Technical Report

Antimicrobial Testing to ISO 22196

For

Forbo Flooring B.V.

Final Report

Work Carried Out By

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PRA Ref: 75221-104A

12 October 2011

Global Surface Coatings Covered

Final Report

PRA Ref. Number	75221-104A
Date Received	19 September 2011
Date Issued	12 October 2011
Client	Forbo Flooring B.V. Industrieweg 12 1566 JP Assendelft Netherlands FAO: Jose Jak
Work Requested	Antimicrobial Testing to ISO 22196
Samples Submitted	1 sample of linoleum floor covering

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1 Materials Submitted For Testing

A sample of linoleum floor covering labelled Marmoleum, batch 30218, was submitted to be tested for antimicrobial activity according to ISO 22196: 2007 (Plastics – Measurement of antibacterial activity on plastic surfaces). This standard replaces JIS Z 2801: 2000.

Antimicrobial activity was to be determined against 2 bacterial species.

2 Test Procedure

Testing was carried out using a procedure based on ISO 22196: 2007 (formerly JIS Z 2801: 2000).

2 separate tests were carried out using the following organisms:

Staphylococcus aureus ATCC6538P

Staphylococcus aureus ATCC43300 (MRSA)

For each test organism 0.1 ml of a suspension containing approx. 5×10^5 cells was placed on the upper surface of triplicate test pieces (60mm x 60mm) of the sample and on triplicate samples of polystyrene sheet (used as the PRA control and known to have no antimicrobial activity). The suspension was held in intimate contact with the test and control surfaces using a polyethylene film rectangle, 20mm x 20mm in size.

To provide a time zero inoculation level, an additional triplicate set of PRA control samples (polypropylene film) were similarly inoculated, and the inoculum then immediately recovered from the surface using the method described below. The remaining replicates were incubated at 21°C and relative humidity of not less than 90%. After 24 hours incubation the inoculum was removed from the test surfaces (again using the method described below) and bacterial counts determined.

Owing to the porosity of the underlying surface of the test sample a modified procedure developed by the International Biodeterioration Research Group (IBRG) was used to recover the inoculum from the surfaces of the control pieces (at time zero and after 24 hours) and the test pieces (after 24 hours).

The polyethylene film covering the inoculum was removed with sterile forceps and placed in 10 ml of sterile neutralising medium. The surface which had been covered by the film was then thoroughly cleaned using a sterile cotton swab, and the untouched portion of this broken off into the neutralising medium. After vigorous agitation bacterial counts were determined on the washings.

3 Results and Observations

The microbial counts obtained (shown as a geometric mean), together with the antimicrobial activity (shown as a Log_{10} reduction) and the % kill, are given in the Tables. The antimicrobial activity was calculated as follows:

$$R = [\log (B/A) - \log (C/A) = [\log (B/C)]$$

where, R = antimicrobial activity

A = mean microbial count on PRA control sample at time zero

B = mean microbial count on PRA control sample after 24 hours

C = mean microbial count on test piece after 24 hours

Table 1: Antimicrobial activity against *Staphylococcus aureus*

Test Sample	Mean Count		Antimicrobial Activity	% Kill
	Initial count	24 hr count		
PRA Control	5.5×10^5	4.6×10^5	-	-
Marmoleum, batch 30218	-	1.5×10^2	3.5	> 99.9

Table 2: Antimicrobial activity against MRSA

Test Sample	Mean Count		Antimicrobial Activity	% Kill
	Initial count	24 hr count		
PRA Control	6.4×10^5	3.9×10^5	-	-
Marmoleum, batch 30218	-	< 10	> 4.6	> 99.9

4 Conclusion

The ISO standard 22196: 2007 specifies a method of evaluating the antimicrobial activity of antimicrobial-treated materials.

The predecessor to this ISO standard, JIS Z 2801: 2000, stated that for a coating to demonstrate antimicrobial efficacy the value of the antimicrobial activity shall not be less than 2.0. The ISO standard provides a means of quantifying the antimicrobial effectiveness of a surface in terms of antimicrobial activity, but no longer specifies a value for determining antimicrobial efficacy.

As a pass/ fail criterion is not defined in the current standard, PRA uses the following criterion to comment on the level of activity determined.

Antibacterial Activity	% Kill	Comment
<1.5	<96.8	poor
1.5 – 2.0	96.8 - 99.0	borderline
2.0 - 3.0	>99.0 - 99.9	good
>3.0	>99.9	excellent

Referring to the Tables, the test sample Marmoleum, batch 30218 demonstrated excellent antimicrobial activity against the 2 test organisms.

End of Report

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