Study No.: **733/005**

Study Title: **Skin Irritation Test in New Zealand White rabbits**

Test Item: **Prima Medix- 3 Ply Disposable Surgical Face Mask**

Manufacturer: **PT Prima Medix Nusantara**

STUDY REPORT

Study Title	Skin Irritation Test in New Zealand White rabbits
Test Item	Prima Medix- 3 Ply Disposable Surgical Face Mask Manufacturer: PT Prima Medix Nusantara
Study Conducted by	Mr. V. Rajasekar, MTech (Biotech)
Sponsor/ Test Facility	Integrated Assessment Services Pvt Ltd 1495/1, Manasarovar, 16th Main Road Anna Nagar West Chennai - 600040
Study Number	733/005
Regulatory Guideline	Biological Evaluation of Medical Devices - Part 23, Tests for Irritation, ISO 10993-23:2021(E).
Report Issued	07 May 2021
Total Number of Pages	25

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STUDY DIRECTOR AUTHENTICATION STATEMENT

Study No.: 733/005

Study Title: Skin Irritation Test in New Zealand White rabbits

Test Item: **Prima Medix- 3 Ply Disposable Surgical Face Mask**

Manufacturer: PT Prima Medix Nusantara

This study was performed in accordance with the mutually agreed study plan and IAS Associated Laboratory's standard operating procedures, unless otherwise stated, and the study objective was achieved. I accept overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results. This report provides a true and accurate record of the results obtained.

This study was performed in compliance with OECD Principles of Good Laboratory Practice* ENV/MC/CHEM (98)17 (Revised 1997, issued January 1998) and applicable regulatory requirements including the US Food and Drug Administration's GLP regulations, 21 CFR 58 (subparts B to G and J).

29 April 2021

Mrs. Jeya Latha
Technical Head

Study Completion Date

*with an exception of the identity and composition of the test item, which are the responsibilities of the Sponsor.

QUALITY ASSURANCE STATEMENT

Study No.: 733/005

Study Title: **Skin Irritation Test in New Zealand White rabbits**

Test Item: **Prima Medix- 3 Ply Disposable Surgical Face Mask**

Manufacturer: PT Prima Medix Nusantara

The Quality Assurance (QA) of IAS Associated Laboratory verified the Study Plan, including any amendments, inspected the critical study phases, audited the raw data, and report of this Study as per in-house Standard Operating Procedures (SOPs) for compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) [ENV/MC/CHEM(98)17], and for compliance with relevant regulatory requirements.

During the Study, the following study-related inspections/audits were performed on the following dates and reported to the Study Director and Test Facility Management. Besides the below, process and facility inspections were also carried out periodically at this Test Facility by auditor(s) of the QA, as per in-house SOPs, which may have relevance to this study.

S. No.	Type(s) of Study Inspection/Audit	Date(s) of Inspection/Audit	Phase(s) of Study inspected/audited	Date(s) of Reporting to Management and Study Director (Inspection No.)
1	Study Plan Verification	16 March 2021	Draft Study Plan	16 March 2021 (SBI/733/005/001)
2	In-life Phase Inspection	26 March 2021	Test Item Extracts Application	26 March 2021 (SBI/733/005/002)
3	In-life Phase Inspection	29 March 2021	Grading of Skin Reactions	29 March 2021 (SBI/733/005/003)
4	Report Audit	19 April 2021	Draft Report	19 April 2021 (SBI/733/005/004)
5	Report Audit	29 April 2021	Final Report	29 April 2021 (SBI/733/005/005)

The QA has determined that the methods, procedures, observations, and reported results are accurately and completely described and that the reported results are based on the Study Plan and the pertinent raw data generated during the course of the Study. The Study Director's GLP compliance statement is supported.

05 May 2021

Mrs.Jeya Latha
Technical Head

Date

TEST FACILITY MANAGEMENT STATEMENT

Study No.: 733/005

Study Title: **Skin Irritation Test in New Zealand White Rabbits**

Test Item: **Prima Medix- 3 Ply Disposable Surgical Face Mask**

Manufacturer: PT Prima Medix Nusantara

This is to certify that, the Test Facility Management appointed the Study Director and provided all necessary facilities and resources for the proper conduct of this study, in compliance with the Principles of OECD Good Laboratory Practice (GLP), as per the recommendations of the OECD (Council Act [C (97) 186 (Final)]) and as adopted in the procedures promulgated by the National GLP Compliance Monitoring Authority, Government of India.

05 May 2021

Mrs.Jeya Latha
Technical Head

Date

SUMMARY

Skin irritation potential of Prima Medix- 3 Ply Disposable Surgical Face Mask, supplied by Integrated Assessment Services Pvt Ltd., was evaluated in male New Zealand White rabbits.

The test item, Prima Medix- 3 Ply Disposable Surgical Face Mask is a protective device worn over nose and mouth to protect against pollutants, dust, bacteria and virus. It is a surface device which comes in contact with skin. The duration of contact is less than 24 hours (limited).

The test item was extracted at the ratio of 0.1 g/mL (since the test item is a porous material) in physiological saline (polar solvent) and cottonseed oil (non-polar solvent) at 37 ± 1 °C for 72 h and 13 min under sterile condition. Polar extract was freshly prepared by extracting 2.87 g of test item in 86.1 mL of physiological saline (0.1 g of test item absorbs 2 mL of physiological saline, additionally 57.4 mL was added to the extraction volume). Similarly, non-polar extract was prepared by extracting 2.91 g of test item in 58.2 mL of cottonseed oil (0.1 g of test item absorbs 1 mL of cottonseed oil, additionally 29.1 mL was added to the extraction volume). Solvent controls were also subjected to same extraction conditions. This fulfils the requirement of ISO 10993- 12:2012(E).

At the end of extraction, minute strands were found and no colour change were observed in both polar and non-polar extract. Solvent controls were clear without any colour change or particulates. Hence, no additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. The extracts and solvent controls were transferred to sterile containers and stored at room temperature. All extracts and solvent controls were used within 2 h and 22 min of preparation and were considered stable during this time.

About 18 h and 10 min prior to application, fur on all the rabbits were closely clipped off their backs, allowing sufficient distance on both sides of the spine for application of test item extracts and solvent controls. The test item extracts (0.5 mL) was loaded on the absorbent gauze measuring 6.25 cm2 (2.5 cm x 2.5 cm) and placed topically on the fur clipped test sites of six male rabbits (3 animals each for polar and non-polar extracts). Similarly, 0.5 mL of solvent controls was loaded on the absorbent gauze measuring 6.25 cm2 (2.5 cm x 2.5 cm) and placed topically on the fur clipped control sites. The patches were loosely held in contact with the skin by semi-occlusive dressing with means of non-irritant adhesive tape for 4 h. After 4 h the patch was removed. No test item extract residues were observed. The test sites were marked with a non-irritant permanent ink.

Animals were observed for three consecutive days for morbidity, mortality, skin reactions and abnormal clinical signs and symptoms following the patch removal. The skin reactions were visually scored according to ISO 10993 10:2010(E) at 24, 48 and 72 h.

Positive control trials for irritation are carried out once in three months in IAS Associated Laboratory. The trial completed on 31 December 2020 gave a “moderate irritant” reaction.

No response was observed in solvent control treated animals. Therefore, the assay was considered valid.

No mortality or morbidity was observed in the experimental animals. A gradual increase in body weight was observed in all the animals at the end of the experiment. No signs of clinical toxicity or overt toxicity was observed in any of the animals. Hence, gross pathology and histopathology was not performed. No local skin irritation was observed at the test site in any of the animals and the primary irritation index obtained was '0'.

Based upon the results obtained in this study and in line with ISO 10993-10:2010(E), the given test item, Prima Medix- 3 Ply Disposable Surgical Face Mask, supplied by Integrated Assessment Services Pvt Ltd., is considered as non-irritant in New Zealand White rabbits.

INTRODUCTION

Biocompatibility testing is a regulatory requirement for demonstrating the preclinical safety of medical devices. This is evaluated in line with the standard, ISO 10993-1: 2018 (E), Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process. This standard describes the test selection necessary to evaluate biocompatibility.

Skin irritation is a key toxicity endpoint to assess biocompatibility of medical devices. An assessment is made to know the potential of the material under test to produce dermal irritation in rabbits following topical application.

OBJECTIVE

To determine the skin irritation potential of the test item in New Zealand White rabbits.

STUDY DATES

Study Start Date	18 March 2021
Experiment Start Date	19 March 2021
Experiment Completion Date	29 April 2021

The study completion date is the date the final report is signed by the Study Director.

TEST ITEM DETAILS

The test item, Prima Medix- 3 Ply Disposable Surgical Face Mask was received at IAS Associated Laboratory on 15 March 2021 and stored at room temperature (20 to 30 °C) until used.

The following test item information provided by the sponsor were considered an adequate description of the characterisation, purity and stability of the test item. No analysis was performed at IAS Associated Laboratory, to confirm it.

Test Item	Prima Medix- 3 Ply Disposable Surgical Face
Batch/ Lot No.	BL21030301
Manufacture Date	03 March 2021
Expiry Date	03 March 2023
Appearance	Blue face mask
Ingredients	Non Woven Fabric
Temperature Stability	37 °C
Sterility	Non-Sterile

CONTROL ITEM DETAILS

Positive Control	20% w/v Sodium Lauryl Sulphate (SLS)	
Manufacturer	Sigma Aldrich	
Batch No.	0000009635	
Expiry date	August 2022	
	Positive control trials for irritation are conducted once in three months in IAS Associated Laboratory. The trial completed on 31 December 2020 gave a “moderate irritant” reaction (Appendix 1).	
Solvent Controls	<u>Physiological saline</u>	
	Manufacturer	Eurolife Healthcare Pvt. Ltd.
	Batch No.	10200632B
	Expiry Date	September 2023
	Appearance	Colourless clear solution
	<u>Cottonseed oil</u>	
	Manufacturer	Sigma-Aldrich
	Lot No.	MKCD7646

Expiry Date	October 2024
Appearance	Yellow coloured viscous liquid

The test item was handled with necessary protective clothing and all recommended safety and sterile measures were followed. Determinations of stability and characteristics of the test item were the responsibility of the sponsor.

DESCRIPTION OF THE TEST ITEM

The test item, Prima Medix- 3 Ply Disposable Surgical Face Mask is a protective device worn over nose and mouth to protect against pollutants, dust, bacteria and virus. It is a surface device which comes in contact with skin. The duration of contact is less than 24 hours (limited).

TEST SYSTEM

Species	*Oryctolagus cuniculus* (Rabbit)
Strain	New Zealand White
Weight range (g) (at the time of dosing)	2159.5 to 2519.6
Sex	Male
Source	VAB Bio Sciences, Hyderabad, India. This supplier is approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India for breeding laboratory animals.
Number of animals used	6
Acclimatization period	7 days
Justification for animal use	Rabbits were selected because there is a large volume of background data on this species. Recommended in ISO 10993-10:2010(E) standard as an appropriate species to evaluate skin irritation of medical devices and recommended by various regulatory authorities.

The test system was approved by Institutional Animal Ethics Committee (IAEC) of IAS Associated Laboratory.

ANIMAL HUSBANDRY

Test room no.	03
Test room temperature (°C)	19.3 to 21.8
Relative humidity (%)	39 to 58
Housing	Animals were housed individually in standard rabbit cages.
Method of identification	Animals were identified using cage cards indicating cage no., study no., species, strain, animal no., sex, body weight, group no., dose, signature and individual earmarking.
Feed	Commercial rabbit pellet feed (VRK Nutritional)
Water	Purified drinking water was provided *ad libitum*
Bedding material	No bedding materials were used as the rabbits were housed in stainless steel cages with mesh floors. Sterilized paddy husk were used to collect the excreta and urine, was changed every day. This was not contact to the rabbits directly.
Photoperiod	12 h light and 12 h dark cycle
Contaminants	Contaminants reasonably expected in feed or water supplies are not believed to influence the outcome of the study.
Personnel	Appropriately qualified and trained associates were involved in this study.
Selection of animals	Only healthy, previously unused young adult animals were selected for this study.

TEST METHOD

Preparation of the test item and control item

The test item was extracted at the ratio of 0.1 g/mL (since the test item is a porous material) in physiological saline (polar solvent) and cottonseed oil (non-polar solvent) at 37 °C for 72 h and 13 min under sterile condition. This fulfils the requirement of ISO 10993 part 12:2012(E).

Extract	Extraction vehicle	Weight of the test item (g)	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Appearance of extracts
Extract 1	Physiological saline	2.87	86.1	09:20 a.m. on 23 Mar 2021	09:40 a.m. on 26 Mar 2021	Colourless clear solution with minute strands
Extract 2	Physiological saline	NA	10			Colourless clear solution no particulates
Extract 3	Cottonseed oil	2.91	58.2			Yellow viscous liquid, with minute strands
Extract 4	Cottonseed oil	NA	10			Yellow viscous liquid, no particulates

NA-Not applicable; Extraction time: 72 h and 13 min. 0.1 g of test item absorbs 2.0 mL of physiological saline and 1.0 mL of cottonseed oil, additionally 57.4 mL of physiological saline and 29.1 mL of cottonseed oil was added to the extraction volume.

No additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. The extracts and solvent controls were transferred to sterile containers and stored at room temperature. All extracts and solvent controls were used within 2 h and 22 min of preparation and were considered stable during this time.

Test procedure

Justification for method of application	Specified in ISO 10993-10:2010(E) standard, skin irritation in rabbit is recommended as a suitable method to determine the biocompatibility of medical devices.

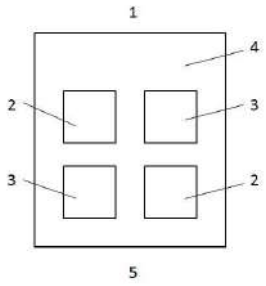
The animals with healthy intact skin were selected for this study. About 18 h and 10 min prior to application, fur on all the rabbits were closely clipped off their backs for an area of 10 cm x 15 cm on both sides of the spine.

Topical Application

About 0.5 mL of test item extracts was loaded on the absorbent gauze measuring

cm2 (2.5 cm x 2.5 cm) which was placed as such on to the fur clipped area of rabbit skin, in the dorsal region on the left cranial end and right caudal end. Similarly, 0.5 mL of solvent controls was loaded on the absorbent gauze (Make: The Ramaraju Surgical Cotton Mills Ltd; Batch. No: 578/19; Expiry Date: July 2022) measuring 6.25 cm2 (2.5 cm x 2.5 cm) which was placed as such on to the fur clipped area of rabbit skin, the right cranial end and left caudal end as shown in the following figure.

The patches were loosely held in contact with the skin by semi-occlusive dressing with means of non-irritant adhesive tape [Make: 3M India Limited; Batch No.: R05190315; Expiry Date: April 2024] for the duration of the 4 h. No test item extracts residues were observed. The test sites were marked with non-irritant permanent ink.



1.Cranial end; 2. Test site; 3. Control site; 4. Clipped dorsal region; 5. Caudal end. Source: ISO 10993-10: 2010(E).

OBSERVATIONS

Mortality & Morbidity

Animals were observed for mortality and morbidity daily throughout the experiment.

Body Weight

Body weight of each animal were recorded prior to dosing and at the end of experiment.

Clinical Observations

Animals were examined for signs of erythema and oedema. The responses were scored at 1 h, and then at 24 h, 48 h and 72 h following the patch removal.

Grading of skin reactions

Animals were macroscopically examined for signs of erythema and oedema, visually

with naked eyes. Skin reactions were graded and recorded at 1 h, and then at 24 h, 48 h and 72 h following the patch removal according to ISO 10993-10:2010(E).

Skin reactions were recorded at each examination as shown in the table below.

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
Maximum possible score for irritation: 8	

Source: ISO 10993-23:2021(E)

In addition to the observation of irritation, all local toxic effects, such as defatting of the skin, and any systemic adverse effects (e.g., effects on clinical signs of toxicity and body weight), were recorded.

Euthanasia

Animals were euthanized by overdose of thiopental sodium injection at the end of the experiment.

Necropsy and Gross pathology

None of the animals were found dead or in moribund condition hence necropsy and gross pathology was not performed.

DATA EVALUATION

The skin irritation scores were evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated.

After 72 h grading, all erythema grades plus oedema grades at 24 h, 48 h and 72 h were totalled separately for test item and control for each animal. The primary irritation score for an animal was calculated by dividing the sum of all the scores by 6 (two test/observation sites, three time points). The primary irritation index (PII) of the test item and control was obtained by adding the scores of the individual animals and dividing it by the total number of animals. The results were evaluated by calculating the difference between the primary irritation score of control and test item.

Based on the observations and primary irritation response, the test item was categorised as per the primary irritation index (Appendix 2).

ACCEPTANCE CRITERIA

The assay is to be considered valid since all the following criteria are met:

1. Positive control trial conducted within the test facility indicated a clear positive result.

2. Solvent control used in the study should gave a mean irritation score of 0 to 0.4.

RESULTS

Mortality & Morbidity
No mortality and morbidity were observed in any of the animals used in this study.

Body Weight
A gradual increase in body weight was observed in all the animals at the end of experiment. Individual body weight of the animals is given in Table 1.

Clinical Observations
No signs of ill health or overt toxicity were observed in the experimental animals.

Grading of skin reactions
The individual score for erythema/eschar and oedema of the test site and control site after 1 h, 24 h, 48 h and 72 h following patch removal are given in Table 2 and Table 3 for all the animals. Mean irritation scores of grading and the difference in primary irritation index of test and control sites are given in Table 4, 5 and Table 6. The observations were not extended to 14 days since no reversibility of the effects was found. Histopathological examination was not performed since unequivocal responses were observed.

CONCLUSION

Based upon the results obtained in this study and in line with ISO ISO 10993 23:2021(E), the given test item, Prima Medix- 3 Ply Disposable Surgical Face Mask, supplied by Integrated Assessment Services Pvt Ltd., is considered as non-irritant in New Zealand White rabbits.

REFERENCES

1. Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process, ISO 10993 1:2018(E).
2. Biological Evaluation of Medical Devices - Part 2, Animal Welfare Requirements, ISO 10993-2:2006(E).
3. Biological Evaluation of Medical Devices - Part 10, Tests for Irritation and Skin Sensitization, ISO 10993-10:2010(E).
4. Biological Evaluation of Medical Devices - Part 12, Sample Preparation and Reference Materials, ISO 10993-12:2012(E).
5. OECD Principles of Good Laboratory Practice. OECD Environmental Health and Safety Publications, Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1. ENV/MC/CHEM (98)17.
6. General Requirements for the Competence of Testing and Calibration Laboratories, ISO/IEC 17025:2017(E).
7. Use of International Standard ISO 10993-1, “Biological Evaluation of Medical Devices, ISO 10993 - Part 1. Evaluation and Testing Within a Risk Management Process. Guidance for Industry and Food and Drug Administration Staff. September 04, 2020

Table 1: Individual body weights

Animal number	Sex	Individual body weights (grams)	
		At the time of dosing	At the end of experiment
1	Male	2209.5	2233.1
2		2237.8	2264.1
3		2519.6	2544.7
4		2159.5	2182.5
5		2503.3	2528.5
6		2466.8	2490.0

Table 2: Individual grades of skin reactions

	Observation Time (h)	Individual score																
		Animal number 1						Animal number 2						Animal number 3				
		T_1	T_2	T	C_1	C_2	T	T_1	T_2	T	C_1	C_2	T	T_1	T_2	T	C_1	C_2	T
Erythema and Eschar formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C_1-First control site; C_2-Second control site; T-Total (Sum of C_1 & C_2)
T_1- First test site; T_2- Second test site; T-Total (Sum of T_1 & T_2)
Source: ISO 10993-23:2021 (E) Clause 7.2.6

Table 3: Individual grades of skin reactions

	Observation Time (h)	Individual score																
		Animal number 4						Animal number 5						Animal number 6				
		T_1	T_2	T	C_1	C_2	T	T_1	T_2	T	C_1	C_2	T	T_1	T_2	T	C_1	C_2	T
Erythema and Eschar formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C_1-First control site; C_2-Second control site; T-Total (Sum of C_1 & C_2)
T_1- First test site; T_2- Second test site; T-Total (Sum of T_1 & T_2)
Source: ISO 10993-23:2021 (E) Clause 7.2.6

Table 4: Calculation of primary irritation score at three time points

		Observation Time (h)	Individual score								
			Animal number 1			Animal number 2			Animal number 3		
			Score	Total Score	PI Score	Score	Total Score	PI Score	Score	Total Score	PI Score
Test (T)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		
Control (C)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		

Total score = Sum of all the scores at test site (or) control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-23:2021(E) Clause 7.2.6

Table 5: Calculation of primary irritation score at three time points

		Observation Time (h)	Individual score								
			Animal number 4			Animal number 5			Animal number 6		
			Score	Total Score	PI Score	Score	Total Score	PI Score	Score	Total Score	PI Score
Test (T)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		
Control (C)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		

Total score = Sum of all the scores at test site (or) control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-23:2021(E) Clause 7.2.6

Table 6: Calculation for Primary Irritation Index and Primary Irritation difference by using Primary Irritation Score

Animal number	1	2	3	PII*	PII difference#	4	5	6	PII*	PII difference#
Negative control site	0	0	0	0	0	0	0	0	0	0
Test item site	0	0	0	0		0	0	0	0	

*Primary irritation index (PII) = Sum of all primary irritation scores divided by 3
#PII difference = PII of test site - PII of control site
Source: ISO 10993-23:2021(E) Clause 7.2.6

APPENDIX 1

CONCISE POSITIVE CONTROL STUDY DATA

Study number	000/048
Study title	Skin Irritation Test in New Zealand White Rabbits
Study start date	23 November 2020
Experiment start date	30 November 2020
Experiment completion date	18 December 2020
Study completion date	31 December 2020

INTRODUCTION

Skin irritation is a key toxicity endpoint to assess biocompatibility of medical devices. An assessment is made for testing the potential of the material under test to produce dermal irritation in rabbits following topical application. This study is a positive control trial, which is conducted once in every three months in IAS Associated Laboratory, to validate our routine procedures.

OBJECTIVE

This skin irritation test was conducted to demonstrate the positive response of Sodium Lauryl Sulphate in New Zealand White Rabbits.

DETAILS OF POSITIVE CONTROL ITEM [Sodium Lauryl Sulphate]

Appearance/Colour	Form: Rods, Colour: White
Manufacturer	Sigma Aldrich
Batch No.	0000009635
Manufacture Date	Not available
Expiry Date	August 2022
Concentration used in study	20% w/v Sodium Lauryl Sulphate

METHODOLOGY

This study was performed based on ISO 10993-10:2010(E) and OECD 404 standard.
One gram of Sodium Lauryl Sulphate was dissolved in distilled water and made up to 5 mL to obtain 20% w/v Sodium Lauryl Sulphate solution. Three male rabbits were clipped free of fur on dorsal side from an area of approximately 10 cm x 15 cm on both sides of the spinal cord approximately 17 h and 05 min prior to commencement of the experiment. The test item (0.5 mL) was applied onto the gauze measuring 6.25 cm2 (2.5 cm x 2.5 cm) and placed on the test site in the dorsal region on the left cranial end and right caudal end of rabbit skin. Similarly, 0.5 mL of the negative control (distilled water) was applied onto the gauze measuring 6.25 cm2 (2.5 cm x 2.5 cm) and placed in

the right cranial end and left caudal end on the control site.

The application sites were covered with a gauze patch (Make.: The Ramaraju Surgical Cotton Mills Limited; Batch No: 578/19; Expiry Date: July 2022) which was loosely held in contact with the skin by means of a suitable semi-occlusive dressing and non- irritant adhesive tape (Make.: 3M India Limited; Batch No.: R05190315; Expiry Date: April 2024) for all the animals. The patches were removed, 4 hours after the test item application and the test sites were marked with non-irritant permanent ink. No residues of the test item were found at the test site after patch removal.

STUDY RESULTS

Table 1: Individual grades of skin reactions

	Observation Time (h)	Individual score Animal number 1						Animal number 2						Animal number 3					
		T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C
Ervthema	1	0	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0
, Eschar formation	24	1	1	2	0	0	0	1	1	2	0	0	0	1	1	2	0	0	0
	48	2	1	3	0	0	0	1	2	3	0	0	0	1	2	3	0	0	0
	72	2	1	3	0	0	0	2	2	4	0	0	0	1	2	3	0	0	0
	Day 7	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema formation	24	1	1	2	0	0	0	1	1	2	0	0	0	1	1	2	0	0	0
	48	2	1	3	0	0	0	2	2	4	0	0	0	1	2	3	0	0	0
	72	2	1	3	0	0	0	2	2	4	0	0	0	2	2	4	0	0	0
	Day 7	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0

C_1-First control site; C_2-Second control site; C-Sum of C_1 & C_2
T_1- First test site; T_2- Second test site; T-Sum of T_1 & T_2
Source: ISO 10993-10:2010(E) Clause 6.3.6

Calculation of primary irritation score at three time points

Sites	Skin Reaction	Observation Time (h)	Individual score Animal number 1 Score	Animal number 1 Total Score	Animal number 1 PI Score	Animal number 2 Score	Animal number 2 Total Score	Animal number 2 PI Score	Animal number 3 Score	Animal number 3 Total Score	Animal number 3 PI Score
Test (T)	Erythema and Eschar formation	24	2	16	2.7	2	19	3.2	2	17	2.8
		48	3			3			3		
		72	3			4			3		
	Oedema formation	24	2			2			2		
		48	3			4			3		
		72	3			4			4		
Control (C)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		

Total score = Sum of all the scores at test site (or) negative control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-10:2010(E) Clause 6.3.6

Calculation for Primary Irritation Index and Primary Irritation difference by using Primary Irritation Score

Animal number	1	2	3	PII	PII difference
Negative control site	0	0	0	0	2.9
Test item site	2.7	3.2	2.8	2.9	

Primary irritation index (PII) = Sum of all primary irritation scores divided by 3
PII difference = PII of test site - PII of negative control site
Source: ISO 10993-10:2010 (E) Clause 6.3.6

DISCUSSION

Based on the primary irritation index obtained, 20% w/v Sodium Lauryl Sulphate is considered as an irritant to rabbit skin. Given that the mucosal membranes are more prone to irritant effects of chemicals, than the skin, it can be considered that 20% Sodium Lauryl Sulphate may induce irritation in mucosal membranes. Therefore, no separate animal experiments were performed in view of 3R's principles of animal testing.

CONCLUSION

Based on the results obtained, 20% w/v Sodium Lauryl Sulphate induced a primary irritation score of 2.9 and hence concluded as a moderate irritant under the conditions of the present study.

SUMMARY OF POSITIVE CONTROL TRIAL (STUDY NUMBER 000/048)

Study number	Study start date	Experiment start date	Experiment completion date	Study completion date	Agent used	Result
000/048	23 November 2020	30 November 2020	18 December 2020	31 December 2020	20% Sodium Lauryl Sulphate	Moderate irritant

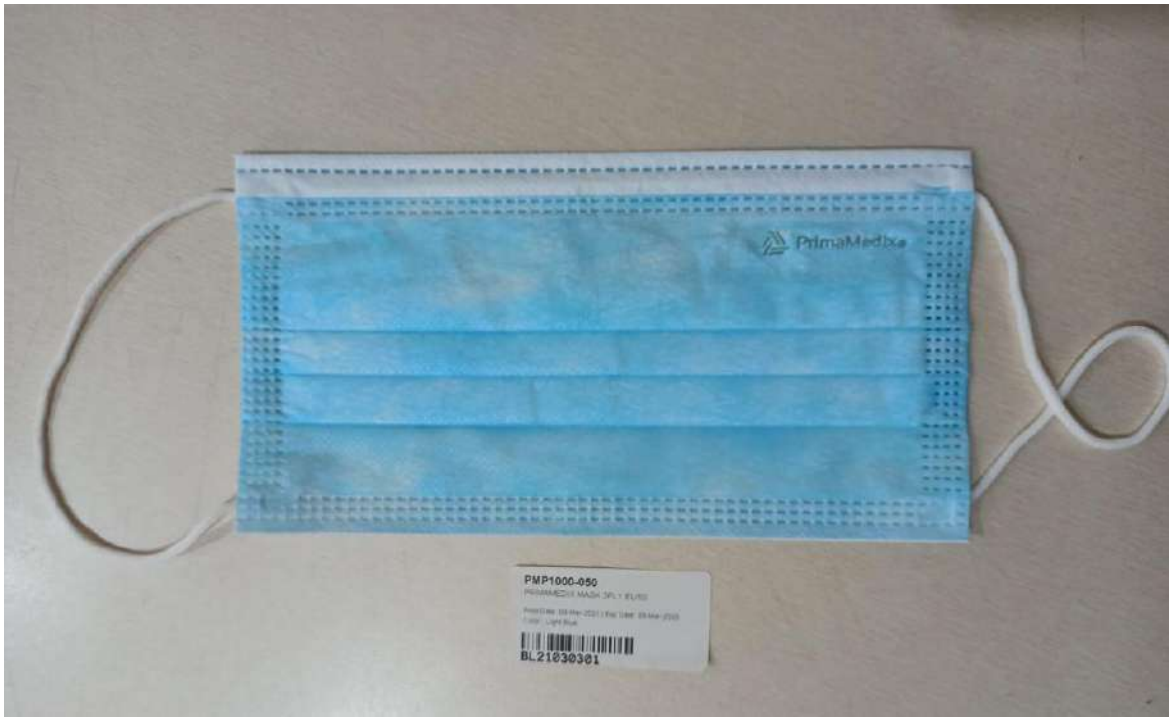
APPENDIX 2

Primary Irritation Index (PII)

Mean Score	Response category
0 to 0.4	Negligible
0.5 to 1.9	Slight
2 to 4.9	Moderate
5 to 8	Severe

Source: ISO 10993-10:2010(E)

PHTOTOGRAPH OF THE TEST ITEM



STATEMENT OF STUDY COMPLIANCE

This study was performed in compliance with:

- OECD Principles of Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17 and
- ISO/IEC 17025:2017(E) (general requirements for the competence of testing and calibration laboratories).

All procedures were performed in accordance with IAS Associated Laboratory Standard Operating Procedures (SOPs). The study was subjected to Quality Assurance evaluation by the IAS Associated Laboratory Quality Assurance Unit (QAU) in accordance with SOPs.

STUDY PLAN AMENDMENT

No study plan amendment was made during the conduct of the study.

STUDY PLAN DEVIATION

No study plan deviation occurred during the conduct of the study.

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, a sample test item, study plan and the final report will be retained for a period of 9 years in the IAS Associated Laboratory archives, after issue of the final report. At the end of the specified archive period the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

DISTRIBUTION OF REPORTS

Three originals of the study report are prepared and distributed as mentioned below:

1. Client.
2. IAS Archive
3. Laboratory Archive

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