ACUTE ORAL MUCOSA IRRITATION TEST

"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

*REPORT N.*2976-12

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

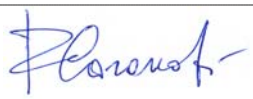
TIME SCHEDULE

Acceptance N.:	12-2454
Samples receiving date:	01/10/12
Start test date:	11/10/12
End test date:	30/11/12

TEST LABORATORY

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

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

Coronati Consulting sas -Tel. 0535-611533 Fax 0535-410441- E-mail: info@coronaticonsulting.it Web: www.coronaticonsulting.it

REFERENCE DOCUMENTS

- ISO 10993-10:2010 Tests for irritation and sensitization

SAMPLES

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

SUMMARY

On the test product a toxicological study aimed to evaluate the irritating effects was carried out. The following test was performed:

- oral mucosa irritation test according to ISO 10993-10:2010

the oral mucosa irritation test was performed for 1 day, one application at hour for four hours on a group consisting of 6 male Hamster, 3 (treated group) treated with the test product and 3 (control group) treated with polyethylene low density.
The test product was inserted directly on the right oral cavity mucosa of every animal.
During the study the oral cavity mucosa of all the animals has been observed before and after every treatment, in order to record the presence of any possible irritative event, such as erythema and/or eschar.

24 hours after the last treatment, all the animals were sacrificied; then the oral cavity mucosa was taken to perform the histological examination.

The results obtained during the study can be summarised as follows:

TREATED GROUP

Macroscopic examination

No eschar and/or erythema case was observed in the animals treated with the test product.

Microscopic examination
Average irritation capacity: 0.67

CONTROL GROUP
Macroscopic examination
No eschar and/or erythema case was observed in the animals treated with the test product.
Microscopic examination
Average irritation capacity: 0.33

IRRITATION INDEX: 0,34
On the basis of the results, interpreted according to ISO 10993-10:2010, the test product must be considered NOT IRRITANT for oral mucosa.

TEST METHOD

Characterization
Specie: Hamster
Breed: Syrian
No: 6 (3 treated and control)
Sex: male
Supplier: Charles River
Food: VRF1 Batch:9099
Bedding Lignocell Batch: 03018120702

Housing
Animals have been put in cages of trasparent polycarbonate with sizes of cm 42.5×26.6×18h separated by sex so that in each cage there were 5 animals of the same sex.
Stabiling rooms have been lighted with fluorescent lamps and kept with cycles of 12 hours of light and 12 hours of darkness.
Temperature and humidity, controlled by air conditioning system, have been continuously registered; registrations are stored in Eurofins Biolab srl archives.

Cleaning and disinfection
Cages and stabling room have been cleaned before housing and periodically disinfected.

Feeding
Animals have been fed with complete pelletized diet.

Watering
Animals have been watered by means of feeding bottles with filtered water derived from municipal waterworks.

Animal identification
Animal selected for the study have been marked with indelible colouring in different parts of the body, as follows.
No sign 1
Head 2
Back 3

Cages have been identified via a tag.

Quarantine
Purchased animals, before being used for this study, have been put in quarantine for 5 days.
During the quarantine period they have been daily observed.
At the end of the quarantine the animals were carefully examined to verify their qualification to the study.

Animal selection
The animals used for this study have been haphazardly selected among the ones eligible available at the time of the study.

ASSAY SAMPLE PREPARATION
Test product was used as neat.

EXPERIMENTAL DESIGN
6 male Hamster have been used for the test, 3 treated with the test product (treated group) and 3 treated with polyethylene low density (control group).

TREATMENT
0.5 ml of the test product was inserted directly into the right oral cavity mucosa of each animal.
The animals were treated for 5 consecutive days.
Control group animals were treated in the same conditions using polyethylene low density instead the test sample.

OBSERVATIONS

Macroscopic examination
The mucosae of oral cavity of all the animals of the study has been daily observed before the first treatment and after the last one, to detect any irritating phenomenon such as erythema and/or eschar.
The reactions occurred on the treated area were evaluated on the basis of the following table:

Scoring system for oral mucosa irritation

(Erythema- Eschar / Excoriations)	***(Score)***	***(Oedema)***	***(Score)***	***(Exudate)***	***(Score)***
(No erythema)	0	*(No oedema)*	0	*(No pus)*	0
(Discrete opacthy erythema, barely perceptible)	1	*(oedema barely perceptible)*	1	*(Few globules of pus)*	1
(Moderate and confluent erythema)	2	*(Well-defined Oedema)*	2	*(Large globules of pus)*	2
(Intense erythema and swelling)	3	*Oedema moderate in relief 1 mm)*	3	*(Large globules of pus)*	3
(Erythema severe red beet type to eschar formation)	4	*(Oedema serious in relief >1 mm which extends beyond the area of exposure)*	4	*(Large amounts of pus exuding)*	4
Possible total score of irritation: **12**					
***Note**: no other adverse changes of the injection sites has been noted*					

Microscopic examination
24 hours after the last treatment the animals of the study have been sacrificed and the mucosa of the oral cavity has been taken to perform the histological examination.
The detected microscopical reactions have been evaluated on the basis of the data of the following table:

REACTION	**Punteggio *Score***
1. Epithelium	
Normal, intact	0
Cellular de generation or flattering	1
Metaplasia	2
Focal wash	3
Generalized wash	4

2. Leukocytic Infiltration	
No irritation	0
Minimal (< 25)	1
Light (26 ÷ 50)	2
Moderate (51 ÷100)	3
Severe (> 100)	4

3. Vascular congestion	
No irritation	0
Minimal	1
Light	2
Moderate	3
Severe, with vascular injury	4

4. Oedema	
No irritation	0
Minimal	1
Light	2
Moderate	3
Severe	4

RESULTS INTERPRETATION

Macroscopic examination
Compare the inflammatory reactions observed in applications area of animals treated with the test substance with that observed in control animals.
The scores for each observation are added together and divided by the number of observations to determine the average score per animal.

Microscopic examination
The scores of the microscopical evaluations for all the animals treated with the test substance have been summed up and divided by the number of the animals, thus value is subtracted from the irritating capacity of the treated group.
The highest irritation score should be 16.

The IRRITATION INDEX is in table below:

Average Score	***Category Response***
0.0	*No Irritation*
From 1 to 4	*Negligible irritation*
From 5 to 8	*Medium irritation*
From 9 to 11	*Moderate irritation*
From 12 to 16	*Severe irritation*

The values of oral mucosa reactions to different periods of observation are shown in table.

RESULTS
EVALUATIONS OF THE ORAL MUCOSA TREATED WITH THE TEST PRODUCT (TREATED GROUP)

OBSERVATION DATE	Time during the study	HAMSTER N.			AVERAGE (X)
		1	2	3	
12-11-2012	Before	-	-	-	-
	After	0	0	0	0
13-11-2012	Before	0	0	0	0
	After	0	0	0	0
14-11-2012	Before	0	0	0	0
	After	0	0	0	0
15-11-2012	Before	0	0	0	0
	After	0	0	0	0
16-11-2012	Before	0	0	0	0
	After	0	0	0	0
17-11-2012	Before	0	0	0	0
	Explantation				

Macroscopic examination
No eschar or erythema case was observed in the animals treated.
Average irritation capacity: 0.00

RESULTS

	ANIMAL N.		
Animal	*Animal 1*	*Animal 2*	*Animal 3*
Epithelium	0	2	0
Leukocytes	0	0	0
Congestion	0	0	0
Oedema	0	0	0
Total	**0**	**0**	**0**

Microscopic examination
Average irritation capacity: 0.67

IRRITATION INDEX: 0,00

EVALUATIONS OF THE ORAL MUCOSA TREATED WITH SODIUM CHLORIDE INJECTION (CONTROL GROUP)

OBSERVATION DATE	Time during the study	HAMSTER N.			AVERAGE (X)
		1	2	3	
12-11-2012	Before	-	-	-	
	After	**0**	**0**	**0**	**0**
13-11-2012	Before	**0**	**0**	**0**	**0**
	After	**0**	**0**	**0**	**0**
14-11-2012	Before	**0**	**0**	**0**	**0**
	After	**0**	**0**	**0**	**0**
15-11-2012	Before	**0**	**0**	**0**	**0**
	After	**0**	**0**	**0**	**0**
16-11-2012	Before	**0**	**0**	**0**	**0**
	After	**0**	**0**	**0**	**0**
17-11-2012	Before	**0**	**0**	**0**	**0**
	Explantation				

Macroscopic examination
No eschar or erythema case was observed in the animals treated.
Average irritation capacity: 0.00

	ANIMAL N.		
Animal	*Animal 1*	*Animal 2*	*Animal 3*
Epithelium	0	0	1
Leukocytes	0	0	0
Congestion	0	0	0
Oedema	0	0	0
Total	**0**	**0**	**0**

Microscopic examination
Average irritation capacity: 0.33

IRRITATION INDEX: 0,34

DEVIATIONS
No deviation has been detected during the study.

CONCLUSIONS
On the basis of the results, interpreted according to ISO 10993-10: 2010, the test product must be considered NOT IRRITANT for oral mucosa.

RECORD FILING: The study program and all raw data will be retained in Biolab's archives for a period of 10 years from the issue of the final report. No.2012/1813-1 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.*