Study No.: **733/006**

Study Title: **Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test)**

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

Manufacturer: **PT Prima Medix Nusantara**

STUDY REPORT

Study Title	Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test)
Test Item	Prima Medix- 3 Ply Disposable Surgical Face Mask Manufacturer: PT Prima Medix Nusantara
Study Conducted by	Mr. G. Santhakumar, MSc (Biotech)
Sponsor/ Test Facility	Integrated Assessment Services Pvt Ltd 1495/1, Manasarovar, 16th Main Road Anna Nagar West Chennai - 600040
Study Number	733/006
Regulatory Guideline	Biological Evaluation of Medical Devices - Part 10, Tests for Irritation and Skin Sensitization, ISO 10993-10:2010(E).
Report Issued	07 May 2021
Total Number of Pages	27

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STUDY DIRECTOR AUTHENTICATION STATEMENT

Study No.: **733/006**

Study Title: **Skin Sensitization Test in Guinea Pigs**

(Guinea Pig Maximization Test)

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 planned 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.: 733/006

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/733/006/001)
2	Study Plan Verification	17 March 2021	Definitive Study Plan	17 March 2021 (SBI/733/006/002)
3	In Life Phase Inspection	17 March 2021	Test Item Extract Administration - Intradermal Phase	17 March 2021 (SBI/733/006/003)
4	In Life Phase Inspection	22 March 2021	Test Item Extract Application - Topical Phase	22 March 2021 (SBI/733/006/004)
5	In Life Phase Inspection	05 April 2021	Test Item Extract Application - Challenge Phase	05 April 2021 (SBI/733/006/005)
6	In Life Phase Inspection	08 April 2021	Grading of Skin Reactions	08 April 2021 (SBI/733/006/006)
7	Report Audit	29 April 2021	Draft Report	29 April 2021 (SBI/733/006/007)

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.: 733/006

Study Title: : Skin Sensitization Test in Guinea Pigs

(Guinea Pig Maximization Test)

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 sensitization potential of Prima Medix- 3 Ply Disposable Surgical Face Mask Manufacturer: PT Prima Medix Nusantara, supplied by Integrated Assessment Services Pvt Ltd, was evaluated in male guinea pigs using guinea pig maximization test (GPMT).

The test item, Prima Medix- 3 Ply Disposable Surgical Face Mask Manufacturer: PT Prima Medix Nusantara is used for respiratory protection. The dimensions of the test item are length: 17.6 cm height: 9.5 cm and thickness: less than 2 mm (as stated by sponsor). It is a surface device which comes in contact with skin. The duration of contact is less than or equal to 24 hours.

Test item was extracted at a ratio of 0.1 g/mL (since the test item is a low-density porous material) in physiological saline (polar solvent) and cottonseed oil (non-polar solvent) at 37 ± 1 °C for 72 ± 2 h (intradermal induction - 72 h and 25 min, topical application - 72 h and 10 min & challenge phase - 72 h and 13 min) under sterile conditions. The test item weighing 2 g was used for extraction. For each intradermal induction, topical application and challenge phase, the polar extract was prepared by extracting 2 g of test item in 20 mL of physiological saline. Similarly, non-polar extract was prepared by extracting 2 g of test item in 20 mL of cottonseed oil. Solvent controls were also subjected to same extraction conditions. This fulfils the requirement of ISO 10993- 12:2021(E).

At the end of extraction, minute strands were observed in both polar and non-polar extracts. Solvent controls were clear without any colour change or particulates. 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 a maximum of 6 h and 25 min of preparation and were considered stable during this time.

Animals were divided into four groups; G1 – five guinea pigs for polar solvent control, G2 – ten guinea pigs for polar test item extract, G3 – five guinea pigs for non-polar solvent control and G4 – ten guinea pigs for non-polar test item extract. The fur over the treatment sites was clipped and shaved on the day of treatment, prior to dosing on all the animals. Induction of sensitization was a two-stage procedure with intradermal injections on day 0 (with Freund's complete adjuvant (FCA), solvent controls and extracts), followed by a topical patch exposure on day 7 for 48 h.

On day 21, challenge patches were applied for 24 h. Skin reaction grading was performed using Magnusson and Kligman scale at 24 h and 48 h, after removing the challenge patches according to ISO 10993-10:2010(E).

Positive control trials for sensitization are carried out once in three months at IAS Associated laboratory in guinea pigs using 2,4-Dinitrochlorobenzene. The trial completed on 07 January 2021 gave a clear sensitizing reaction in 90% of treated animals. No response was observed in solvent controls treated animals. Therefore, the assay was considered valid.

No mortality or morbidity were observed in any of the animals used in this study. A gradual increase in body weight was observed in all the animals at the end of the experiment. No skin sensitization reactions were observed in both control and test sites of all the animals. Therefore, no gross and histopathological examination were conducted.

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 Manufacturer: PT Prima Medix Nusantara, supplied by Integrated Assessment Services Pvt Ltd, is considered as non-sensitizer to Guinea Pigs.

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.

Sensitization (Type IV hypersensitivity reaction) is a key toxicity endpoint to assess the biocompatibility of medical devices. Guinea pig maximization test is a sensitive method to determine the sensitization potential of medical devices, both in terms of induction and elicitation

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	22 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 Manufacturer: PT Prima Medix Nusantara was received at IAS Associated laboratory on 10 February 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

Test Item	Inno Medical - Medical Disposable face Mask Manufacturer: PT Prima Medix Nusantara
Batch \ Lot No.	BL21030301
Manufacture Date	03 March 2021
Expiry Date	03 March 2023
Appearance	Blue face mask
Ingredients	Not provided by sponsor
Temperature Stability	37°C
Sterility	Non-Sterile
Positive Control	2,4-Dinitrochlorobenzene Positive control trials for sensitization are carried out once in three months at IAS Associated laboratory in guinea pigs using 2,4-Dinitrochlorobenzene. The trial completed on 07 January 2021 gave a clear sensitizing reaction in 90% of treated animals (Appendix 3).
Solvent Controls	Physiological saline (0.9% w/v sodium chloride solution)

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. No analysis was performed at IAS Associated laboratory, to confirm it.

Description of the test item

The test item, Prima Medix- 3 Ply Disposable Surgical Face Mask Manufacturer: Inno Medical Pte Ltd is used for respiratory protection. The dimensions of the test item are length: 17.6 cm height: 9.5 cm and thickness: less than 2 mm (as stated by sponsor). It is a surface device which comes in contact with skin. The duration of contact is less than or equal to 24 hours

TEST SYSTEM

Species	*Cavia porcellus* (Guinea pig)
Strain	Dunkin – Hartley
Weight range (g) (at the time of dosing)	313.69 to 408.41
Sex	Male
Source	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 and were quarantined for 7 days.

Number of animals	30
Number of groups	4
Number of animals per group	Physiological saline control: 5 Physiological saline extract: 10 Cottonseed oil control: 5 Cottonseed oil extract: 10
Acclimatization period	5 days
Justification for animal use	Guinea pigs were selected because there is a large volume of background data on this species. Recommended in ISO 10993-10:2010(E) standard guideline as an appropriate species to evaluate the skin sensitization of medical devices and by various regulatory authorities.

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

ANIMAL HUSBANDRY

Test room no.	04
Test room temperature (°C)	19.2 to 21.9
Relative humidity (%)	37 to 60
Housing	Animals were housed individually in polypropylene 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 and signature and individual ear marking.
Feed	Commercial guinea pig pellet feed.
Water	Purified drinking water supplemented with vitamin C was provided *ad libitum*.
Bedding material	Sterilized paddy husk.
Photoperiod	12 h light and 12 h dark cycle
Contaminants	Contaminants, reasonably expected in feed and/or water supplies were not believed to influence the outcome of the study. Analysis of feed, water and bedding materials are carried out once in every 6 months and the results of the most recent analysis were placed in the study file.
Personnel	Appropriately qualified and trained associates were involved in this study.

Selection of animals	Only healthy young adults, previously unused animals were selected for this study.

TEST METHOD

Preparation of test item extracts

Test item was extracted at a ratio of 0.1 g /mL (since the test item is a low-density porous material) in physiological saline (polar solvent) and cottonseed oil (non-polar solvent) at 37 ± 1 °C for 72 ± 2 h. Solvent controls were also subjected to the same temperature and time conditions. This fulfils the requirement of ISO 10993-12:2021(E). The test item weighing 2 g was used for extraction.

Day 0: Intradermal induction phase

The required volume of extract was prepared freshly prior to dosing as given below.

Extracts	Extraction vehicle	Weight of the test item (g)	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts
Extract 1	Physiological saline	NA	10	09:00 am on 12 Mar 2021	09:25 am on 15 Mar 2021	Colourless clear liquid; no particulates
Extract 2	Physiological saline	2	20			Colourless liquid with minute strands
Extract 3	Cottonseed oil	NA	10			Yellow viscous liquid; no particulates
Extract 4	Cottonseed oil	2	20			Yellow viscous liquid with minute strands

NA-Not applicable. Extraction time: 72 h and 25 min.

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 6 h and 13 min of preparation and were considered stable during this time

Day 7: Topical application

The required volume of extract was prepared freshly prior to dosing as given below.

Extracts	Extraction vehicle	Weight of the test item (g)	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts
Extract 1	Physiological saline	NA	10	09:05 am on 19 Mar 2021	09:15 am on 22 Mar 2021	Colourless clear liquid; no particulates
Extract 2	Physiological saline	2	20			Colourless liquid with minute strands
Extract 3	Cottonseed oil	NA	10			Yellow viscous liquid; no particulates
Extract 4	Cottonseed oil	2	20			Yellow viscous liquid with minute strands

NA-Not applicable, Extraction time: 72 h and 10 min.

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 6 h and 25 min of preparation and were considered stable during this time.

Day 21: Challenge phase

The required volume of extract was prepared freshly prior to dosing as given below.

Extracts	Extraction vehicle	Weight of the test item (g)	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts
Extract 1	Physiological saline	NA	10	09:12 am on 02 Apr 2021	09:25 am on 05 Apr 2021	Colourless clear liquid; no particulates
Extract 2	Physiological saline	2	20			Colourless liquid with minute strands
Extract 3	Cottonseed oil	NA	10			Yellow viscous liquid; no particulates
Extract 4	Cottonseed oil	2	20			Yellow viscous liquid with minute strands

NA-Not applicable. Extraction time: 72 h and 13 min.

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 6 h and 13 min of preparation and were considered stable during this time.

Test Procedure

Justification for method of administration The method of administration is in line with the ISO 10993- 10:2010(E) standard. For the induction phase, intradermal injections and the topical application were employed. The challenge phase was accomplished by topical applications.

Animals were divided into four groups; G1 - five guinea pigs for polar solvent control, G2 - ten guinea pigs for polar test item extract, G3 - five guinea pigs for non-polar solvent control and G4 - ten guinea pigs for non-polar test item extract. The fur over the treatment sites was clipped and shaved on the day of treatment, prior to dosing on all the animals. Induction of sensitization was a two-stage procedure with initial administration of intradermal injections followed by a topical patch exposure on day 7.

Intradermal Induction phase
About 0.1 mL intradermal injections (1 mL; Make: Hindustan Syringes and Medical Devices, Batch No: 004011AG32, Exp. Date: December 2024) of the test item extracts, Solvent controls and Freund's Complete Adjuvant (Sigma-Aldrich; Lot No. SLBZ9885; Expiry date: May 2024) in various mixtures were administered to the solvent control and test groups (Appendix 1).

Control group:
Site A: 1: 1 mixture (v/v) Freund's Complete Adjuvant + (solvent) (solution A) Site B: Polar solvent or non-polar solvent (solution B)
Site C: 1: 1 mixture of solution A and solution B

Test group:
Site A: 1: 1 mixture (v/v) Freund's Complete Adjuvant + (solvent) (solution A) Site B: Polar extract of test item or non-polar extract of test item (solution B) Site C: 1: 1 mixture of solution A and solution B

Topical Induction Phase
Since no irritation was observed in day 6, the test area was treated with 10% Sodium Lauryl Sulphate (Avantor Performance Material India Ltd; Batch No.: J159K18; Expiry date: November 2023) in petroleum jelly (Make: HiMedia Laboratories Pvt Ltd; Lot. No: 0000314448; Expiry date: Nov 2022) to create local irritation.

On day 7, an absorbent gauze (Make: The Ramaraju Surgical Cotton Mills Ltd; Batch No.: 578/19; Expiry date: Jul 2022) measuring 8 cm2 loaded with 0.5 mL of test item extract and solvent, respectively was placed topically on respective groups of guinea pigs, on the same site as that of intradermal injections. The over patch was covered loosely with an occlusive dressing which was held in place for 48 h.

Challenge phase
On day 21, the challenge exposure was administered as a topical patch. Absorbent gauze patch measuring 8 cm2 loaded with 0.5 mL of test item extract was placed on the left side and the patch with 0.5 mL of the solvent was placed on the right side of each animal in respective groups for 24 h at sites other than those used for intradermal injections/topical

applications and the application sites were marked with a non-irritant permanent marker ink. The details of the experiment are summarized in Appendix 1

OBSERVATIONS

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

Body weight
Body weight of each animal was recorded at the time of dosing and at the end of experiment.

Grading of skin reactions
Grading of skin reactions was performed visually at 24 and 48 h after removing the challenge patch. The challenge application sites were assessed for erythema and oedema using Magnusson and Kligman scale (Appendix 2).

Euthanasia
Animals were euthanized by carbon dioxide exposure at the end of the experiment.

Necropsy and Gross pathology
Since no abnormality was observed in any of the animals, necropsy and gross pathology was not performed.

DATA EVALUATION

A comparison of the biological responses seen following skin sensitization of the test item extracts and solvents were reported and interpreted, using good scientific judgement.

Skin reactions elicited in terms of incidence and severity of reactions between the test item extracts treated and solvent control groups were compared.

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, re-challenge may be performed to confirm the results from the first challenge. A re-challenge will be carried out 1 week to 2 weeks after the first challenge (induction phase) on the naive side of the animals. The outcome of the results will be presented as the frequency of positive challenge results in test and control animals. If equivocal responses remain, the study will be re-conducted using a minimum of 20 test and 10 control animals.

ACCEPTANCE CRITERIA

The study is considered valid, as the following criteria are met:

1. Positive control trial conducted within the test facility gave clear positive results.

2. No response was observed in solvent control treated animals.

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 the experiment. Body weight of all animals recorded at the time of dosing and end of the experiment is given in Table 1.

Grading of skin reactions
Grading of skin reactions performed at 24 h and 48 h after removing the challenge patch are given in Table 2. No sensitization reactions were observed in animals treated with the solvent control. No evidence of sensitization was seen in any of the test item extracts treated animals.

Gross Pathology and Histopathology
No gross and histopathological examination were found necessary in this study.

CONCLUSION

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 Manufacturer: PT Prima Medix Nusantara, supplied by Integrated Assessment Services Pvt Ltd, is considered as non-sensitizer to Guinea Pigs.

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

PHOTOGRAPH OF THE TEST ITEM

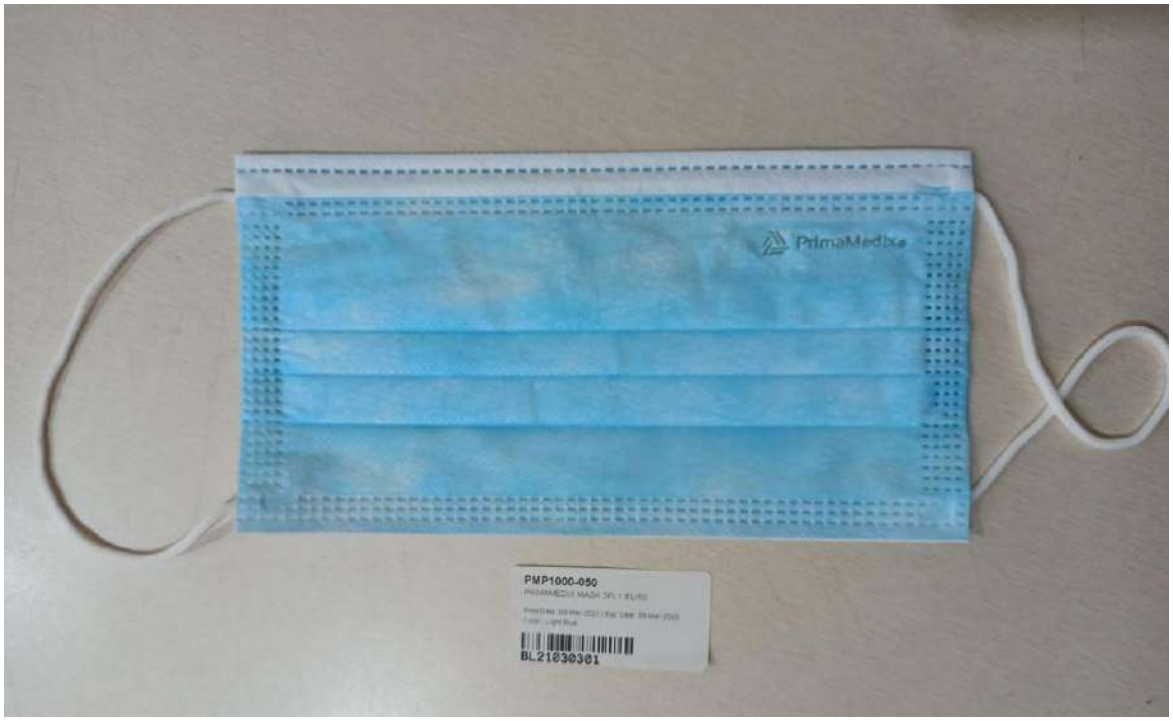


Table 1: Individual body weights of all animals

Group	Sex	Animal No.	Weight (in grams)		
			At the time of dosing	At the end of experiment	Increase in weight
G1	M	1	408.41	445.93	37.52
		2	352.57	383.28	30.71
		3	332.16	369.52	37.36
		4	325.56	359.21	33.65
		5	387.50	423.41	35.91
		Mean ± SD	**361.24 ± 35.74**	**396.27 ± 36.95**	**35.03 ± 2.87**
G2	M	6	363.49	399.24	35.75
		7	353.35	386.61	33.26
		8	387.30	422.23	34.93
		9	360.26	395.50	35.24
		10	405.88	440.08	34.20
		11	356.09	392.88	36.79
		12	394.14	427.85	33.71
		13	401.56	434.21	32.65
		14	321.69	359.13	37.44
		15	320.48	357.65	37.17
		Mean ± SD	**366.42 ± 30.61**	**401.54 ± 29.32**	**35.11 ± 1.67**
G3	M	16	320.64	354.22	33.58
		17	315.68	354.12	38.44
		18	371.32	407.98	36.66
		19	404.26	439.61	35.35
		20	395.57	431.91	36.34
		Mean ± SD	**361.49 ± 41.4**	**397.57 ± 41.3**	**36.07 ± 1.79**
G4	M	21	402.89	440.82	37.93
		22	378.34	414.09	35.75
		23	358.81	392.93	34.12
		24	372.41	405.30	32.89
		25	400.31	433.56	33.25
		26	376.62	414.04	37.42
		27	313.69	349.86	36.17
		28	361.65	392.99	31.34
		29	347.85	379.41	31.56
		30	349.31	384.81	35.50
		Mean ± SD	**366.19 ± 26.37**	**400.78 ± 26.83**	**34.59 ± 2.32**

Table 2: Grading of skin reaction after removal of the challenge patch

Group	Sex	Animal No.	Magnusson and Kligman Scale			
			24 h		48 h	
			C	T	C	T
G1	M	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	0	0	0
		5	0	0	0	0
G2	M	6	0	0	0	0
		7	0	0	0	0
		8	0	0	0	0
		9	0	0	0	0
		10	0	0	0	0
		11	0	0	0	0
		12	0	0	0	0
		13	0	0	0	0
		14	0	0	0	0
		15	0	0	0	0
G3	M	16	0	0	0	0
		17	0	0	0	0
		18	0	0	0	0
		19	0	0	0	0
		20	0	0	0	0
G4	M	21	0	0	0	0
		22	0	0	0	0
		23	0	0	0	0
		24	0	0	0	0
		25	0	0	0	0
		26	0	0	0	0
		27	0	0	0	0
		28	0	0	0	0
		29	0	0	0	0
		30	0	0	0	0

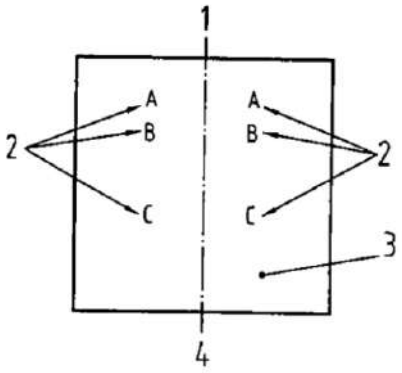
M - Male; C- Control site; T- Treated site; h- hour

APPENDIX I

Test Procedure

Group	Animal No.	Sex	Treatment Group	Intradermal Induction Phase (0.1 mL)			Topical induction phase (0.5 mL per patch) *		Challenge phase # (0.5 mL per patch) *
				Injection I (Solution A)	Injection II (Solution B)	Injection III (Solution C)	10% SLS	Treatment	
G1	1-5	M	Polar solvent control	1: 1 mixture of FCA and polar solvent	Polar solvent	1: 1 mixture of sol. A and sol. B	Yes	Polar solvent	Polar solvent & Polar extract of test item
G2	6-15	M	Test item in polar solvent	1: 1 mixture of FCA and polar solvent	Polar extract of test item	1: 1 mixture of sol. A and sol. B		Polar extract of Test item	Polar solvent & Polar extract of test item
G3	16-20	M	Non-polar solvent control	1: 1 mixture of FCA and non-polar solvent	Non-polar solvent	1: 1 mixture of sol. A and sol. B		Non-polar solvent	Non-polar solvent & Non-polar extract of test item
G4	21-30	M	Test item in non-polar solvent	1: 1 mixture of FCA and non-polar solvent	Non-polar extract of test item	1: 1 mixture of sol. A and sol. B		Non-polar extract of Test item	Non-polar solvent & Non-polar extract of test item

M - Male; FCA - Freund's Complete Adjuvant; SLS-Sodium Lauryl Sulphate;
* Gauze patch size = 8 cm2 approximately
# Two challenge patches are placed on upper flank, one on left side and other on right side
Intradermal Injection was administered on Day 0 at sites A, B and C
Topical application was applied on Day 7
Challenge dose was applied on Day 21
Sites A, B and C are shown below:



1 - Cranial end; 2 - 0.1 mL intradermal injection sites; 3 - Clipped intrascapular region; 4 - Caudal end
Source: ISO 10993-10:2010(E).

APPENDIX 2

Magnusson and Kligman scale

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

Source: ISO 10993-10:2010(E).

APPENDIX 3

CONCISE POSITIVE CONTROL STUDY DATA

Study Number	000/047
Study Title	Skin sensitization test in guinea pigs (Guinea Pig Maximization Test)
Study Start Date	07 November 2020
Experiment Start Date	10 November 2020
Experiment Completion Date	10 December 2020
Study Completion Date	07 January 2021

OBJECTIVE

To ensure the reproducibility and sensitivity of the test procedure at the test facility, skin sensitization assay with positive control 2,4-Dinitrochlorobenzene is performed. This data will serve as a positive control for all the sensitization test conducted at the test facility for three months from the date of issue of the final report for this study.

CONTROL ITEM DETAILS [2,4-Dinitrochlorobenzene (DNCB)]

Manufacturer:	Sigma-Aldrich
Appearance \ Colour:	Crystalline \ Faint yellow
Batch No.:	BCBS4201V
CAS No.:	97-00-7
Molecular Formula:	C6H3ClN2O4
Molecular Weight:	202.55 g/mol
Date of Receipt:	05 March 2018
Expiry date:	04 March 2023

METHODOLOGY

This study was performed based on OECD 406 and ISO 10993-10:2010(E) standard.

Induction: Intradermal Injections
On day 0, 0.1 mL intradermal injection of the following were given to the fur clipped animals in the treated group. Injection 1: a 1:1 mixture (v/v) of FCA & physiological saline (Solution A), injection 2: a 0.025% w/v of DNCB in 1:4 v/v acetone: sesame oil (Solution B) and injection 3: a 1:1 mixture of solution A and solution B.

Similarly, 0.1 mL intradermal injection of the following were given to the fur clipped animals in the control group. Injection 1: a 1:1 mixture (v/v) of FCA & physiological saline (Solution A), injection 2: a 1:4 v/v acetone: sesame oil (Solution B) and injection 3: a 1:1 mixture of solution A and solution B.

Induction: Topical Application

Since DNCB induced skin reaction, Sodium Lauryl Sulphate was not applied on the day before thetopical treatment. On day 7, the test and control area were again cleared of fur and an absorbent gauze patch (2 x 4 cm) loaded with 0.2 mL of 0.25 % w/v of DNCB in a 1:4 v/v acetone: sesame oil and 0.2 mL of 1:4 v/v acetone: sesame oil, was placed on respective groups. The patch was then held in contact by an occlusive dressing for 48 h.

Challenge: Topical Application

On day 21, the flanks of treated and control animals were cleared of fur. Absorbent gauze patch loaded with 0.2 mL of 0.1 % w/v of DNCB in 1:4 v/v acetone: sesame oil was applied on left side and absorbent gauze patch loaded with 0.2 mL of 1:4 v/v acetone: sesame oil was applied on right side of each animal in respective groups at the sites other than those used for intradermal injections/topical applications. The gauze patches were held in contact by an occlusive dressing for 24 h, then the patch was removed and the application sites were marked with non-irritant marker ink. At 21 hours, after removing the patch, the challenge area was cleaned and closely-clipped. The application sites were scored at 24 and 48 h after patch removal using a Magnusson and Kligman grading scale.

Magnusson and Kligman Grading Scale (For evaluation of Challenge patch test reactions)

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

STUDY RESULTS

Grading of skin reaction after removal of the challenge patch

Group	Animal No.	Magnusson and Kligman Scale			
		At control site		At treated site	
		24 h	48 h	24 h	48 h
G1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
G2	6	0	0	2	2
	7	0	0	1	1
	8	0	0	1	1
	9	0	0	2	1
	10	0	0	2	2
	11	0	0	0	0
	12	0	0	2	2
	13	0	0	2	1
	14	0	0	2	1
	15	0	0	1	1

CONCLUSION

The results indicated that animals treated with 2,4-Dinitrochlorobenzene (Batch No. BCBS4201V), induced sensitization reactions in 90% of treated animals. Therefore, according to OECD Guidelines for Testing of Chemicals, 406 and ISO 10993-10:2010(E), 2,4-Dinitrochlorobenzene (Batch No. BCBS4201V) is categorized as a strong sensitizer under the conditions of the present study.

Summary of positive control trial for skin sensitization, GPMT (000/047)

Study start date	Experiment Start Date	Experiment Completion Date	Study Completion date	Concentration of 2,4-Dinitrochlorobenzene			Vehicle used	Result	
				Induction Phase 1 (Intradermal)	Induction Phase 2 (Topical)	Challenge Phase		No of animals +ve	Maximum reaction grading
07 November 2020	10 November 2020	10 December 2020	07 January 2021	0.025% w/v	0.25% w/v	0.1% w/v	1:4 v/v acetone: sesame oil	+ve in 9/10 animals	Grade 2 - Moderate and confluent erythema

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.
- US Food and Drug Administration's GLP regulations, 21 CFR Part 58 (subparts B to G and J).
- 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

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

1. Sponsor.
2. Archive – Laboratory
3. IAS Archeive

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