Nelson Labs™
A Sotera Health company

Sponsor:
Julia Madison
Armbrust Inc.
3813A Helios Way #290
Pflugerville, TX 78660

Intracutaneous Injection Test – ISO (GLP)

Test Article:	Model: USA Made Surgical Mask Part ID: AA-US-SURGICAL-01
Study Number:	1297408-S01.1 Amended
Study Received Date:	08 May 2020
Testing Facility:	Toxikon USA
Deviations:	None

Summary: Enclosed is the final report for the testing we coordinated for you. The information is retained by the testing laboratory.

Amendment Justification: The final report was amended to update the test article name, per sponsor request.

If you have any questions, please feel free to call or email any of our Subcontracting personnel at 801-290-7500 or subcontracting@nelsonlabs.com. Thank you for testing with Nelson Laboratories, LLC.

Mindy Schvaneveldt
Reviewed By Mindy Schvaneveldt, A.S.

30 NOV 2021
Amended Report Date

1297408-S01

TOXIKON
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FINAL GLP REPORT: 20-01829-G1
AMENDED

Nelson Report Number: NL # 1297408

INTRACUTANEOUS INJECTION TEST – ISO

Test Article
Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

21 CFR Part 58 Compliance
Good Laboratory Practice for Nonclinical Laboratory Studies

Final Report Date
6/11/2020

Amended Final Report Date
11/8/2021

Study Director
Sarah Goulet, M.S.

Sponsor
Nelson Laboratories, LLC
A Sotera Health Company
6280 South Redwood Road
Salt Lake City, UT 84123
USA

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STUDY SUMMARY

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, Model: USA Made Surgical Mask Part ID: AA-US-SURGICAL-01, were evaluated for their potential to produce irritation after intracutaneous injection in New Zealand White rabbits. The test article sites did not show a significantly greater biological reaction than the sites injected with the control article.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–10 guidelines.

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon's Management.

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase	Inspection Date	Date Reported to Study Director	Date Reported to Management
SCORING	6/4/2020	6/4/2020	6/4/2020
DATA	6/11/2020	6/11/2020	6/11/2020
FINAL REPORT	6/11/2020	6/11/2020	6/11/2020
AMENDED REPORT	11/8/2021	11/8/2021	11/8/2021

Stephanie McHugh

Stephanie McHugh, M.S.
Quality Assurance

11-8-21
Date

GLP COMPLIANCE STATEMENT

This study meets the technical requirements of the protocol.

This study was conducted in compliance with the current U.S. Food and Drug Administration 21 CFR, Part 58 Good Laboratory Practices for Nonclinical Laboratory Studies.

The sections of the regulations not performed by or under the direction of Toxikon Corporation, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article, 21 CFR, Part 58.105, and its mixture with carriers, 21 CFR, Part 58.113.

SIGNATURES

Signature Information	
Protocol Number	p19-1787-00d
Study Director	Sarah Goulet, M.S.
Study Supervisor	Allan Sleger, A.S., LAT
Company	Toxikon Corporation

VERIFICATION DATES

The study initiation day is the date the protocol is signed by the Study Director.

Verification Dates	
Test Article Receipt	5/22/2020
Project Log	5/22/2020
Study Initiation	5/26/2020
Study Completion	6/11/2020

Sarah Goulet
Sarah Goulet, M.S.
Study Director

11/8/21
Date

1.0 PURPOSE

The purpose of the study was to determine the potential irritation effects of the test article extract as a result of an intracutaneous injection in New Zealand White rabbits.

2.0 REFERENCES

The study was based upon the following references:

- ISO 10993–10, 2010, Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Skin Sensitization.
- ISO 10993–12, 2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.
- ISO/IEC 17025, 2017, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

The study conformed to the current FDA 21 CFR, Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a Test Requisition Form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor was responsible for all test article characterization data as specified in the GLP regulations.

4.1 Test Article:

Name: Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

CAS/Code Number: Not Supplied by Sponsor (N/S)

Lot/Batch Number: 0506200101

Physical State: Insoluble

Color: N/S

Expiration Date: N/S

Density: Unknown

Stability: Unknown

Sterility: Not Sterile

Sterilization Conditions: N/S

Storage Condition: Room Temperature

Safety Precautions: Unknown

Intended Use: N/S

4.2 Negative Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article 1:

Name: USP 0.9% Sodium Chloride for Injection (NaCl)

Toxikon QC Number: CSC-20-03-00179

4.2.2 Negative Control Article 2:

Name: Cottonseed Oil (CSO)

Toxikon QC Number: CSC-20-05-00112

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

Number and Species: 3 New Zealand White rabbits (*Oryctolagus cuniculus*)

Sex: female (females were non–pregnant and nulliparous)

Weight/Age Range: 2.21 – 2.51 kilograms / at least 10 weeks old (adult) weighed to the nearest 10 g

Health Status: healthy, not previously used in other experimental procedures

Animal Purchase: Envigo Global Services, Denver, PA

Animal Identification: ear tattoo

Acclimation: minimum 5 days, under same conditions as for the actual test

Animal Selection: selected from larger pool and examined to ensure lack of adverse clinical signs

5.2 Animal Care and Maintenance:

Animal Room Target Temperature: 68 ± 5 °F

Animal Room Target Relative Humidity: 30–70%

Air Exchanges per Hour: a minimum of 10 changes per hour

Lights: 12–hour light/dark cycle, full spectrum fluorescent lights

Housing: individually housed

Cages: suspended stainless steel

Bedding: Alfa Cobs, ScottPharma Solutions, Marlborough, MA (non-contact)

Animal Rations: Teklad Global High Fiber Rabbit Diet 2031, Envigo, Madison, WI, *ad libitum*

Water: tap water, *ad libitum*

There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

The laboratory and animal rooms were maintained as limited–access facilities.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Justification of Test System:

Historically, New Zealand White rabbits have been used in intracutaneous safety evaluation studies because the guidelines have no alternative (non–animal) methods. The animal species, number, and route of test article administration are recommended by the ISO 10993–10 guidelines.

6.2 Route of Administration:

Animals were treated by intracutaneous injections. The test article was extracted and administered *in vivo* through a medium compatible with the test system, as indicated on the Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Preparation, Extraction Medium, and Extraction Conditions:

The test article (307.58 cm^2 as per Sponsor) was combined with 102.5 mL of vehicle following an ISO 10993–12 ratio of 3 cm^2 per 1 mL. The test article was separately extracted in NaCl and CSO at 50 ± 2 °C for 72 ± 2 hours under dynamic conditions. A total of 2 units were used for testing.

7.1.2 Addition of Extraction Medium:

Properly prepared test articles were placed in separate extraction vessels, and to each vessel the appropriate medium was added. The extraction medium completely covered the test article.

7.1.3 Control Conditions:

An untreated control (blank) was prepared for parallel treatment and comparison. The untreated control was the extraction medium that was subjected to the same temperature and for the same duration as the test article.

7.1.4 Extract Agitation:

Each extract was agitated vigorously prior to administration.

7.1.5 Extract Examination:

The test article appeared unchanged by the extraction procedure. The extracts were clear and free of particulates and the color of the vehicle unchanged.

7.1.6 Extract Manipulation:

The extracts were not filtered, centrifuged, or pH adjusted.

7.1.7 Extract Storage:

Following extraction, the vessel containing each test or control article was cooled to room temperature.

After the completion of the extraction, the extracts were kept at room temperature and were used the same day the extraction was completed. No storage of the extracts occurred.

7.1.8 Other Test Article Preparation:

All other test article preparation was as specified by the Sponsor.

7.2 Pre–Dose Procedure:

7.2.1 Pre–Treatment Screening Procedure:

Animals selected for the study were examined to ensure that their skin was free from irritation, trauma, and disease.

7.2.2 Body Weights:

Each animal was weighed on the day of the study prior to injection.

7.2.3 Fur Clipping:

Each animal was clipped free of fur on the dorsal side within 4 to 18 hours prior to injection.

7.3 Dose Administration:

A volume of 0.2 mL per site of one extract was injected intracutaneously at one side of each of three rabbits, five sites for the test article extract and five posterior sites for the control.

Similarly, at the other side of each rabbit, the other extract was injected.

The maximum injections per rabbit was limited to 2 test articles and 2 corresponding control articles.

Extracts prepared with NaCl and CSO were tested at 100% (neat) concentration.

7.4 Post–Dose Procedure:

The injection sites on each animal were observed for signs of erythema and edema immediately following injection and at 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours after injection of the test article. Observations were scored according to the Classification System for Scoring Skin Reactions (see Appendix I).

7.4.1 Clinical Observations:

Observations conducted also included all clinical and toxicologic signs.

7.4.2 Body Weights:

At the end of the observation period, the animals were weighed.

7.4.3 Euthanasia:

At the end of the study, the animals were returned to the general colony.

8.0 EVALUATION CRITERIA

8.1 Evaluation of Data:

After the 72 ± 2 hours grading, all erythema grades plus edema grades from 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours were totaled separately for each test article or vehicle control for each individual animal. To calculate the score of a test article or vehicle control on each individual animal, divide each of the totals by 15 (3 scoring time points × 5 test or vehicle control injection sites). To determine the overall mean score for each test article and each corresponding vehicle control, add the scores for the three animals and divide by three. The final test article score was obtained by subtracting the score of the vehicle control from the test article score. The requirements of the test will be met if the difference between the test article mean score and the vehicle control mean score is 1.0 or less. If at any observation period the average reaction to the test article is questionably greater than the average reaction to the vehicle control, the test will be repeated using three additional rabbits.

8.2 Control of Bias Statement:

The study as designed employed methodology to minimize uncertainty of measurement and to control bias for data collection and analysis, which included but was not limited to: concurrent control data, system suitability assessment, randomization, and method controls such as blanks and replicates.

9.0 RESULTS

9.1 Animal Weights:

All of the test animals increased in weight (Table 1).

9.2 Clinical Observations:

None of the animals exhibited overt signs of toxicity at any of the observation points (Table 1).

The sites injected with the test article did not show a significantly greater biological reaction than the sites treated with the control article (Table 2). The difference of the overall mean score between the test article and the control article was 0.0.

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, Model: USA Made Surgical Mask Part ID: AA-US-SURGICAL-01, were evaluated for their potential to produce irritation after intracutaneous injection in New Zealand White rabbits. The test article sites did not show a significantly greater biological reaction than the sites injected with the control article.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–10 guidelines.

11.0 RECORDS

- Original raw data will be archived by Toxikon Corporation.
- The original final report and any report amendments will be archived by Toxikon Corporation.

- A copy of the final report and a copy of any protocol amendments or deviations will be forwarded to the Sponsor.
- The test article will be disposed by Toxikon.
- Test article retention upon study completion is the responsibility of the Sponsor.

12.0 CONFIDENTIALITY AGREEMENT

Per corporate policy, confidentiality shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between Toxikon and the Sponsor.

13.0 ANIMAL WELFARE STATEMENT

The Sponsor assured that, to the best of their knowledge, this study did not unnecessarily duplicate previous testing and that there were no non–animal alternatives acceptable for the evaluation of this test article as defined by the protocol.

No evidence of pain and distress was reported to the Veterinarian and/or Study Director during the course of this study.

Toxikon strictly adheres to the following standards in maintaining the animal care and use program:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service, 9 CFR Ch. 1, Subchapter A–Animal Welfare.

"Guide for the Care and Use of Laboratory Animals," National Research Council, 2011.

Office for Laboratory Animal Welfare (OLAW), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99–158 November 20, 1985), revised 2015.

ISO 10993–2, 2006, Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements.

AAALAC International accreditation.

14.0 UNFORESEEN CIRCUMSTANCES

Any unforeseen circumstances were documented in the raw data. However, no unforeseen circumstances that affected the integrity of the study were noted.

15.0 PROTOCOL AMENDMENTS/DEVIATIONS

There were no protocol amendments or deviations. No changes to the protocol were required.

TABLE 1:
Animal Weights and Clinical Observations

Group	Animal #	Sex	Body Weight (kg)			Signs of Toxicity*
			Day 0 6/3/2020	Day 3 6/6/2020	Weight Change	
NaCl & CSO	00730	Female	2.51	2.52	0.01	None
	00732	Female	2.21	2.30	0.09	None
	00734	Female	2.41	2.49	0.08	None

* Summary of Clinical Observations at 24, 48, and 72 hours excluding skin reactions.

TABLE 2:
Intracutaneous Test Skin Reaction Scores

NaCl Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
00730	NaCl	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
00732	NaCl	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
00734	NaCl	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

† = Immediately after injection, not used for the evaluation criteria.

Animal #	Vehicle	Total Scores (ER + ED)		*Individual Score	
		Test	Control	Test	Control
00730	NaCl	0	0	0.0	0.0
00732	NaCl	0	0	0.0	0.0
00734	NaCl	0	0	0.0	0.0
			**Overall Mean Score	0.0	0.0

*Individual Score = Total (ER + ED) divided by 15 (3 grading periods × 5 test or control sites)

** Overall Mean Score = Total Individual Scores divided by 3 animals
Overall Mean Score for Test Article = 0.0
Overall Mean Score for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0 – 0.0 = 0.0

ER = Erythema T = Test Site
ED = Edema C = Control Site

TABLE 2:
Intracutaneous Test Skin Reaction Scores (Cont.)

CSO Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–6	T–7	T–8	T–9	T–10	C–6	C–7	C–8	C–9	C–10
00730	CSO	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
00732	CSO	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
00734	CSO	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

† = Immediately after injection, not used for the evaluation criteria.

Animal #	Vehicle	Total Scores (ER + ED)		*Individual Score	
		Test	Control	Test	Control
00730	CSO	0	0	0.0	0.0
00732	CSO	0	0	0.0	0.0
00734	CSO	0	0	0.0	0.0
			**Overall Mean Score	0.0	0.0

*Individual Score = Total (ER + ED) divided by 15 (3 grading periods × 5 test or control sites)

** Overall Mean Score = Total Individual Scores divided by 3 animals
Overall Mean Score for Test Article = 0.0
Overall Mean Score for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0 – 0.0 = 0.0

ER = Erythema T = Test Site
ED = Edema C = Control Site

REPORT AMENDMENT PAGE

SPONSOR: Nelson Laboratories, LLC
A Sotera Health Company
6280 South Redwood Road
Salt Lake City, UT 84123
USA

TESTING LABORATORY: Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Test Article Name: Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

CAS/Code #: Not Supplied by Sponsor (N/S)

Lot/Batch Number: 0506200101

AMENDMENT:

Per Sponsor request, Test Article Name and Lot/Batch Number have been changed from:

Name: Lot # 7

Lot/Batch Number: 7

To:

Name: Model: USA Made Surgical Mask
Part ID: AA-US-SURGICAL-01

Lot/Batch Number: 0506200101

This amendment does not affect the integrity of the study.

AUTHORIZED PERSONNEL:

Sarah Goulet

Sarah Goulet, M.S.
Study Director

11/8/21
Date

APPENDIX I:
Classification System for Scoring Skin Reactions

Erythema and Eschar Formation	Value
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

Total possible erythema score = 4

Edema Formation	Value
No edema	0
Very slight edema (barely perceptible)	1
Well–defined edema (edges are well–defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Total possible edema score = 4

Total possible score for irritation = 8

APPENDIX II:
Software Systems

Software	Use	21 CFR Part 11 Status	Publisher/ Vendor	Location
Adobe Acrobat 8, 9, and 10 Professional	Document preparation	Not Applicable	Adobe Systems, Inc.	San José, CA
Matrix Gemini 5.3.19	Laboratory Information Management System	Compliant	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite and higher	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Not Applicable	Microsoft Corporation	Redmond, WA
Rees Scientific Centron Presidio 3.0	Automated Environmental Monitoring	Compliant	Rees Scientific	Trenton, NJ
TMS Web 7	Document management for SOPs and training records management software system	Compliant	Quality Systems Integrators	Eagle, PA
Toxikon Protocol Manager 1.0	Protocol requisition application	Not Applicable	Toxikon Corporation	Bedford, MA