations of the surface layer.

An International Standard Method, **BS ISO 22196:2011** ***Measurement of antibacterial activity on plastics and other non-porous surfaces,*** was used to determine the antimicrobial efficacy. For the purposes of the current test, several modifications to the method were made, including extending the range of challenge bacterial species and the periods of exposure to the sample surface. Any modifications made to the parameters of the test are set out above under Test Method.

In summary, standard sized samples were cut and prepared from the ABLiS devices and regular basin. These were cleaned and sanitized using 70% alcohol. Five bacterial species were cultured and prepared to exact concentrations. These were then applied to the test samples under aseptic conditions in precise amounts and allowed to remain in contact with the sample for a defined period of time, in a controlled environment. The tests were done in triplicate. The bacterial inocula were recovered at time zero, after 24 hours or after 72 hours and the changes in bacterial count were assayed, giving a measure of the antimicrobial efficacy of the respective sample surfaces.

Validation testing was undertaken to establish suitable recovery protocols and to confirm neutralisation efficacy of the recovery medium for up to 3% silver nanoparticles.
Validation testing was repeated on three occasions to confirm reproducibility. When this was satisfactorily completed, the samples were subjected to the test protocols. See Appendix 1.

RESULTS

24 Hour Challenge testing using *Staphylococcus aureus* ATCC 6538P

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 24 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	3.2E+04	2.8E+04	- 0.05	-
ABLiS device (new, unused)	4.4E+04	5.1E+00	- 3.94	>99.9
ABLiS device (after 5 months use)	3.8E+04	1.4E+01	- 3.43	>99.9

72 Hour Challenge testing using *Staphylococcus aureus* ATCC 6538P

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 72 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	5.1E+04	3.8E+04	-0.12	-
ABLiS device (new, unused)	4.7E+04	2.0E+00	-4.37	>99.99
ABLiS device (after 5 months use)	4.4E+04	<1.0	≥4.64	>99.99

24 Hour Challenge testing using MR *Staphylococcus aureus* Clinical isolate

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 24 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	6.2E+04	5.4E+04	-0.06	-
ABLiS device (new, unused)	6.4E+04	2.1E+00	-4.40	>99.99
ABLiS device (after 5 months use)	5.8E+04	2.4E+00	-4.38	>99.99

72 Hour Challenge testing using MR *Staphylococcus aureus* Clinical isolate

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 72hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	3.7E+04	3.5E+04	-0.02	-
ABLiS device (new, unused)	3.9E+04	<1.0	≥4. 59	>99.99
ABLiS device (after 5 months use)	3.5E+04	2.2E+00	-4.20	>99.99

24 Hour Challenge testing using *Pseudomonas aeruginosa* Clinical isolate

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 24 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	4.1E+04	5.2E+04	+0.10	-
ABLiS device (new, unused)	4.4E+04	1.1E+00	-4.60	>99.99
ABLiS device (after 5 months use)	4.0E+04	1.4E+00	-4.45	>99.99

72 Hour Challenge testing using *Pseudomonas aeruginosa* Clinical isolate

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 72 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	5.1E+04	5.7E+04	+0.04	-
ABLiS device (new, unused)	4.9E+04	1.1E+00	-4.65	>99.99
ABLiS device (after 5 months use)	5.1E+04	<1.0	≥4. 70	>99.99

24 Hour Challenge testing using *Escherichia coli* ATCC 8739

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 24 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	3.9E+04	3.4E+04	-0.06	-
ABLiS device (new, unused)	3.6E+04	<1.0	≥4. 55	>99.99
ABLiS device (after 5 months use)	3.6E+04	<1.0	≥4. 55	>99.99

72 Hour Challenge testing using *Escherichia coli* ATCC 8739

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 72 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	3.7E+04	4.0E+04	+0.03	-
ABLiS device (new, unused)	3.5E+04	<1.0	≥4. 54	>99.99
ABLiS device (after 5 months use)	3.7E+04	<1.0	≥4. 57	>99.99

24 Hour Challenge testing using VR *Enterococcus faecalis* Clinical isolate

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 24 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	4.1E+04	3.8E+04	-0.03	-
ABLiS device (new, unused)	4.5E+04	8.1E+01	-2.75	>99.0
ABLiS device (after 5 months use)	3.9E+04	4.4E+01	-2.95	>99.0

72 Hour Challenge testing using VR *Enterococcus faecalis* Clinical isolate

Test sample	Exposure period Time Zero Average colony count/cm^2 from triplicate tests	Exposure period 72 hours Average colony count/cm^2 from triplicate tests	Log change In count from Time Zero	% Reduction
Untreated polypropylene	3.8E+04	3.8E+04	0	0
ABLiS device (new, unused)	3.8E+04	1.4E+00	-4.44	>99.99
ABLiS device (after 5 months use)	4.3E+04	1.1E+00	-4.59	>99.99

Conclusions

The results clearly show that there is little, if any, reduction in bacterial counts on standard polypropylene for any of the bacterial species tested. *E.coli* and *Pseudomonas aeruginosa* counts were seen to increase slightly but not to any significant degree. This shows that under the ISO 22196 test conditions, high counts of bacteria were able to survive in viable form and were recoverable from standard polypropylene surfaces after 3 days.

Results recorded for the ABLiS devices showed a marked contrast to those recorded for polypropylene. The survival rates for all bacterial species tested were very significantly less than on polypropylene and this was regardless of whether the bacteria were in contact for 24 hours or 72 hours.
There was no significant difference between the ABLiS device antimicrobial performance if in new and unused state or after 5 months in use.
All ABLiS device samples were capable of reducing challenge bacterial counts by greater than 99% at 24 hours and by 99.99% at 72 hours.

This antimicrobial performance of the ABLiS device shows definite potential for infection control in clinical usage.

SIGNED: 

R. J. Russell Ph.D.
(Associate Professor of Microbiology)