GLP REPORT

TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619
419.666.9455

SPONSOR

William Kling
Benova RX LLC
11556 Farm to Market Road 428
Aubrey, Texas 76227
United States

CONFIDENTIAL

STUDY TITLE

ISO Guinea Pig Maximization Sensitization Test

TEST ARTICLE NAME

Benova RX Mouth Rinse System Step 1

TEST ARTICLE IDENTIFICATION

Benova RX Mouth Rinse System Step 1: LAB-082021-0

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Summary

The test article, Benova RX Mouth Rinse System Step 1, was evaluated for the potential to cause delayed dermal contact sensitization in a guinea pig maximization test. This study was conducted based on the requirements of ISO 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization. Dose determination was performed to determine a suitable test article concentration for testing. The test solution was intradermally injected and occlusively patched to ten test guinea pigs. The control article was similarly injected and occlusively patched to five control guinea pigs. Following a recovery period, the test and control animals received challenge patches of the test solution and the vehicle control article. All sites were scored for dermal reactions at 24 and 48 hours after patch removal.

The test article solution showed no evidence of causing delayed dermal contact sensitization in the guinea pig. The test article was not considered a sensitizer in the guinea pig maximization test.

Supervisory Personnel: Michelle E. Zdawczyk, MS, ALAT
Senior Manager, In Life Services

Statement of
GLP Compliance: There were no deviations to the provisions of the FDA Good Laboratory Practice (GLP) Regulations (21 CFR, Part 58) noted during the course of the study.

Study Director Approval:

DocuSigned by:
Abby Stone
Signer Name: Abby Stone
Signing Reason: I approve this document
Signing Time: 08-Dec-2021 | 16:50 EST
363AE96CBA6B4131B7BCD8499C77B6CE

Abby L. Stone, BS
Study Director

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the potential of the test article to cause delayed dermal contact sensitization in the guinea pig maximization test. The Magnusson and Kligman method has been effective in identifying a variety of allergens.

1.2 Testing Guidelines

This study was conducted based on the requirements of the International Organization for Standardization 10993-10, Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.

This test was performed in compliance with the ISO 13485 standard, with the test method accredited to the ISO 17025 standard.

1.3 Dates

Test Article Received:	August 31, 2021
Treatment Started:	November 1, 2021 (Dose Determination) November 5, 2021 (Definitive)
Observations Concluded:	November 4, 2021 (Dose Determination) December 1, 2021 (Definitive)

1.4 GLP Compliance

The study initiated by protocol signature on October 4, 2021 was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with this report.

1.5 Duplication of Experimental Work

By signature on the protocol for the study, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

2. Identification of Test and Control Articles

The test article provided by the sponsor was identified and handled as described below:

Table 1: Test Article

Name:	Benova RX Mouth Rinse System Step 1
Identification:	Benova RX Mouth Rinse System Step 1: LAB-082021-0
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Strength, Purity and Composition:	Strength: not applicable because no active ingredients are used to formulate a concentration; Purity: not applicable because the test article does not contain an active ingredient; Composition: Purified Water, Sodium Chlorite, Sodium Bicarbonate, Citric Acid, Disodium Phosphate
Physical Description of the Test Article:	Anti-microbial mouth rinse which kills 99% of bacteria, yeast, and mold in 45 seconds.
Storage Conditions:	Ambient Temperature

Table 2: Control Article

Name:	0.9% sodium chloride (SC)
Stability Testing:	Marketed product, stability characterized by labeling
Strength, Purity, Composition or Other Characteristics:	Purity: Meets requirements of USP Sodium Chloride for Injection and is certified as USP Grade; Composition: 0.9% NaCl ± 5.0% of label claim, balance is water; sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5

Table 3: Ancillary Material

Name:	Freund's Complete Adjuvant (FCA) was mixed 50:50 (v/v) with the chosen vehicle and used at Induction I. A 10% (w/w) sodium lauryl sulfate (SLS) suspension in petrolatum was used prior to Induction II. These materials were provided by the test facility.

3. Test System

3.1 Test System

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Crl:(HA) BR
Source:	Charles River Laboratories
Sex:	Male
Body Weight Range:	343 grams to 391 grams (Dose Determination); 373 grams to 452 grams at study initiation (Definitive)
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Twenty
Identification Method:	Ear tag

3.2 Justification of Test System

The Hartley albino guinea pig (animal) has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at NAMSA with a similar method under lab number 21T_49542_02 completed on September 1, 2021 (see Appendix 3).

4. Animal Management

4.1 Husbandry, Housing and Environment

Conditions conformed to NAMSA Standard Operating Procedures that are based on the "*Guide for the Care and Use of Laboratory Animals*." Animals were housed in groups in plastic suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, and first treatment date.

The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 68-79°F and the relative humidity was set to 30-70%. There were no significant environmental excursions that adversely affected the health of the animals.

The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).

4.2 Food, Water and Contaminants

A commercially available guinea pig feed, PROLAB Guinea Pig - 5P18, was provided daily. Potable water was provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.

No contaminants present in the feed and water impacted the results of this study.

4.3 Accreditation

NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.

4.4 Personnel

Associates involved in this study were appropriately qualified and trained.

4.5 Veterinary Care

Standard veterinary medical care was provided in this study.

4.6 IACUC

The procedures for this study were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC) prior to conduct.

4.7 Selection

Only healthy, previously unused animals were selected.

5. Dose Determination

A dose determination was conducted on stock guinea pigs prior to dosing to find the most suitable concentration of the test solution for injection and topical application.

The test article at full strength and v/v concentrations (dilutions) of 50%, 25%, and 12.5% (in SC solution) was intradermally injected in two guinea pigs and occlusively patched to another three animals. The injected animals were observed for signs of irritation at 24, 48 and 72 hours following injection. The patched animals were unwrapped at 24 hours and the treated sites were observed after patch removal as well as 24 and 48 hours following patch removal. The final preparation was described in this report under Method.

The results of the dose determination are presented in the table below.

Table 4: 24 Hour Dermal Observations

Animal Number/ Treatment Group	Body Weight (g)	Dermal Reactions			
		Test Article Concentration – 100%	Test Article Concentration – 50%	Test Article Concentration – 25%	Test Article Concentration – 12.5%
4456/Injected	343	1	1	0	0
4457/Injected	387	1	0	0	0
4458/Patched	391	0	0	0	0
4459/Patched	388	0	0	0	0
4460/Patched	353	0	0	0	0

Table 5: 48 Hour Dermal Observations

Animal Number/ Treatment Group	Dermal Reactions			
	Test Article Concentration - 100%	Test Article Concentration - 50%	Test Article Concentration - 25%	Test Article Concentration – 12.5%
4456/Injected	0	0	0	0
4457/Injected	1	0	0	0
4458/Patched	0	0	0	0
4459/Patched	0	0	0	0
4460/Patched	0	0	0	0

Table 6: 72 Hour Dermal Observations

Animal Number/ Treatment Group	Dermal Reactions			
	Test Article Concentration - 100%	Test Article Concentration – 50%	Test Article Concentration - 25%	Test Article Concentration – 12.5%
4456/Injected	0	0	0	0
4457/Injected	0	0	0	0
4458/Patched	0	0	0	0
4459/Patched	0	0	0	0
4460/Patched	0	0	0	0

The highest concentration that did not produce apparent systemic toxicity, local necrosis, ulceration, or excessive dermal irritation for each route of exposure was chosen: 100% injection and 100% patch. All animals were clinically normal throughout the dose determination.

6. Method

6.1 Test Article Preparation

The test article was dosed as received. Conditions of test article: Clear, colorless with no particulates. Control Conditions: Clear, colorless and no particulates.

Figure 1: Representative Photograph of the Test Article

Test Article

6.2 Test Procedure

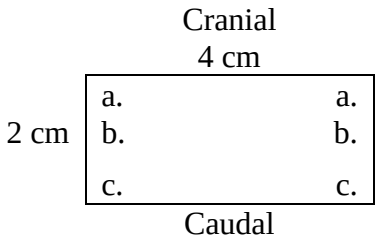
6.2.1 Induction I

On the first day of treatment, the animals were weighed and arbitrarily assigned to a treatment group as shown below.

Table 7: Treatment Group Assignment

Treatment Group	Number of Animals
Test	10
Control	5

The fur over the dorsoscapular region was removed with an electric clipper. The test animals were injected with the test solution and the control animals were injected with the control article. Three rows of intradermal injections (two injections per row) were given to each animal within an approximate 2 cm x 4 cm boundary of the fur clipped area as illustrated below:



Control Animals:

a. 0.1 mL of 50:50 (v/v) mixture of FCA and the control article
b. 0.1 mL of the control article
c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the vehicle

Test Animals:

a. 0.1 mL of 50:50 (v/v) mixture of FCA and the control article
b. 0.1 mL of test article
c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the test article

6.2.2 Induction II

At 6 days after completion of the Induction I injection, the fur over the dorsoscapular region (same area as used during Induction I) of each animal was removed with an electric clipper. The area was treated with the SLS suspension, sufficient to coat the skin. The SLS suspension, applied to provoke a mild acute inflammation, was massaged into the skin over the injection site. The area was left uncovered.

At 24 hours any remaining SLS residue was wiped from the area with dry gauze. Following removal of the SLS suspension, an approximate 2 cm x 4 cm section of filter paper, saturated with 0.3 mL of test solution, was then topically applied to the previously injected sites of the test animals. The control animals were similarly patched with the control article. Each patch was secured with a nonreactive tape, and the trunk of each animal was wrapped with an elastic bandage. At 48 hours the bandages and patches were removed.

6.2.3 Challenge

At 15 days after unwrapping the Induction II wraps, the fur was removed from the sides and flanks with an electric clipper. Nonwoven cotton disks contained in a Hill Top Chamber® were saturated with 0.3 mL of the test solution or control article. The test solution was applied to the right flank of each animal and the control vehicle was applied to the left flank of each animal.

The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites. At 24 hours the wraps and Hill Top Chambers were removed. Any residue remaining at the sites was wiped with dry gauze.

6.2.4 Laboratory Observations

1. Animals were observed daily for general health.
2. Body weights were recorded at pretreatment.
3. Observations for dermal reactions were conducted at 24 and 48 hours after challenge patch removal. If necessary, the sites were wiped with 35% isopropyl alcohol and/or the fur was clipped to facilitate scoring. Dermal reactions were scored in accordance with the criteria shown below:

Table8: Test Scoring

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or NAMSA standard operating procedures.

7. Evaluation

The responses from the challenge phase were compared within the test animal group and between test and control conditions. In the final analysis of data, consideration was given to the overall pattern, intensity, duration and character of reactions of the test as compared to the control conditions. The control conditions are (1) the control vehicle on the test animals, (2) the test on the control animals, and (3) the control vehicle on the control animals. Statistical manipulation of data was not applicable to this study. Grades of 1 or greater in the test group generally indicated sensitization, provided that grades of less than 1 were observed on the control animals. If grades of 1 or greater were noted on control animals, then the reactions of test animals that exceeded the most severe control reaction were considered to be due to sensitization.

8. Results

8.1 Clinical Observations Treatment and Body Weight Data

The following incidental findings were observed: had alopecia on face between eyes, eschar at injection site lesion with erythema and/or edema, healing erosion and/or eschar.

Animal 5144 (Test) alopecia on face between eye and observed with expected dermal reactions associated with intradermal injection of FCA.

Significant reactions to the Induction I injection sites were noted for animal 5146 (Test). Animal 5146 was treated with an analgesic, Buprenorphine SR LAB, at a dose of 0.3 mg/kg

subcutaneously for one treatment on day 14. Healing erosion and eschar was also noted after treatment.

The ability to evaluate the sensitization potential of the test article was not compromised. The observations documented were not associated with the placement of the test or control patches.

Unless otherwise indicated, all animals were observed with the expected dermal reactions associated with intradermal injection of FCA and were clinically normal throughout the study. The clinical observations and individual body weights at pretreatment are presented in Appendix 1.

8.2 Dermal Observations

No evidence of sensitization was observed. Individual results of dermal scoring for the challenge phase are presented in Appendix 2.

9. Conclusion

The test article solution showed no evidence of causing delayed dermal contact sensitization in the guinea pig. The test article was not considered a sensitizer in the guinea pig maximization test.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

10. Quality Assurance

Inspections were conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report was reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities was issued with the report.

11. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files in accordance with NAMSA SOPs.

12. References

Code of Federal Regulations (CFR), Title 9, Parts 1-4, Animal Welfare Act.

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

Frankild S, Basketter DA, Andersen KE. The value and limitations of rechallenge in the guinea pig maximization test. *Contact Dermatitis*. 1996;35:135-140.

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2018).

International Organization for Standardization (ISO) 10993-2, Biological evaluation of medical devices - Part 2: Animal welfare requirements (2006).

International Organization for Standardization (ISO) 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization (2010).

International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2021).

International Organization for Standardization (ISO) 13485, Medical devices - Quality management systems - Requirements for regulatory purposes (2016).

International Organization for Standardization (ISO) 17025, General requirements for the competence of testing and calibration laboratories (2017).

Klecak G. Identification of Contact Allergens: Predictive Tests in Animals. In: F.N. Marzulli and H.I. Maibach, eds. *Dermatotoxicology, Second Edition*. USA: Hemisphere Publishing Corporation; 1983:193-236.

Kligman AM, Basketter DA. A critical commentary and updating of the guinea pig maximization test. *Contact Dermatitis*. 1995;32:129-134.

Magnusson B, Kligman A. *Allergic Contact Dermatitis in the Guinea Pig*. Springfield: C.H. Thomas, 1970.

National Research Council, *Guide for the Care and Use of Laboratory Animals*, Washington, DC: National Academy Press, 2011.

Office of Laboratory Animal Welfare (OLAW), Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Organisation for Economic Co-operation and Development (OECD), Guideline for Testing of Chemicals, Test No. 406, Skin Sensitisation (1992).

Appendix 1 - Clinical Observations and Individual Body Weight Data

Treatment Group	Animal Number	Individual Observation	
		Pretreatment Body Weight (g)	Clinical Observations
Test	5141	400	Normal
	5142	373	Normal
	5143	418	Normal
	5144	393	Days 1 through 25 – Normal Days 26 and 27 – Alopecia on face between eyes
	5145	388	Normal
	5146	450	Days 1 through 13 – Normal Day 14 – Eschar at injection site lesion with erythema and/or edema Day 15 through 17 – Healing erosion and/or eschar Days 18 through 27 – Normal
	5147	380	Normal
	5148	388	Normal
	5149	418	Normal
	5150	420	Normal
Control	5151	417	Normal
	5152	410	Normal
	5153	415	Normal
	5154	399	Normal
	5155	452	Normal

Appendix 2 - Dermal Reactions Following Challenge Exposure

Treatment Group	Animal Number	Dermal Reactions			
		24 Hour Score		48 Hour Score	
		Control Site	Test Solution Site	Control Site	Test Solution Site
Test	5141	0	0	0	0
	5142	0	0	0	0
	5143	0	0	0	0
	5144	0	0	0	0
	5145	0	0	0	0
	5146	0	0	0	0
	5147	0	0	0	0
	5148	0	0	0	0
	5149	0	0	0	0
	5150	0	0	0	0
Control	5151	0	0	0	0
	5152	0	0	0	0
	5153	0	0	0	0
	5154	0	0	0	0
	5155	0	0	0	0

Appendix 3 - Periodic Positive Control Study for the Guinea Pig Maximization Test

What was tested

1-chloro-2,4-dinitrobenzene (DNCB)

Dates

Treatment Started: July 19, 2021 under NAMSA Lab Number: 21T_49542_02
Observations Concluded: August 13, 2021

Purpose

A periodic positive control study was conducted for the Guinea Pig Maximization Test to meet the following objectives: 1) confirm the methodology in ISO 10993-10, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Skin Sensitization, 2) substantiate the potential of DNCB to cause delayed dermal contact sensitization, 3) verify proper training of the technicians performing these studies, and 4) substantiate the susceptibility of the Hartley guinea pig strain to dermal contact sensitization.

Methods

The test utilized young adult, male Hartley albino guinea pigs supplied by Charles River Laboratories. The weight at study initiation ranged from 357 grams to 404 grams. A 0.1% (w/w) concentration of DNCB in propylene glycol was intradermally injected and occlusively patched to ten test guinea pigs in an attempt to induce sensitization. The propylene glycol vehicle was similarly injected and occlusively patched to five control guinea pigs. Following a recovery period, the test and control animals received a challenge patch of 0.01% (w/w) DNCB in propylene glycol and propylene glycol alone. All sites were scored for dermal reactions at 24 and 48 hours after patch removal. The patch sites were graded using the scale:

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Results

Eight of the ten test animals demonstrated a positive sensitization response to the known sensitizer, DNCB. None of the control animals demonstrated a sensitization response. The results are shown below:

Treatment Group	Animal Number	Dermal Reactions				Results (+) or (-)
		24 Hour Score		48 Hour Score		
		Control Site	Test Article Site	Control Site	Test Article Site	
Test	941	0	0	0	0	-
	942	0	2	0	2	+
	943	0	0	0	0	-
	944	0	2	0	2	+
	945	0	2	0	2	+
	946	0	2	0	2	+
	947	0	2	0	2	+
	948	0	2	0	2	+
	949	0	2	0	2	+
	950	0	2	0	2	+
Control	951	0	0	0	0	-
	952	0	0	0	0	-
	953	0	0	0	0	-
	954	0	0	0	0	-
	955	0	0	0	0	-

Conclusion

The known sensitizer DNCB produced evidence of causing delayed dermal contact sensitization in the Hartley strain of guinea pig. Therefore, the following objectives were met: 1) the methodology in ISO 10993-10, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization was confirmed, 2) the potential for DNCB to cause delayed contact sensitization was substantiated, 3) proper training of the technicians performing this study design was verified and 4) the susceptibility of the Hartley guinea pig strain to sensitization was substantiated.

Phase Inspected	Date Inspected	Study Director Notification Date	Management Notification Date
Scoring	November 30, 2021	November 30, 2021	November 30, 2021
Final Report Review	December 8, 2021	December 8, 2021	December 8, 2021

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative:

DocuSigned by:
Heatherbea L. Weirich
Signer Name: Heatherbea L. Weirich
Signing Reason: I have reviewed this document
Signing Time: 08-Dec-2021 | 16:47 EST
45E1CC5E297046A78766054834D95A49

Heatherbea L. Weirich, BS
Auditor, Quality Assurance

Abby Stone

21T_62382 GLP
52274_001 52274

From: OH Order Processing
Sent: Thursday, August 26, 2021 3:50 PM
To: Abby Stone
Cc: Amber McGraw; Madalyn Souders
Subject: FW: Electronic Sample Submission 269629-1

21T_62382

From: NAMSA Connect
Sent: Wednesday, August 25, 2021 6:05 PM
To: bkling@benovarx.com
Subject: Electronic Sample Submission 269629-1

Overview

Submission ID: 269629
Revision #: 1
Date Created: 25 Aug 2021
Testing Location: Northwood
Control Article: No
Proposal Number: 2_Q-46123
Quantity Submitted: 1

Billing and Shipping

Bill To: Benova RX LLC (52274)
11556 Farm to Market Road 428
Aubrey, Texas 76227
United States

Ship To: Benova RX LLC (52274)
11556 Farm to Market Road 428
Aubrey, Texas 76227
United States

Contact: William Kling (52274_001)
Benova RX LLC (52274)
bkling@benovarx.com
940-226-6000

Test Article Information

Name: Benova RX Mouth Rinse System Step 1
Test Article ID: Benova RX Mouth Rinse System Step 1: LAB-082021-0
Physical Description: Anti-microbial mouth rinse which kills 99% of bacteria, yeast, and mold in 45 seconds.
Type: Medical Device
Clinical Use: Step 1 of two-step system to be used by patients with oral cavity sores and pain.
Sterility: Not Sterile
Can Be Cut: Yes
Reported Client Specific Number:

Special Instructions: Shake bottle well before use.

Contains Elastomer: No
Storage Conditions: Ambient (15-30 °C)
Shipping Conditions: Ambient
Disposition: Discard used and unused test article

GLP Information

Stability: Stability testing is in progress and sponsor affirms that test article is stable for duration of intended testing.

Analysis: Analysis is not necessary due to test article being a solid, powder, gel, or liquid being extracted or tested as received (mixture with a carrier not needed).

Has Active Ingredient: No

21T 62382

Composition: Purified Water, Sodium Chlorite, Sodium Bicarbonate, Citric Acid, Disodium Phosphate

Testing Services

Test Code	Qty	Proposal	Grouping	STAT	Regulatory Scope	Draft Report	Comments	Purchase Order	Test Spec	Extracts
V0705-001	1	2_Q-46123		No	GLP	No		Benova RX Step 1 8-25-21		
TI261-306/s	1	2_Q-46123		No	GLP	No	Includes the cost of a dose range finding study	Benova RX Step 1 8-25-21		
TA103-507/s	1	2_Q-46123		No	GLP	No	-6 hamsters (3/group) -Test articles soaked onto cotton pellets -Article extract in left pouch, right remains untreated -Article application for at least 5 min, repeated every 4 hours for 24 hours -Sites scored following removal of pellets, immediately pr	Benova RX Step 1 8-25-21		
TA004-909	1	2_Q-46123		No	GLP	No	Dosage was changed to 10 mg/kg per discussion with Abby Stone and her supervisor.	Benova RX Step 1 8-25-21		

Authorization

Electronically Signed By: bkling@benovarx.com **Date:** 25 Aug 2021

Reviewed By (NAMSA Associate Signature):_____ Date:_____

GLP PROTOCOL

TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619

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William Kling
Benova RX LLC
11556 Farm to Market Road 428
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United States

STUDY TITLE

ISO Guinea Pig Maximization Sensitization Test

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Approvals

Sponsor Representative: Bill King

Date Approved: 9/20/21

Study Director (NAMSA): Abby Stone

Abby L. Stone, BS
Study Director

Date Initiated: 10-4-21

1. Introduction

1.1 Purpose

The purpose of this study is to evaluate the potential of the test article to cause delayed dermal contact sensitization in the guinea pig maximization test. The Magnusson and Kligman method has been effective in identifying a variety of allergens.

1.2 Testing Guidelines

This study will be conducted based on the International Organization for Standardization 10993-10, Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.

1.3 GLP Compliance

Good Laboratory Practice - This nonclinical laboratory study will be conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

1.4 Duplication of Experimental Work

By signature on this protocol, the sponsor confirms that the conduct of this study does not unnecessarily duplicate previous experiments.

2. Identification of Test and Control Articles

2.1 Test Article

The sponsor will submit the test article, Benova RX Mouth Rinse Step 1, to be evaluated. The sponsor provided detailed information about the test article to NAMSA on the sample submission form.

2.2 Control Article

Any diluent or vehicle used with the test article in this study will serve as the control. If a diluent or a vehicle is not used, 0.9% sodium chloride USP solution will be used as the control article. Untreated skin will serve as an additional control reference for scoring dermal reactions during the challenge phase.

2.3 Ancillary Material

Freund's Complete Adjuvant (FCA) will be used at Induction I, and 10% (w/w) sodium lauryl sulfate (SLS) suspension in petrolatum will be used for Induction II. These materials will be provided by the test facility.

3. Test System

3.1 Test System

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Hartley
Source:	NAMSA approved supplier
Sex:	No particular sex is prescribed for this test; females will be nulliparous and nonpregnant
Body Weight Range:	300-500 grams at study initiation
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Minimum of fifteen
Identification Method:	Ear tag and/or marking

3.2 Justification of Test System

The Hartley albino guinea pig (animal) has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at NAMSA with this method.

4. Animal Management

4.1 Husbandry, Housing and Environment

Conditions will conform to NAMSA Standard Operating Procedures that are based on the "*Guide for the Care and Use of Laboratory Animals*." Animals will be housed in groups in plastic suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, and first treatment date.

The animal housing room temperature and relative humidity will be monitored daily. The recommended temperature range for the room is 68-79°F. The recommended humidity range for the room is 30-70%.

The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).

4.2 Food, Water and Contaminants

A commercially available guinea pig feed will be provided daily. Potable water will be provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.

No contaminants present in the feed and water are expected to impact the results of this study.

4.3 Accreditation

NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.

4.4 Personnel

Associates involved in this study will be appropriately qualified and trained.

4.5 Sedation, Analgesia or Anesthesia

It has been determined that the use of analgesics may be necessary during the routine course of this procedure. Intradermal injections with FCA may cause minimal lesions which may subsequently become covered by eschar. Such reactions are anticipated and self-limiting. However, more extreme reactions or significant self-induced trauma to the skin at the Induction I injection sites will not be considered an appropriate reaction. These animals will be treated with an analgesic, buprenorphine, as outlined in NAMSA TM_00135.

4.6 Veterinary Care

All anesthetics, analgesics, and other medications may be given or altered at the discretion of the attending veterinarian in accordance with standard veterinary practice and the study objectives. This applies to specific medication, dose, and dosing intervals. In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Guidelines on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.

4.7 IACUC

This test method has been approved by the NAMSA Institutional Animal Care and Use Committee (IACUC), and is reviewed at least annually by the same committee. Any changes to the test method that are incorporated into this protocol that pertain to the care and use of animals must be approved by the IACUC prior to conduct.

4.8 Selection

Only healthy, previously unused animals will be selected.

5. Method

5.1 Test Article Preparation

The following information was completed based on the sponsor providing the information to NAMSA. Further instructions may be attached to the protocol.

The sample will be prepared as follows:

Test Article Form: Liquid

5.2 Test Procedure

A trial dose determination on at least five stock animals will be conducted before the first Induction treatment to determine the concentration of the test article that will not produce apparent systemic toxicity, local necrosis, ulceration, or excessive dermal irritation. As intended for the actual study, the material will be injected and patched topically at various concentrations to the fur clipped flank or dorsum.

On the first day of treatment, fifteen guinea pigs (ten test, five control) will be weighed. The fur from the dorsoscapular area of the animals will be removed with an electric clipper.

5.2.1 Induction I

Three pair of intradermal injections will be administered to the animals within an approximate 2 cm x 4 cm area over the dorsoscapular region as follows:

Control Animals

a. 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 mL of vehicle
c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the vehicle

Test Animals

a. 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 mL of test article
c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the test article

5.2.2 Induction II

At 6 days (±1 day) after completion of the Induction I injection, the fur over the dorsoscapular region (same area as used during Induction I) of each animal will be removed with an electric clipper. The area will be treated with the SLS suspension, sufficient to coat the skin unless the animals exhibit excessive redness and/or swelling at site b. The area will be left uncovered.

At 24 hours any remaining SLS residue will be wiped from the area with dry gauze. Following removal of the SLS, an approximate 2 cm x 4 cm section of filter paper, saturated with approximately 0.3 mL of the article preparation (test animals) or vehicle (control animals) will be applied over the same injection area. Each patch will be secured with nonreactive tape, and the trunk of each animal will be wrapped with an elastic bandage. At 48 hours the bandages and patches will be removed.

5.2.3 Challenge

At 14 days (±1 day) after unwrapping Induction II wraps, the fur will be removed from the sides and flanks with an electric clipper. A nonwoven cotton disk backed by a flexible chamber (e.g. Hill Top Chamber®) and semiocclusive hypoallergenic tape will be saturated with approximately 0.3 mL of freshly prepared test article and applied to the right flank or dorsum of each animal. In addition, the control article will be similarly patched to the left flank or dorsum of each animal.

The trunk of each animal will be wrapped to maintain well-occluded sites. At 24 hours the wraps and Hill Top Chambers® will be removed. Any residue remaining at the sites will be wiped with dry gauze.

5.2.4 Laboratory Observations

1. Animals will be observed daily for health.
2. Body weights will be recorded at pretreatment.
3. Observations for dermal reactions will be conducted at 24 and 48 hours after patch removal. If necessary, the sites will be wiped with 35% isopropyl alcohol and/or the fur will be clipped to facilitate scoring. Dermal reactions will be scored in accordance with the criteria shown below:

Table 1: Grading Scale

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

5.2.5 Rechallenge

Should the original challenge results be equivocal, the animals may be rechallenged with a fresh test article and vehicle control approximately 1-2 weeks after the first challenge patch application. All animals are to be rechallenged. The rechallenge will be conducted in the same manner as the challenge but at virgin sites on the opposite flank. After the test is completed, all animals will be handled in accordance with IACUC approved NAMSA procedures.

6. Evaluation

The responses from the challenge phase will be compared within the test animal group and between test and control conditions. In the final analysis of data, consideration will be given to the overall pattern, intensity, duration, and character of reactions of the test as compared to the control conditions. The control conditions are (1) the control article on the test animals, (2) the test on the control animals, and (3) the control article on the control animals. Statistical manipulation of data is not applicable to this study. Grades of 1 or greater observed in the test group generally indicate sensitization, provided that grades of less than 1 are observed on the control animals. If grades of 1 or greater are noted on control animals, then the reactions of test animals that exceeded the most severe control reaction will be considered to be due to sensitization.

For rechallenge results, the overall pattern, intensity, duration and character of reactions seen will be compared between the challenge and rechallenge. Recurring observations in at least one of the same animals will be considered as verification of earlier findings.

7. Protocol Changes

Any necessary changes to the protocol after sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

8. Report

A final report will be issued to include a description of the methods, the resulting data in tabular format and conclusions.

9. Quality Assurance

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities will be provided with the final report.

10. Records

All raw data pertaining to this study and a copy of the final report will be retained in designated NAMSA archive files in accordance with NAMSA SOPs.

11. References

Code of Federal Regulations (CFR), Title 9, Parts 1-4, Animal Welfare Act.

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

Frankild S, Basketter DA, Andersen KE. The value and limitations of rechallenge in the guinea pig maximization test. *Contact Dermatitis*. 1996;35:135-140.

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2018).

International Organization for Standardization (ISO) 10993-2, Biological evaluation of medical devices - Part 2: Animal welfare requirements (2006).

International Organization for Standardization (ISO) 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization (2010).

International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2021).

Klecak G. Identification of Contact Allergens: Predictive Tests in Animals. In: F.N. Marzulli and H.I. Maibach, eds. *Dermatotoxicology, Second Edition*. USA: Hemisphere Publishing Corporation; 1983:193-236.

Kligman AM, Basketter DA. A critical commentary and updating of the guinea pig maximization test. *Contact Dermatitis*. 1995;32:129-134.

Magnusson B, Kligman A. *Allergic Contact Dermatitis in the Guinea Pig*. Springfield: C.H. Thomas, 1970.

National Research Council, *Guide for the Care and Use of Laboratory Animals*, Washington, DC: National Academy Press, 2011.

Office of Laboratory Animal Welfare (OLAW), Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Organisation for Economic Co-operation and Development (OECD), Guideline for Testing of Chemicals, Test No. 406, Skin Sensitisation (1992).

GLP PROTOCOL AMENDMENT 1

SPONSOR:

William Kling
Benova RX LLC
11556 Farm to Market Road 428
Aubrey, Texas 76227
United States

TEST FACILITY:

NAMSA
6750 Wales Road
Northwood, OH 43619

STUDY TITLE:
ISO Guinea Pig Maximization Sensitization Test

TEST ARTICLE:
Benova RX Mouth Rinse System Step 1

This amendment is being issued to make the following change(s) to the indicated section(s) of the protocol:

1) **Part to be Changed/Amended:** Test Article

   Change/Addition: Benova RX Mouth Rinse **System** Step 1

   Reason for Change/Addition: This amendment is being written to correct the test article name. There is no impact to the study.

Study Director Approval: Abby Stone Date: 12.8.21
Abby L. Stone, BS
Study Director
cc: Sponsor, QA, IACUC

DocuSign

Certificate Of Completion

Envelope Id: E1B961844C2D4B37841168BA4F84A97E
Status: Completed
Subject: Please DocuSign: SKM_C65821120814260.pdf, 62382-03.doc
Source Envelope:
Document Pages: 28
Signatures: 2
Envelope Originator:
Certificate Pages: 2
Initials: 0
Abby Stone
AutoNav: Enabled
astone@namsa.com
EnvelopeId Stamping: Disabled
IP Address: 12.208.56.162
Time Zone: (UTC-05:00) Eastern Time (US & Canada)

Record Tracking

Status: Original
12/8/2021 2:04:59 PM
Holder: Abby Stone
astone@namsa.com
Location: DocuSign

Status: Original
12/8/2021 4:50:13 PM
Holder: NAMSA Archivist DocuSign
docusignarchive@namsa.com
Location: DocuSign

Signer Events	Signature	Timestamp
Heatherbea L. Weirich hweirich@namsa.com Senior Auditor, Quality Assurance North American Science Associates, Inc. Security Level: Email, Account Authentication (Required)	 Signature Adoption: Pre-selected Style Signature ID: 45E1CC5E-2970-46A7-8766-054834D95A49 Using IP Address: 72.241.200.62 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I have reviewed this document	Sent: 12/8/2021 2:35:18 PM Resent: 12/8/2021 4:28:27 PM Viewed: 12/8/2021 4:42:04 PM Signed: 12/8/2021 4:47:46 PM
Electronic Record and Signature Disclosure: Not Offered via DocuSign		
Abby Stone astone@namsa.com Associate Study Dir North American Science Associates, Inc. Security Level: Email, Account Authentication (Required)	 Signature Adoption: Pre-selected Style Signature ID: 363AE96C-BA6B-4131-B7BC-D8499C77B6CE Using IP Address: 12.208.56.162 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 12/8/2021 4:47:48 PM Viewed: 12/8/2021 4:49:50 PM Signed: 12/8/2021 4:50:09 PM
Electronic Record and Signature Disclosure: Not Offered via DocuSign		

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	12/8/2021 2:35:18 PM
Certified Delivered	Security Checked	12/8/2021 4:49:50 PM
Signing Complete	Security Checked	12/8/2021 4:50:09 PM
Completed	Security Checked	12/8/2021 4:50:09 PM

Payment Events	Status	Timestamps