Consulenza e Servizi di Laboratorio per la Qualità Biomedicale

DELAYED HIPERSENSITIVITY TEST
Guinea-Pig Maximization Test (GPMT)

"TACKI GEN SIRINGHE DA 2,5 ml – CAMPIONATURA
(TACKI GEN 2,5 ml SYRINGE – SAMPLING),
gel orale per uso dentistico *(oral gel for dental use)"*

According to ISO 10993-10:2010

REPORT N. 2975-12

Customer: **BEST MICRO S.R.L.**
S. P. Piansanese, 11 - 01018 Valentano (VT)

TIME SCHEDULE

Acceptance N.	12-2453
Samples receiving date:	01/10/12
Start test date:	11/10/12
End test date:	30/11/12

TEST MANAGEMENT
Coronati Consulting sas Via L. Gavioli, 3 I-41037 Mirandola (MO)
Certified ISO 9001/ ISO 13485 and Accredited by M.I.U.R.

The test was performed at:
Eurofins Biolab S.r.l.
Via B. Buozzi, 2 – 20090 Vimodrone, (MI) Italy

REFERENCE DOCUMENTS
- ISO 10993-10: 2010 Biological evaluation of medical devices Part 10: Tests for irritation and delayed-type hypersesivity

Mirandola	Prepared by: Dr. C. Pellegrini	Verified and Approved by: Dr. R. Coronati
30/11/12		

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SUMMARY
On the test product “TACKI GEN SIRINGHE DA 2,5 ML - CAMPIONATURA” a toxicological study was carried out to evaluate the possible sensitizing effects, throughout the following test:

- Guinea Pig Maximization Test according to ISO 10993-10: 2010

In the Guinea Pig Maximization test 15 guinea pigs were used: 10 of which were treated with the test material and 5 were used as a control group.

The maximization test is composed of an induction phase and a challenge phase.

Induction phase
During the induction phase, guinea pigs were treated with 3 pairs of intradermal injections (each dose of 0,1 ml) thus subdivided.

1. Stable emulsion of Freund's complete adjuvant (FCA) in physiological solution 50:50 (v:v);
2. test sample for treated animals; physiological solution for the control animals;
3. test sample diluted 50:50 (v:v) with stable emulsion of FCA and physiological solution (50%) for treated animals, physiological solution diluted 50:50 (v:v) with stable emulsion of FCA and physiological solution (50%) for control animals.

After 6 days from performing the intradermal injections on all the animals – both treated and control ones – a local application was performed by massaging 1 ml of Lauryl Sulfatum at 10 %.

After 7 days from the intradermal injections, a test sample in the volume of 1 ml/animal was applied to the skin of the 10 treated animals for a period of 48 hours.
The same treatment was performed on control guinea pigs using physiological solution.

Challenge phase
After 21 days from the beginning of the treatment on all animals, both treated and control ones, the challenge phase was carried out by applying on the right side of the back 1 ml of the test sample and on the left side 1 ml of physiological solution. Bandaging was left on for 24 hours.

After 48 and 72 hours from the beginning of this phase, the reactions of both the treated and the control animals were evaluated.

No abnormalities were observed in treated and control animals.

On the basis of the results, interpreted according to ISO 10993-10: 2010, the test product “TACKI GEN SIRINGHE DA 2,5 ML - CAMPIONATURA” must be considered **NOT SENSITIZING**.

TESTED SAMPLE

Sample:	TACKI GEN SIRINGHE DA 2,5 ml – CAMPIONATURA *(TACKI GEN 2,5 ml SYRINGE – SAMPLING)*,gel orale per uso dentistico *(oral gel for dental use)"*
Composition:	- olio di Iperico *(Hypericum iol)*; - olio di Calendula *(Calendula oil)*; - olio di mandorle dolci *(sweet almond oil)*; - silice *(silica)*; - olio di oliva *(olive oil)*; - vitamina e acetato *(vitamin and acetate)*; - vitamina F *(vitamin F);* - fenossietanolo *(phenoxyethanol)*; - limone olio essenziale *(lemon essential oil);* - Lavanda olio essenziale *(Lavender essential oil);* - olio di Equiseto *(Horsetail oil)*; - olio di Echinacea *(Echinacea oil)*; - profumo Vaniglia *(Vanilla fragrance)*; - Camomilla olio essenziale *(Chamomile essential oil)*; - Geranio olio essenziale *(Geranium essential oil)*; - pompelmo olio essenziale *(grapefruit essential oil)*; - solfato di calcio emidrato *(Calcium sulphate hemihydrate).*
Code (REF):	TGEN/200
LOT	not provided
Manufacture date:	not provided
Expiry date:	not provided
Sterilization Method:	EtO
Sterilization lot:	not provided

PROCEDURE

All procedures used during this study are recorded in the Biolab Procedures Manual.

TEST METHOD

Characterisation

Species:	Albino guinea pigs
Strain:	Hartley
Number:	15+2
Weight:	300÷400 g at the arrival at the centre
Sex:	Female
Supplier:	Bettinardi-Momo (No)
Food:	Altromi MSK; batch: 1206
Bedding:	Lignocell Batch: 03018120702 (from November 11th 2012 to November 22nd)
	Lignocell Batch: 03018120901 (from November 23th 2012 to November 29nd)

Caging

The animals were caged, in group of ten, in transparent polycarbonate cages (dimensions: 59×38.5×20h cm).
The housing room was lighted with fluorescent lamps and kept with cycles of 12 hours of light and 12 hours of dark.
Room Temperature and humidity were regulated by a air conditioning plant and were monitored daily.
Continuous recordings of the housing conditions are being retained in Eurofins Biolab srl files.

Cleaning and disinfection

The cages and the housing room were cleaned before the animals were accommodated, then disinfected periodically.

Feeding

Animals have been fed with standard pellet complete diet supplied by the authorised breeder.

Watering

Purified water was supplied ad libitum.

Quarantine

Before allocation to the study, the animals were kept in quarantine for five days. During this period they were observed daily.
At the end of the quarantine period the animals were carefully examined in order to evaluate their suitability for the study.

Animals identification

The animals were identified with an indelible colouring in different areas of the body as:

No sign	(B)	1
Head	(T)	2
Tail	(C)	3
Head-tail	(TC)	4
Right forepaw	(ZAD)	5
Left forepaw	(ZAS)	6
Right hind leg	(ZPD)	7
Left hind leg	(ZPS)	8
Abdomen	(P)	9
Head. Abdomen	(TP)	10

PRELIMINARY-TEST

A preliminary test was intended to determine the concentration of the test sample to be used in the main test.
For the topical induction phase, select the highest concentration that causes mild to moderate erythema but does not otherwise adversely affect the animal. For the challenge phase, select the highest concentration that produces no erythema.
To select the sample dilution three occlusive patches with 1 ml of the undiluted sample and diluted sample (1:2 and 1:5 in sodium chloride injection) were applied to the dorsum of two additional animals.

Animal N.	24 hours after patch removal		
	Neat	1:2 dilution	1:5 dilution
1	0	0	0
2	0	0	0

0= no symptoms
After 24 hours of bendage removal, no erythema was observed in any treated sites.

PREPARATION OF THE ASSAY SAMPLE

The test sample was used neat for al the test phases.

EXPERIMENTAL DESIGN

Experimental design consisted of two groups (treated) of 10 animals treated with the extract in vegetable oil and sodium chloride injection of the substance (group 1-2) and two groups (control) of 5 control animals treated with only vegetable oil and sodium chloride injection (group 3-4).
The animals were divided in groups as follows:

GROUP	INDUCTION		CHALLENGE TOPIC REACTION
	Intradermal injection	Topic application	Challenge
1	1. Stable emulsion of Freund's complete adjuvant (FCA) in physiological solution 50:50 (v:v); 2. test sample 3. test sample diluted 50:50 (v:v) with stable emulsion of FCA and physiological solution (50%)	Test product	Test product
2	1. Stable emulsion of Freund's complete adjuvant (FCA) in physiological solution 50:50 (v:v); 2. physiological solution for the control animals; 3. physiological solution diluted 50:50 (v:v) with stable emulsion of FCA and physiological solution (50%)	Physiological solution	Test product

The animals allocated to the study were selected randomly from those suitable, available at that time.
Animals were logged in group of 5 for cage; each cage was identify by a tag

TREATMENT

Skin preparation

24 hours before testing, fur was removed by shaving a 50 cm^2 wide area on the back of the animals.

Administration
The test consisted of an induction phase and a challenge phase.

Induction phase
Day 0 treated and control group
Three pairs of 0,1 ml intradermal injections were made in the intrascapolar region of each animal, on each side of the midline, according to the experimental design.

Day 6 treated and control group
After 6 days the beginning of the treatment on the alls animals was made a topical application, with slight massage of 1 ml of Sodium Lauril Solfatum 10 % was made.

Day 7- treated and control groups
Seven days after the intradermal injections 1 ml of the assay sample was applied to each animal and held in place with an occlusive patch. The application was made on area localised caudally to the area of injection.
The dressing was left in place for 48 hours.
The same treatment was performed on the control group, using physiological solution instead of the test substance.

Challenge
Day 21- treated and control groups
An occlusive patch with 1 ml of assay sample was applied to the right flank of all 15 guinea pigs wwhile physiological solution were applied on the left side. The dressing was left in place for 24 hours.

OBSERVATIONS
On the 23rd day (24 hours after removal the patch), and the 24th day (48 hours after removal the patch) of tests all the animals treated and controlled were evaluated for a skin reaction.
The intensity of erythema and/or oedema were evaluated according to the following scale (Magnusson and Kligman):

REACTION	GRADING SCALE
No visible change	**0**
Discrete o pacthy erythema	**1**
Moderate and confluent erythema	**2**
Intense erythema and swelling	**3**

INTERPRETATION OF RESULTS
Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animals. If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization.
If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge.
The outcome of the test is presented as the frequency of positive challenge results in the test and control animals.

RESULTS

SKIN REACTION IN TREATED ANIMLAS

ANIMAL N.	TIME AFTER REMOVAL OF THE PATCH	
	24 hours	48 hours
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

0: No Symptoms

No abnormalities were observed in treated animals

SKIN REACTION IN CONTROL ANIMLAS

ANIMAL N.	TIME AFTER REMOVAL OF THE PATCH	
	24 hours	48 hours
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0

0: No Symptoms

No abnormalities were observed in control animals

% **sensitising treated guinea pigs: 0%**

DEVIATION

No deviation has been recorded from study program.

CONCLUSIONS

On the basis of the results, interpreted according to ISO 10993-10:2010 the test product “TACKI GEN SIRINGHE DA 2,5 ML - CAMPIONATURA”must be considered **NON SENSITIZING.**

RECORD FILING

The study program and all raw data will be retained in Eurofins Biolab’s archives for a period of 10 years from the issue of the final report. No. 2012/1813-2 SAMi dated 30-11-12. An original copy of the report is available at Coronati Consulting.

⇒ The present test report refers only to the samples examined.