Final report 2012/757 SAM

MEASUREMENT OF ANTIBACTERIAL ACTIVITY on BETACRYL (ISO22196:2007)

Study Program n.: 2012/757SAM

Contract n: PLTO2012015001

Sponsor: BTS S.r.l.
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Test Product: BETACRYL

Study Director: ..... Date May 28th, 2012
(F. Faccioli)

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SUMMARY

A study has been conducted on the test product "BETACRYL", in order to determine its antibacterial effectiveness against bacterial strain, according to International Standard ISO 22196:2007 and according to Sponsor's requirements.
To this purpose, the Sponsor provide to Eurofins Biolab S.r.L. specimens treated with antibacterial agent "BETACRYL", the negative control (specimens untreated) was provided by Eurofins Biolab S.r.L..
Microorganism used to verify antimicrobial effectiveness:
Escherichia coli ATCC 8739
Staphylococcus aureus ATCC6538P

On the basis of the obtained results, in compliance with the assay validity criteria, the test product "BETACRYL" **has antibacterial activity** since it causes, after the applied contact time of 24h, a reduction of the bacterial viability greater than 1 Log (% reduction >90%) compared to negative control (untreated specimens).

INTRODUCTION

A study has been conducted on behalf of BTS S.R.L. in order to determine its antibacterial effectiveness against bacterial strain, according to International Standard ISO 22196:2007 and according to Sponsor's requirements.
The study was performed at the Test Facility Eurofins Biolab S.r.L. of Vimodrone (MI) –via B. Buozzi n. 2.

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Antibacterial effectiveness	May 15th, 2012	May 18th, 2012	D. Donvito

REFERENCES

- JIS Z 2801:2000 Antimicrobial products-Test for antimicrobial activity and efficacy
- ISO22196, October 2007: Plastics. Measurement of antibacterial activity on plastics surfaces

FILING

The study program with possible amendments, raw data with possible deviations and a copy of the final report with possible revisions, are stored in Eurofins Biolab S.r.L. archives for a period of 10 years starting from the end of the study.
At the end of the study the samples have not been kept.
The Sponsor, drawing up of a suitable contract, may request an extension of the conservation of all or part of the materials for a further period or their restitution.

PROCEDURES

All procedures used during this study are recorded in the Eurofins Biolab S.r.L. Procedures Manual.

PRODUCT

Product	BETACRYL
Stability	Not provided
Composition	Not provided
CoA	Not provided

SAMPLE

Batch	N01-2
Manufacturing date	Not provided
Expiry date	Not provided
Receiving	EUITVI-25450
Date	April 26th, 2012
#ID	12.1071-S

The characterization of the test product is under Sponsor responsibility.

Experimental Report 2012/757 – Measurement of antibacterial activity

EXPERIMENTAL PROCEDURES

ASSAY SYSTEM

Microorganisms

Identification and preservation of the strains

Escherichia coli ATCC 8739 and Staphylococcus aureus ATCC6538P strains were used.
The bacterial strain is kept frozen; before the use it will be transplanted on TSA slants for a maximum of 4 passages; after the growth it has been kept in a refrigerator at 5 ± 3°C.

Preparation of test suspension

The bacterial strain was grown in TSA at 35°C±1°C for 18-24 hours. Within 2 h from beginning of the test, the culture was diluted with TSB to obtain a test suspension with a concentration between $2.5x10^5$ and $1x10^6$ 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.

MEDIA AND REAGENTS

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

- *Tryptone water*		
NaCl	8,5 g	Merck
Tryptone	1 g	Merck
Water to	1000 ml	
- *Saboraud Dextrose Agar (SDA)*		Merck
- *Tryptone Soy Agar (TSA)*		Merck
- *Neutralizer CEN*		AES

INSTRUMENTS

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

Thermostat @ 37°±1°C	MEMMERT
Refrigerator	BOSH
pHmeter	CRISON

Ordinary microbiology laboratory equipment

EXPERIMENTAL DESIGN

Six specimens, for each strains, measured about 50 x 50 x 12 mm of treated material and six specimens of the untreated material (negative control) were used.
Each test specimen was placed into a separate sterile Petri dish and the exposed outer surface of test material was inoculated with 0.4 ml of the bacterial test suspensions. The test inoculum was then covered and gently pressed with a PET sterile film that measured about 40 x 40 mm.
Immediately after inoculum (T0), 3 specimens of untreated and 3 specimens of treated material have been processed by adding 50 ml of neutralizer and, in order to remove microorganism, subjected to mechanical agitation for 2 minutes.
The remaining specimens have been incubated at 37°C±1°C for 24h.
At the end of the contact time (T24), the specimens have been processed like T0 specimens.
The viable bacterial count was verified by proper serial decimal dilution (1:10) in Tryptone water: a double count by inclusion in Agar Medium (TSA) was performed.
The plates have been incubated at 37°C±1°C for 48 h.
After incubation, the colonies in the Petri dishes have been counted and the final results were expressed as antibacterial activity of the test product compared to the negative control.

ASSAY VALIDITY CRITERIA

The logarithmic value of the number of viable recovered immediately after inoculation from the untreated test specimens shall satisfy the following requirement:

$$(Lmax - Lmin)/Lmean \leq 0.2$$

where:
Lmax: is the common logarithm (i.e. base 10 logarithm) of the maximum number of viable bacteria found on a specimen;
Lmin: is the common logarithm of the minimum number of viable bacteria found on a specimen;
Lmean: is the common logarithm of the mean number of viable bacteria found on a specimen;

-the average number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall be within the range $6.2x10^3$ cfu/cm^2 to $2.5x10^4$ cfu/cm^2;

-the number of viable bacteria recovered from each untreated test specimens after incubation for 24 hours shall not be less than $6.2x10^1$ cfu/cm^2;

CALCULATION AND EXPRESSION OF THE RESULTS

The results were expressed as antibacterial activity using the following equations:

- for log expression: $\mathbf{R = (Ut - U_0) - (At - U_0) = Ut - At}$

Where:
R: is the antibacterial activity
U_0: is the average of the common logarithm of the number of viable bacteria, in cfu/cm^2, recovered from the untreated test specimens immediately after inoculation;
U_t: is the average of the common logarithm of the number of viable bacteria, recovered from the untreated test specimens after 24 h;
A_t: is the average of the common logarithm of the number of viable bacteria, recovered from the treated test specimens after 24 h;

The antibacterial activity of the test substance is considered effective if R is higher or equal to 2, according to JIS Z 2801:2001 provisions.
Viability reduction of microorganism at the end of the contact time is also expressed as percent according to the following equation:

$$R(\%) = \frac{(N_u - N_a)}{N_u} \times 100$$

Where:
N_u: is the average of the number of viable bacteria, in cfu/cm^2, recovered from the untreated test specimens after 24 h;
N_a: is the average of the number of viable bacteria, in cfu/cm^2, recovered from the treated test specimens after 24 h.

The antimicrobial activity is considered effective if, at the end if the contact time, the vitality reduction of microorganisms is at least equal to 99% compared to the negative control.

RESULTS

The conditions for the validity of the assay were satisfied.
The obtained logarithmic reduction and % reduction values are shown below:

Test strain	*Log Reduction*	*% Reduction*
Staphylococcus aureus ATCC6538P	1.05	91.09
Escherichia coli ATCC 8739	1.38	95.67

DEVIATIONS

No deviation was found during the study.

CONCLUSION

On the basis of the obtained results, in compliance with the assay validity criteria, the test product "BETACRYL" **has antibacterial activity** since it causes, after the applied contact time, a reduction of the bacterial viability greater than 1 Log (% reduction >99%) compared to negative control (untreated specimens).

ADDENDA

ADDENDUM	TITLE	NUMBER OF PAGES
N.1	*Copy of Raw data*	1

ID. studio 2012/757 SAM

ID. campione: 12.1071-S

Data inizio *(Started on)*: 15/05/12

Data fine *(Finished on)*: 18/05/12

Table 1: COUNT OF MICROBIAL STRAINS (cfu/ml)

Test Microrganism	RESULT cfu/ml	INOCULUM cfu/mm^2
Staphylococcus aureus ATCC 6538P	9,4E+08	5,9E+04
Escherichia coli ATCC8739	1,0E+08	6,3E+03

NC = non contabile

Table n. 2: AVERAGE COUNT OF LIVING MICRORGANISMS

Test strains	*Specimens*	*Contact times*	
		t 0	t 24 hours
		cfu/ml	*cfu/ml*
Staphylococcus aureus ATCC 6538P	Untreated sample	1,7E+03	1,1E+03
	Treated sample	2,1E+03	9,8E+01
Escherichia coli ATCC8739	Untreated sample	3,6E+03	3,0E+03
	Treated sample	3,4E+03	1,3E+02

Table n. 3: AVERAGE COUNT OF LIVING MICRORGANISMS

Test strains	*Specimens*	*Contact times*	
		t 0	t 24 hours
		cfu/cm^2	*cfu/cm^2*
Staphylococcus aureus ATCC 6538P	Untreated sample	5,3E+03	3,4E+03
	Treated sample	6,5E+03	3,1E+02
Escherichia coli ATCC8739	Untreated sample	2,3E+02	9,4E+03
	Treated sample	1,1E+04	4,0E+02

Table n. 4: LOG EXPRESSION OF THE AVERAGE NUMBER OF MICRORGANISMS

Test strains	*Specimens*	*Contact times*	
		t 0	t 24 hours
		cfu/cm^2	*cfu/cm^2*
Staphylococcus aureus ATCC 6538P	Untreated sample	3,72	3,53
	Treated sample	3,80	2,48
Escherichia coli ATCC8739	Untreated sample	4,04	3,97
	Treated sample	4,03	2,59

Table n. 5: LOG REDUCTION AND % REDUCTION OF THE NUMBER OF MICRORGANISMS

Test strains	*ANTIBACTERIAL ACTIVITY (R)*	*% REDUCTION*
Staphylococcus aureus ATCC 6538P	1,05	91,09
Escherichia coli ATCC8739	*1,38*	95,67

Technician signature: _____ Date: 21/05/12

Responsible signature: _____ Date: 21/05/12