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FINAL REPORT 2010/202 SAM

**EVALUATION OF ACTIVITY OF MICROORGANISM
(AATCC 30 - Test III)**

Study Program n.: 2010/202SAM

Contract n.: PANC2010009901

Sponsor: UNION FOAM SPA
Via Dell'Industria, 11
20040 Bellusco (MI)

Test Product: EUROBATE X

Study Director:
(Dr. S.Viganò)

S. Viganò

Date

16/03/2010

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SUMMARY

A study has been conducted on the test product EUROBATE X in order to determine its antimicrobial effectiveness according to AATCC Test Method 30-2004 and Sponsor's requirements.

Microorganisms used to verify antimicrobial effectiveness:

Microorganism	Supplier code
<i>Staphylococcus aureus</i>	ATCC 6538
<i>K. pneumoniae</i>	ATCC 10031
<i>Escherichia coli</i>	ATCC 10536
<i>Salmonella abony</i>	NCTC 6017
<i>Listeria monocytogenes</i>	ATCC19117

For each test strain, three Petri dishes containing culture medium agar (TSA) have been inoculated with 1 ml of test suspension., A specimen of test material, previously moistened with a solution of 0.05% in WFI of a non ionic wetting agent (Triton X-100) was placed, at the center of each plate.

The specimen was then inoculated with 0.2 ml of test suspension.

The plates have been incubated at 37 ° C ± 1 ° C for 2 days.

After incubation, the microbial growth developed in the Petri dishes was examined and the final result was expressed as presence or absence of zones of inhibition around the test specimen.

The following table summarizes the obtained results

Test strain	Repetition		
	Specimen 1	Specimen 2	Specimen 3
<i>S. aureus</i>	+	+	+
<i>K. pneumoniae</i>	+	+	+
<i>E. coli</i>	+	+	+
<i>S. abony</i>	+	+	+
<i>L. monocytogenes</i>	+	+	+

+ presence of inhibition zone - absence of inhibition zone

On the basis of the obtained results, the test product EUROBATE X causes inhibition of growth of test strains since, after incubation, the inhibition zone of about 1cm around the test material is present.

INTRODUCTION



A study has been conducted on behalf of UNION FOAM SPA in order to determine antimicrobial effectiveness of test product, according to AATCC Test Method 30-2004 and Sponsor's requirements.

The study has been conducted in the Biolab S.p.A. Test Facility located in Vimodrone (MI), via Bruno Buozzi, 2.

The experimentations started on 11/03/2010 and finished on 12/03/2010.

REFERENCES

AATCC Test method 30-2004 - Antifungal activity, Assessment on textile materials: mildew and rot resistance of textile materials

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TERMS AND DEFINITIONS

Antibacterial agent formulation chemical compound, or product manufactured to prevent growth of microorganisms on the surface of a product by inhibiting growth or by destroying the micro-organism

ARCHIVE

All the raw data will be filed in the archives of Biolab SpA for ten years after the issuing of the report. No retained sample has been kept. At the end of the period, the Client may request an extension of the conservation of all or part of the materials for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used are recorded in Biolab SpA Manual.

PRODUCT

<i>Name</i>	<i>Composition</i>
EUROBATE X	Not applicable

SAMPLE

The sample, representative of the entire batch, is a black round specimens with diameter about 3.8cm:

<i>Batch</i>	L24.02.2010
<i>Preparation Date</i>	24/02/2010
<i>Expiry date</i>	02/2015
<i>Stability</i>	5 years
<i>Receiving N.</i>	EUITVI7500
<i>Receiving Date</i>	03/03/2010
<i>#ID</i>	10.306-S

The characterization of the test product is under Sponsor responsibility.

MEDIA AND REAGENTS

The validity of media and reagents has been checked before starting the analyses

Reagent		Supplier	Amount
Tryptone water	NaCl	Merck	8,5 g
	Tryptone	Merck	1 g
	Water	-	to 1000 ml
Tryptone Soya Agar (TSA)		Merck	-
Triton X-100		Sigma	-

INSTRUMENTS

The validity of instruments and equipments have been assessed before starting the analyses

Equipments	Supplier	Model	Serial no	Reference
Thermostat @ 37°±1°C	MEMMERT	B80	850182	Qual. 58/08 Man. 05/10
Refrigerator	BOSH	KGV36X00F F/IE	1051	Qual. 109/08
Ordinary microbiology laboratory equipment				

ASSAY SYSTEM

The validity of bacterial strains and reference microorganism has been assessed before starting the analyses

Microorganism	Supplier
<i>Staphylococcus aureus</i>	ATCC 6538
<i>K. pneumoniae</i>	ATCC 10031
<i>Escherichia coli</i>	ATCC 10536
<i>Salmonella abony</i>	NCTC 6017
<i>Listeria monocytogenes</i>	ATCC19117

Strain maintenance

The bacterial strains are kept frozen; before the use they will be transplanted on TSA slants for a maximum of 4 passages; after the growth they have been kept in a refrigerator at 5 ± 3°C.

Preparation of test suspension

Each bacterial strain was grown in TSA at 37°C±1°C for 18-24 hours. Each culture was diluted with tryptone water to obtain a test suspension with a concentration about 10⁷ cfu/ml.

The number of cfu/ml of each microbial suspension has been counted by serial decimal dilutions and by seeding of 1 ml per plate, performed in duplicate.

EXPERIMENTAL PROCEDURE

-Test

For each test strain, three Petri dishes containing culture medium agar (TSA) have been inoculated with 1 ml of test suspension., A specimen of test material, previously moistened with a solution of 0.05% in WFI of a non ionic wetting agent (Triton X-100) was placed, at the centre of each plate. The specimen was then inoculated with 0.2 ml of test suspension.

The plates have been incubated at 37 ° C ± 1 ° C for 2 days.

After incubation, the microbial growth developed in the Petri dishes was examined and the final result was expressed as presence or absence of zones of inhibition around the test specimen.

-Negative control

Five specimens of test product have been separately placed in Petri dishes containing culture medium agar (TSA).

The plates have been incubated at 37 ° C ± 1 ° C for 2 days.

After incubation, the microbial growth developed in the Petri dishes was examined

RESULTS

-Test

Test strain	Repetition		
	Specimen 1	Specimen 2	Specimen 3
<i>S. aureus</i>	+	+	+
<i>K. pneumoniae</i>	+	+	+
<i>E. coli</i>	+	+	+
<i>S. abony</i>	+	+	+
<i>L. monocytogenes</i>	+	+	+

+ presence of inhibition zone - absence of inhibition zone

-Negative control

Repetition				
Specimen 1	Specimen 2	Specimen 3	Specimen 4	Specimen 5
-	-	-	-	-

+ presence of growth - absence of growth

DEVIATIONS

No deviation was found during the study.

CONCLUSION

On the basis of the obtained results, the test product EUROBATE X causes inhibition of growth of test strains since, after incubation, the inhibition zone of about 1cm around the test material is present.