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IN VIVO SKIN IRRITATION TEST IN ALBINO RABBIT - Single Exposure - ON "3ply non woven disposable surgical masks"

Contract n: PO9YPH200562-01

Sponsor:
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Test item: 3ply non woven disposable surgical masks

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SUMMARY

On the test item “3ply non woven disposable surgical masks” a biological evaluation was carried out aimed to identify its irritant effects by means of following test:

- Skin irritation according to ISO 10993-10:2010.

The **skin irritation test** was carried out through a semi-occlusive application; the test sample was applied on the intact skin of 3 rabbits, in the dorsal region both on the left and on the right side.
Each animal had the right caudal region and left cranial region treated with the test sample (25 mm x 25 mm patches humidified with sodium chloride injection).
The right cranial region and the left caudal region were treated with a no irritant humidified gauze (25 mm x 25 mm), humidified with sodium chloride injection, used as control.

Reactions were evaluated (1 ± 0.1) h following the removal of the patches and were evaluated again after (24 ± 2) h, (48 ± 2) h and (72 ± 2) h after the treatment.

No signs of erythema and oedema were detected in all treated sites of all animals during the study.

No signs of erythema and oedema were detected in all control sites of all animals during the study.

PRIMARY SKIN IRRITATION INDEX (TREATED - CONTROL): 0.00

On the basis of the results, interpreted according to ISO 10993-10:2010, the test item “3ply non woven disposable surgical masks” must be considered **NEGLIGIBLY IRRITANT** for skin.

INTRODUCTION

This study has been carried out at the Test Facility Eurofins Biolab S.r.l. on behalf of the Sponsor on the test item “3ply non woven disposable surgical masks”.

TEST	*START*	*END*	*RESEARCHER*
Skin irritation	28/07/2020	01/08/2020	E. M. Zeni G. Mauro

BIBLIOGRAPHY

ISO 10993-10:2010 Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization.
ISO 10993-2:2006 Biological evaluation of medical devices - Part 2: Animal welfare requirements.

FILING

All raw data are filed in the archives of Eurofins Biolab S.r.l. for 10 years after the issuing of the Final Report.
Retained sample has not been kept.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the documents/products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in Test Facility Eurofins Biolab S.r.l.

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TEST ITEM

The test item consists of a medical device.

Name	3ply non woven disposable surgical masks
Code	Not Provided
Stability	5 years
Sterilization	None
Type of material	100% PP

ANALYSED SAMPLE

The test sample, representative of the test item, consists of a surgical face masks.

Batch	L201851
Manufacturing date	04/07/2020
Expiry date	Not provided (Sponsor declared under his responsibility that sample was considered stable for aim of the study for at least 1 month)
CoA	Not provided
Parcel Registration Number	IP-LV-2020190-AEN
Storage	Room temperature
Receiving date	08/07/2020
Material item aliquot	LV-MAT-PMRO-20-210-0F56:a

The test item and the information concerning the test item were provided by the Sponsor.
All data related to the test item are under the responsibility of the Sponsor and have not been verified by the test facility.

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Experimental Report

TEST METHOD

Characterization

Specie:	White rabbits
Strain:	New Zealand
No.:	3
Sex:	Male
Weight:	3435-3570 g at the beginning of the test
Supplier:	Charles River- Calco (LC) – Italy
Receiving number	080 (Animals N.0737, N.0755) 084 (Animal N.0771)
Food:	2RB15 Batch: 364701

Justification of the assay system

Rabbit has been used because of recommendation of ISO 10993-10 – current edition.

Quarantine

Before being used in this study, the animals were kept in quarantine according to internal procedure. During this period they were daily observed by a veterinarian. At the end of the quarantine period the animals were carefully examined in order to evaluate their suitability for the study.

Animal selection

The animals used for this study were randomly selected from those suitable, available at that time.

Caging

The animals were caged according to internal procedure.
The housing room are lighted with fluorescent lamps 12 hours for day.
The room temperature and humidity have been regulated by an air conditioning unit and have been continuously monitored. Recordings of the housing conditions are retained in Eurofins Biolab S.r.l. files.

Cleaning and disinfection

The cages and the housing room were cleaned before animal accommodation, then cleaning and disinfection were periodically performed.

Feeding

The animals were fed with standard pellet complete diet supplied by the authorized breeder.

Watering

Filtered tap water from local network was supplied ad libitum.

Animal identification

A numbered tag placed through the edge of the right ear identified the animals selected for the study.
A label identified the cages.

Test materials

Sodium Chloride Injection Supplier: Eurospital Batch: 19I2602

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SAMPLE PREPARATION

The test sample was cut in 25 mm x 25 mm patches.

EXPERIMENTAL DESIGN

Three white rabbits were used.
The dorsum of each animal was divided in four parts and the right caudal region and the left cranial region has been treated with the test sample (25 mm x 25 mm patches humidified with Sodium Chloride Injection).
The right cranial region and the left caudal region were treated with a no irritant gauze (25 mm x 25 mm) humidified with Sodium Chloride Injection, used as control. The reactions in the treated area has been compared with those of the control area.

TREATMENT

Skin preparation

About 20 hours before the test, the fur was removed from an area approximately 240 cm^2 wide by clipping and shaving the dorsal and flank zones of the animals.
An area of the back, about 6 cm^2 wide, was designed for the application of the test sample.

Application

25 mm x 25 mm patch of the test sample humidified with Sodium Chloride Injection was applied directly to the skin on the two sites of each rabbit and covered with non-occlusive dressing, then the trunk was then protected with a semi-occlusive bandage.

Removal of the patches

The patches were removed 4 hours after the application and residual sample was removed cleaning the skin with sodium chloride injection.

OBSERVATIONS

General conditions of the animals were daily verified. Reactions were evaluated at (1 ± 0.1) h, (24 ± 2) h, (48 ± 2) h and (72 ± 2) h following removal of the patches.
Skin irritation was scored and recorded according to the scores reported in the following table.

Reaction	Irritation score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

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INTERPRETATION OF RESULTS

For acute exposure, determine the Primary Irritation Index (PII) as follows.
For each animal, add together the Primary Irritation Scores for the test substance for both erythema and oedema at each time specified and divide by the total number of observations. When vehicle controls are used, calculate the Primary Irritation Score for the vehicle controls and subtract that score from the score for the test substance to obtain the Primary Irritation Score.
Only use 24 hours, 48 hours and 72 hours observations for calculations. Observations made prior dosing or after 72 hours, to monitor recovery, are not used in the determination.
Add the scores for each animal and divide the total by number of animals. This value is the Primary Irritation Index.
Number and description in follow table characterise the Primary Irritation Index:

Mean score	Response category
0 to 0,4	Negligible
0,5 to 1,9	Slight
2 to 4,9	Moderate
5 to 8	Severe

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RESULTS

TIME AFTER REMOVAL OF PATCHES	REACTION	RABBIT N.											
		0737				0755				0771			
		Treated		Control		Treated		Control		Treated		Control	
		Ca dx	Cr sx	Ca sx	Cr dx	Ca dx	Cr sx	Ca sx	Cr dx	Ca dx	Cr sx	Ca sx	Cr dx
60 minutes	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	0
24 hours	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	0
48 hours	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	0
72 hours	Erythema	0	0	0	0	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	0

Ca: caudal *Cr:cranial* *dx: right* *sx:left*

No signs of erythema and oedema were detected in all treated sites of all animals during the study.

No signs of erythema and oedema were detected in all control sites of all animals during the study.

PRIMARY SKIN IRRITATION INDEX (TREATED - CONTROL): 0.00

DEVIATION

No deviation has been recorded during the study.

CONCLUSIONS

On the basis of the results, interpreted according to ISO 10993-10:2010, the test item “3ply non woven disposable surgical masks” must be considered **NEGLIGIBLY IRRITANT** for skin.

Reviewed and electronically signed for Study Technical Supervisor Approval by
Alessandro Moschetti, Employee
for Eurofins Biolab Srl, on 05-Aug-2020 10:45:49 UTC+02:00