

STUDY REPORT:	Determination of the Antibacterial Activity of Shield Sprayed Plastic Formulations against <i>Escherichia coli</i> and <i>Staphylococcus aureus</i> using ISO 22196: 2011.
CLIENT:	Chamco International Station House Station Road Shankill Dublin 18
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Study: Determination of the Antibacterial Activity of Shield Sprayed Plastics Formulations against *Escherichia coli* and *Staphylococcus aureus* using ISO 22196: 2011 (JIS Z 2801).

Number: IMSL 2017/11/019.1A

Client: Chamco International Station House Station Road Shankill Dubln 18

The above study was conducted in the laboratories of Industrial Microbiological Services Ltd at Pale Lane Hartley Wintney, Hants, RG27 8DH, UK. This report represents a true and accurate account of the results obtained.

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## 1 Introduction

This report summarises a study performed to compare the antibacterial performance of shield sprayed plastic formulations supplied by Chamco International using the method described in ISO 22196: 2011.

## 2 Test Materials

Samples of unfortified plastic plaques and spray concentrate AEGIS Microshield AEM 5700 were supplied by Chamco International. All samples were stored in the dark at 20°C prior to analysis. An unfortified polypropylene was suppled by IMSL to act as a reference material.

Prior to testing the spray solution was prepared using 3% AEGIS Microshield AEM 5700 and sterile distilled water. The spray solution was allowed to hydrolyse for 30 minutes prior to use. Replicate (6) plastics were sprayed and air dried for 4 weeks. Replicate (6) samples were sprayed and air dried for 7 days in sterile distilled water prior to testing. Replicate (6) samples were sprayed and air dried for 24 hours, and leached for 24 hours, and leached for 24 hours in sterile distilled water prior to testing. Replicate (6) samples were sprayed and air dried for 24 hours, and leached for 24 hours in sterile distilled water prior to testing. Replicate (6) samples were sprayed and air dried for 24 hours in sterile distilled water prior to testing.

#### 3 Methods

Antibacterial activity was determined using the method described in ISO 22196: 2011 (Ref 1).

#### 3.1 Determination of Antibacterial Activity

An aliquot  $(225\mu)$  of a log phase cell suspension of either *Escherichia coli*  $(5.1 \times 10^5 \text{ cells ml}^-)$ ; ATCC 8739) or *Staphylococcus aureus*  $(5.0 \times 10^5 \text{ cells ml}^-)$ ; ATCC 6538p) prepared using the method described in ISO 22196 : 2011 were held in intimate contact with each of 4 replicates of the test surfaces supplied using a 30 x 30 mm polyethylene film (cut from a sterile Stomacher bag) for 24 hours at 35°C. The size of the surviving population was determined using the method described in ISO 22196 : 2011.

The viable cells in the suspension were enumerated by spiral dilution on to Trypcase Soya Agar and by the pour plate method described in ISO 22196 These plates were incubated at 35°C for 24 hours and then counted.



## 4 **Results / Discussion**

The results are shown in Table 1 and 2.

Sample	Contact Time		Reduction From Control	
	0 hours	24 hours	Log	%
IMSL Polypropylene Control	1.1 x 10 <sup>4</sup>	7.4 x 10 <sup>4</sup>	-	-
Unsprayed Plastic	1.1 x 10 <sup>4</sup>	1.4 x 10 <sup>5</sup>	-	-
Air dried 24 hours	1.1 x 10 <sup>4</sup>	<u>&lt;</u> 1.0	<u>&gt;</u> 3.9	<u>&gt;</u> 99.99
Air dried 24 hours / Leached 24 hours	1.1 x 10 <sup>4</sup>	<u>&lt;</u> 1.0	<u>&gt;</u> 3.9	<u>&gt;</u> 99.99
Air dried 24 hours / Leached 7 days	1.1 x 10 <sup>4</sup>	<u>&lt;</u> 1.0	<u>&gt; 3.9</u>	<u>&gt;</u> 99.99
Air dried 4 weeks	1.1 x 10 <sup>4</sup>	<u>&lt;</u> 1.0	<u>&gt; 3.9</u>	<u>&gt;</u> 99.99

 Table 1: Activity Against Escherichia coli

 (Geometric Mean of Replicates as Colony Forming Units cm<sup>-2</sup>)

 $\ddagger$  The theoretical limit of detection is 1 CFU cm<sup>-2</sup>

It can be seen from the data above that the population of *Escherichia coli* exposed to the unsprayed Plastic increased in size by 1.1 orders of magnitude during the 24 hour contact period. This is considered a normal response for this species on an inert surface under the conditions imposed by ISO 22196. In contrast, the population of *Escherichia coli* held in contact with the surface of sprayed plastic, air dried for 24, air dried for 24 hours and leached for 24 hours and 7 days and the samples air dried for 4 weeks all declined by  $\geq$  3.9 orders of magnitude to below the limit of detection.

Sample	Contact Time		Reduction From Control	
	0 hours	24 hours	Log	%
IMSL Polypropylene Control	9.5 x 10 <sup>3</sup>	6.4 x 10 <sup>2</sup>	-	-
Unsprayed Plastic	9.5 x 10 <sup>3</sup>	<u>&lt;</u> 1.0	-	-
Air dried 24 hours	9.5 x 10 <sup>3</sup>	<u>&lt;</u> 1.0	-	-
Air dried 24 hours / Leached 24 hours	9.5 x 10 <sup>3</sup>	<u>&lt;</u> 1.0	-	-
Air dried 24 hours / Leached 7 days	9.5 x 10 <sup>3</sup>	<u>&lt;</u> 1.0	-	-
Air dried 4 weeks	9.5 x 10 <sup>3</sup>	<u>≤</u> 1.0	-	-

# Table 2: Activity Against Staphylococcus aureus (Geometric Mean of Replicates as Colony Forming Units cm<sup>-2</sup>)

 $\ddagger$  The theoretical limit of detection is 1 CFU cm<sup>-2</sup>

It can be seen from the results in Table 2 that the *Staphylococcus aureus* populations exposed to the surface of the unsprayed plastic samples declined to below the limit of detection over the 24 hour contact period. It is therefore not possible to assess the efficacy of AEG Microshield AEM 5700 for these samples against this organism.

## 5 Raw Data

The raw data for this study will be held in file IMSL 2017/11/019.1 in the Archive of IMSL at Pale Lane, Hartley Wintney, Hants, RG27 8DH, UK for 6 years from the date of this report unless other specific instructions are given.

## 6 References

1 ISO 22196:2011 Plastics - Measurement of antibacterial activity on plastics and other non-porous surfaces

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