NELSON
LABORATORIES

07 May 2009

Jodie Alexander
Oerlikon Balzers Coating USA, Inc.
1181 Jansen Farm Court
Elgin, IL 60123

Dear Jodie,

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

NELSON NUMBER: 465458

TESTING LAB: WuXi AppTec, Inc.

TYPE OF TEST: ISO Guinea Pig Maximization Sensitization Test
(Method for Biomaterial Extracts)

SAMPLE IDENTIFICATION:
#4 BaLinit – A – Medical TiN

If you have any questions, please feel free to call any of our Subcontracting personnel at 801-963-2600 or 800-826-2088. Thank you for testing with Nelson Laboratories, Inc.

Jennifer Shaw

Jennifer Shaw, B.S.
Subcontracting Coordinator

07 May 2009
Sign Date

FRM0641 Rev.1

Nelson Laboratories Incorporated
P. O. Box 17557, Salt Lake City, UT 84117-0557 • 6280 South Redwood Road, Salt Lake City, UT 84123-6600
Telephone: 801-963-2600 • Fax: 801-963-2630 • Operator: 800-826-2088 • Auto Attendant: 800-963-6280 or 801-963-5299
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St. Paul

Report

WuXi AppTec

FINAL STUDY REPORT

STUDY TITLE

ISO Guinea Pig Maximization Sensitization Test
(Method for Biomaterial Extracts)

TEST ARTICLE IDENTIFICATION

#4 BaLinit - A - Medical TIN

STUDY COMPLETION DATE

May 6, 2009

PERFORMING LABORATORY

WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

SPONSOR

Nelson Laboratories, Inc.
6280 South Redwood Road
Salt Lake City, UT 84123

PROTOCOL

900850S

PROJECT NUMBER

118179

NLI#

465458

Reference PO # WUX-2009

QUALITY ASSURANCE UNIT SUMMARY

STUDY: ISO Guinea Pig Maximization Sensitization Test (Method for Biomaterial Extracts)

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

Phase Inspected	Date	Study Director	Management
Challenge Phase	04/17/09	04/17/09	05/06/09
Final Report	05/05/09	05/05/09	05/06/09

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Angela Renner Date: 5/6/09
Angela Renner

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Nick Wolner Date: 5/6/09
Nick Wolner

Professional Personnel Involved:

Lisa Olson, BS	Vice President St. Paul Operations
Don Palme, Ph.D.	Vice President of Toxicology and In-Life Testing
Roxanne Miller, AA, CVT	Associate Director, In-Life Studies
Nick Wolner, BA	Study Director
Jean Mesarich, AA	Client Relations Manager

PROJECT NUMBER: 118179

SPONSOR: Nelson Laboratories, Inc.
6280 South Redwood Road
Salt Lake City, UT 84123

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

SAMPLE STORAGE: Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing. Unused test samples remained in the storage area until the study was completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

CHARACTERIZATION: The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

PURPOSE: This test was designed to evaluate the allergenic potential or sensitizing capacity of a test article. The test was used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE SAMPLE RECEIVED: 03/12/09
STUDY INITIATION DATE: 03/12/09
STUDY COMPLETION DATE: 05/06/09
IACUC APPROVAL NUMBER: 98-02D

METHOD: This study was based upon the procedures described in ISO 10993-10: 2002 Standard and Amendment 1, "Biological Evaluation of Medical Devices, Part 10-Tests for Irritation and Delayed-Type Hypersensitivity" pp. 15-18; Magnusson, B. and Kligman, A.M. 1969. "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test;" *J. Invest. Dermatol.* 52:268-276.; Magnusson, B. and Kligman, A.M. 1970. "Allergic Contact Dermatitis in the Guinea Pig," Identification of Contact Allergens; Springfield, Ill.: Thomas.

EXPERIMENTAL METHOD SUMMARY: In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic response. The response generally is due to leachable substances in the test material. Therefore, test material extracts are used for animal administrations. The extraction was performed according to the standard ISO 10993-12 Sample Preparation and Reference Materials in the Sponsor designated vehicle. The use of Freund's Complete Adjuvant (FCA) and sodium lauryl sulfate (SLS) enhances the potential of weak sensitizing agents.

Eleven test guinea pigs (per extract) were injected with the test article extract and FCA, and six guinea pigs (per extract) were injected with the corresponding control blank and FCA. On Day 6, the dorsal site was reshaved and sodium lauryl sulfate (SLS) in mineral oil was applied. The day after the SLS application, the test animals were topically patched with the appropriate test extract and the control animals were patched with the corresponding control blank. The patches were removed after 48 ± 2 hours of exposure. Following an approximate two week rest period, the animals were topically patched with the appropriate test extract and corresponding control blank. The patches were removed after 24 ± 2 hours of exposure. The dermal patch sites were observed for erythema and edema 24 ± 2 and 48 ± 2 after patch removal. Each animal was assessed for a sensitization response based upon the dermal scores. The test results were based upon the percentage of animals exhibiting a sensitization response.

DEVIATIONS/AMENDMENTS: None.

TEST MATERIAL PREPARATION

Test Article Identification :

Test Article Name:	#4 BaLinit - A - Medical TIN
NLI#:	465458
Sterilization Method:	Non-Sterile
Physical State:	Insoluble Material
Stability (Expiration):	Not Applicable
Storage Conditions:	Room Temperature
Safety Precautions:	Standard Precautions
Intended Use/Application:	Unknown

Test Article Preparation: The test article appeared to consist of gold-colored metal. The test article was extracted intact, placed into test tubes and prepared at a ratio of 60 cm^2 to 20 mL of extraction vehicle.

TABLE 1: TEST ARTICLE RECORD

TEST PHASE AND VEHICLE	TEST ARTICLE AREA (CM^2)	VEHICLE AMOUNT (mL)	NUMBER OF TEST ARTICLE DEVICES USED
First Induction Injection Normal Saline (NS)	228.0	76.0	1
First Induction Injection Cottonseed Oil (CSO)	228.0	76.0	1
Second Induction Patch NS	228.0	76.0	1
Second Induction Patch CSO	228.0	76.0	1
Challenge Patch NS	228.0	76.0	1
Challenge Patch CSO	228.0	76.0	1

Test Article Extraction: The extraction mixtures and corresponding control blanks were incubated for 24 ± 2 hours at 70 ± 2 °C. The extracts were agitated during the course of the extraction period. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. After all of the extractions, the gold colored metal component of the test article appeared to have rusted. The NS test extract was cloudy and red with a large amount of small red flakes. The NS test extract for the second induction phase was cloudy and brown with a large amount of small brown flakes. The NS test extract for the challenge phase was cloudy and brown with a large amount of small brown flakes. The particles in the test extracts were allowed to settle prior to use. The extracts were not filtered prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation. See Tables 1-3.

TABLE 2: EXTRACTION RECORD

TEST PHASE AND VEHICLE	CONDITION OF EXTRACT (PRE)	EXTRACTION TEMPERATURE (IN)	DATE/TIME OF EXTRACTION START	EXTRACTION TEMPERATURE (OUT)	DATE/TIME OF EXTRACTION END	CONDITION OF EXTRACT (POST)	DATE/TIME EXTRACT USED FOR TESTING
First Induction Injection NS	Clear	70 °C	03/24/09 0718	70 °C	03/25/09 0633	Cloudy; red in color; large amount of small red flakes	03/25/09 1234
First Induction Injection CSO	Clear	70 °C	03/24/09 0718	70 °C	03/25/09 0633	Clear	03/25/09 1234
Second Induction Patch NS	Clear	70 °C	03/31/09 0757	70 °C	04/01/09 0635	Cloudy; brown in color; large amount of small brown flakes	04/01/09 1120
Second Induction Patch CSO	Clear	70 °C	03/31/09 0757	70 °C	04/01/09 0635	Clear	04/01/09 1120
Challenge Patch NS	Clear	70 °C	04/16/09 0701	70 °C	04/17/09 0624	Cloudy; brown in color; large amount of small brown flakes	04/17/09 1022
Challenge Patch CSO	Clear	70 °C	04/16/09 0701	70 °C	04/17/09 0624	Clear	04/17/09 1022

TABLE 3: VEHICLE RECORD

VEHICLE IDENTIFICATION:	TEST PHASE	LOT #	SUPPLIED BY:	EXPIRATION
0.9% Normal Saline (NS)	First, Second & Challenge	J9B563	Braun	08/2011
Cottonseed Oil NF (CSO)	First, Second & Challenge	YA0319	Spectrum	01/31/10

TABLE 4: ADDITIONAL TEST REAGENT RECORD

ADDITIONAL TEST REAGENTS:	LOT #	SUPPLIED BY:	EXPIRATION
FCA	8353847	Difco	12/16/11
10% SLS	081572	Fisher	09/15/09
0.9% Sterile Saline	J8P502	Braun	05/11
Mineral Oil	G08001613	EM Science	08/25/09

TEST SYSTEM

Species/Strain/Sex: Albino guinea pig, Hartley strain, specific pathogen free, male.

Source: Charles River Laboratories, St. Constant, Quebec, Canada

Animal Body Weight Range: All animals weighed between 300 and 500 g upon assignment to the test.

Age: Not applicable.

Animal Identification: Individually numbered ear tags.

HUSBANDRY

Receipt: Animals were received on 03/18/09. Each animal was examined for signs of disease and injury.

Housing: Animals were housed in solid bottom cages with contact bedding. The test and negative control animals were housed separately. Housing density complied with AAALAC International recommendations.

Environment: Animal rooms were maintained according to AAALAC International recommendations and the "Guide for Care and Use of Laboratory Animals". The laboratory and animal rooms were maintained as limited - access facilities.

Diet: Animals were supplied with certified commercial feed ad libitum. No known contaminants present in the feed were expected to interfere with the test results.

Water: Potable water was obtained from the St. Paul municipal water supply. No known contaminants present in the water were expected to interfere with the test results.

Termination: Animals were euthanized by CO_2 asphyxiation following completion of this test.

Compliance: The care, housing and handling of the animals were in compliance with AAALAC International guidelines as reported in the "Guide for the Care and Use of Laboratory Animals", National Research Council - ILAR, Revised 1996; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, Animal Welfare, Final Rule 1989.

Selection of Animals: Animals were randomly placed in cages upon receipt and were assigned on test as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

Animal Preparation: The application sites were prepared by clipping the fur of the test site using an electric clipper with an appropriate blade. Prior to the induction phases, an approximate 5 x 7 cm area over the shoulder region was shaved. Prior to challenge, an approximate 4 x 4 cm area of the right and left flank was shaved.

TEST ARTICLE ADMINISTRATION

First Induction / Intradermal Injection: Three (3) syringes were prepared for the test animals and three (3) for the negative control animals as indicated in Table 5. The prepared syringes were injected in pairs on each side of the dorsal mid-line. The six (6) injection sites were within the boundaries of a 2 x 4 cm area. Injections that included FCA were made slightly deeper to minimize adverse tissue reactions.

TABLE 5: FIRST INDUCTION SYRINGE PREPARATION AND DOSE VOLUME

PREPARATION	VOLUME INJECTED PER SITE	SYRINGE CONTENTS	RATIO (V / V)
TEST GROUP			
Syringe 1	0.1 mL	FCA + Appropriate Vehicle*	1:1
Syringe 2	0.1 mL	Test Extract	NA
Syringe 3	0.1 mL	FCA + Appropriate Vehicle* (1:1) + Test Extract	1:1
CONTROL GROUP			
Syringe 1	0.1 mL	FCA + Appropriate Vehicle*	1:1
Syringe 2	0.1 mL	Control Vehicle	NA
Syringe 3	0.1 mL	FCA + Appropriate Vehicle* (1:1) + Control Vehicle	1:1

*Appropriate Vehicle = 0.9% Sterile Saline

Second Induction / Topical Application: On Day 6, the injection site area was clipped free of fur and treated with 0.5 mL to 1.0 mL of 10% (w/w) sodium lauryl sulfate (SLS) prepared by mixing solid SLS with mineral oil. The day following the SLS treatment, the remaining SLS residue was gently wiped from the area with gauze.

On Day 7, the test article extracts (0.3 mL) were applied to a 2 x 4 cm piece of filter paper (Whatman) to saturation and applied after SLS removal. The patch was secured to the site with non-permeable tape and the trunk wrapped with Vetrap™ and Transpore™ tape. The control animals received a similar patch with the control vehicles. Freshly prepared extracts were used for this administration. This preparation was removed after 48 ± 2 hours of application.

Challenge Patch / Topical Application: Fourteen days after completion of the topical induction phase, the challenge procedure was initiated on the twenty-two test animals and the twelve negative control animals. A filter paper patch was saturated with 0.3 mL of freshly prepared test article extract and applied to the fur clipped right flank of each test animal. A filter paper patch was saturated with 0.3 mL of freshly prepared control vehicle and applied to the fur clipped left flank of each test animal.

The negative control animals were challenged in an identical fashion with similarly prepared patches. The left side of each animal was patched with a filter paper patch saturated with 0.3 mL of control vehicle. The right side was patched with a filter paper patch saturated with 0.3 mL of the prepared test article extract applied to the fur clipped flank. The trunk of each animal was wrapped for 24 ± 2 hours with an expandable wrapping material and secured with tape.

OBSERVATIONS AND SCORING: The following day (24 ± 2 hours) after challenge exposure, the patches were removed and the site was wiped gently with a 70% isopropyl alcohol soaked gauze sponge prior to each scoring period. The challenge sites were observed for irritation and sensitization reaction, as indicated by erythema and edema. Daily challenge observation scores were recorded 24 ± 2 and 48 ± 2 hours after patch removal in accordance with the classification system for skin reactions in Table 6. Daily animal health observations were recorded throughout the study period.

TABLE 6: DERMAL OBSERVATION 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

Note: Erythema is defined as redness and edema is defined as a swelling at the challenge site. Any other adverse changes at the skin sites were recorded and reported.

EVALUATION CRITERIA

Main Test Interpretation: Individual animal scores of '1' or greater in the test group generally indicate sensitization, provided scores of less than '1' are observed on the control animals. If scores of '1' or greater are noted on control animals, then the reactions of the test animals which exceed the most severe control reaction are presumed to be due to sensitization.

Occasionally, the test group has a greater number of animals showing a response than the controls, although the intensity of the reaction is not greater than that observed on the controls. In these instances, a rechallenge may be necessary to define the response clearly. If necessary, a rechallenge shall be carried out 1-2 weeks after the first challenge. The method used shall be as described for the first challenge.

RESULTS

Clinical Observations: None of the animals in the study showed abnormal clinical signs during the test period.

Main Test Results: None of the negative control animals challenged with the control vehicles were observed with a sensitization response greater than '0'. Since animal 68813 from the NS negative control group was not observed with any redness during the 48 hour score, the redness observed for this animal during the 24 hour score was irritation. For this reason, the sensitization response result for animal 68813 was negative. See Tables 7 and 8 for individual animal scores.

None of the test animals challenged with the test article extracts were observed with a sensitization response greater than '0'. Since animal 68802 from the NS test group was not observed with any redness during the 48 hour score, the redness observed for this animal during the 24 hour score was irritation. For this reason, the sensitization response result for animal 68802 was negative. A negative sensitization incidence was interpreted for all test animals. See Tables 7 and 8 for individual animal scores.

Positive Control: WuXi AppTec completes positive controls every 3 months. A positive control was completed on 02/28/09 (see Table 9 for individual animal scores). The methods for the positive control assay are identical to the methods described above in the "Experimental Method Summary." Guinea pigs utilized for positive control studies are of the Hartley strain and are supplied by the same vendor as animals used for general testing (Charles River Laboratories). For the Induction I and Induction II phases, 0.3% dinitrochlorobenzene (DNCB), a known sensitizer, in ethanol is used. For the challenge phase, 0.15% DNCB in acetone is used. The negative control animals are exposed to the appropriate vehicle (acetone is used for the challenge and ethanol is used for the Inductions I and II) only.

Animals in the positive control test group exhibited moderate and confluent to intense erythema and swelling reactions at the challenge sites treated with the 0.15% w/v mixture of DNCB in acetone. Since there was no redness observed during the 48-hour score for negative control animals 65847 and 65851, the redness observed during the 24-hour score was an irritation response and therefore the sensitization results are negative. All reactions in the positive control test group (score of 2–3 during 48 hour score) are considered to be sensitization reactions. Based on the results obtained, this test methodology demonstrated dermal sensitization in guinea pigs using DNCB, a known sensitizer.

ANALYSIS AND CONCLUSION: None of the negative control animals challenged with the control vehicle were observed with a sensitization response greater than '0'. None of the animals challenged with the test article extract were observed with a sensitization response greater than '0'. The Normal Saline extract of the test material had a 0% sensitization response under valid test conditions. The Cottonseed Oil extract of the test material had a 0% sensitization response under valid test conditions. Under the conditions of this protocol, #4 BaLinit - A - Medical TIN, did not elicit a sensitization response.

STATISTICAL METHODS: None applied.

TECHNICAL REFERENCES:

ASTM Designation: F720-81 (Reapproved 2002) Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test. Section 13, Volume 13.01, pp. 268-270.

ISO 10993-10: 2002 Standard and Amendment 1, 2006. Biological Evaluation of Medical Devices, Part 10-Tests for Irritation and Delayed-Type Hypersensitivity. pp. 15-18.

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

Magnusson, B. and Kligman, A.M. "Allergic Contact Dermatitis in the Guinea Pig, Identification of Contact Allergens." Springfield, Ill.: Thomas. 1970.

Magnusson, B. and Kligman, A.M. "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test." *J. Invest. Dermatol.* 52:268-276. 1969.

WuXi AppTec Reference Library Contents, Form ALS-4650-1, (current revision).

TABLE 7: DAILY CHALLENGE OBSERVATIONS (NS)

ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
TEST GROUP					
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
68801	0	0	0	0	-
68802	1*	0	0	0	-
68803	0	0	0	0	-
68804	0	0	0	0	-
68805	0	0	0	0	-
68806	0	0	0	0	-
68807	0	0	0	0	-
68808	0	0	0	0	-
68809	0	0	0	0	-
68810	0	0	0	0	-
68811	0	0	0	0	-
NEGATIVE CONTROL GROUP					
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
68812	0	0	0	0	-
68813	0	1*	0	0	-
68814	0	0	0	0	-
68815	0	0	0	0	-
68816	0	0	0	0	-
68817	0	0	0	0	-

*=See Main Test Results section.

TABLE 8: DAILY CHALLENGE OBSERVATIONS (CSO)

ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
TEST GROUP					
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
67489	0	0	0	0	-
68819	0	0	0	0	-
68820	0	0	0	0	-
68821	0	0	0	0	-
68822	0	0	0	0	-
68823	0	0	0	0	-
68824	0	0	0	0	-
68825	0	0	0	0	-
68826	0	0	0	0	-
68827	0	0	0	0	-
68828	0	0	0	0	-
NEGATIVE CONTROL GROUP					
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
68829	0	0	0	0	-
68830	0	0	0	0	-
68831	0	0	0	0	-
68832	0	0	0	0	-
68833	0	0	0	0	-
68834	0	0	0	0	-

TABLE 9: POSITIVE (DNCB) AND NEGATIVE CONTROL DAILY CHALLENGE OBSERVATIONS

ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS
	TEST GROUP				
	CONTROL VEHICLE	DNCB SOLUTION	CONTROL VEHICLE	DNCB SOLUTION	
65835	0	3	0	2	+
65836	0	2	0	3	+
65837	0	2	0	2	+
65838	0	3	0	2	+
65839	0	2	0	2	+
65840	0	2	0	3	+
65841	0	2	0	2	+
65842	0	2	0	2	+
65843	0	2	0	3	+
64982	0	2	0	2	+
65346	0	2	0	3	+
	CONTROL VEHICLE	DNCB SOLUTION	CONTROL VEHICLE	DNCB SOLUTION	
65846	0	0	0	0	-
65847	0	3*	0	0	-
65848	0	0	0	0	-
65849	0	0	0	0	-
65850	0	0	0	0	-
65851	0	1*	0	0	-

DNCB= dinitrochlorobenzene
*See Positive Control section.

(For Laboratory Use Only)

WuXi AppTec Study # 118179

WuXi AppTec

PROTOCOL TITLE: **ISO GUINEA PIG MAXIMIZATION SENSITIZATION TEST (Method for Biomaterial Extracts)**

TEST CODE: **900850**

PERFORMING LABORATORY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

EFFECTIVE DATE: 17 February 2009

GLP PROTOCOL: **900850S**

Quality Assurance has reviewed this protocol for compliance with applicable regulatory requirements and internal procedures.

Exact Copy
Initals: SD Date: 5/6/09

2540 Executive Drive • St. Paul, MN 55120 • 888.794.0077 • 651.675.2000 • Fax: 651.675.2005

ISO Guinea Pig Maximization Sensitization Test (Method for Biomaterial Extracts)

1.0 PURPOSE

This test is designed to evaluate the allergenic potential or sensitizing capacity of a test article. The test is used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization.

In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic reaction. The reaction generally is due to substances that may leach out of a material. Therefore, this practice provides for the use of material extracts. The use of Freund's Complete Adjuvant and sodium lauryl sulfate tend to enhance the potential of weak sensitizing agents. While this test does not ensure that test materials are completely non-allergenic, it is the most sensitive animal test in common use today.

2.0 TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

3.0 SCHEDULING AND DISCLAIMER OF WARRANTY

3.1 Test protocol initiation is generally within 10 working days after receipt of the test article, a signed Client Protocol Approval form, and a signed test request form. The Client Protocol Approval form and the test request form serve as addenda to this protocol. Written notification of the proposed initiation and completion dates will be provided at the time the test article and signed protocol are received by the laboratory. The estimated testing time is 32 - 35 days. Verbal results will be available from the Study Director upon completion of the study with the written quality assurance audited report to follow approximately 10 working days after completion of the study.

3.2 If a test, or a portion of it, must be repeated due to failure by WuXi AppTec to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls or failure to meet assay validity requirements, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test article and test system require modifications due to complexity and difficulty of testing.

3.3 If the Sponsor requests a repeat test, they will be charged for an additional test.

3.4 Neither the name of WuXi AppTec nor any of its employees are to be used in advertising or other promotion without written consent from WuXi AppTec.

3.5 The Sponsor is responsible for any rejection of the final report by the regulatory agency concerning report format, pagination, etc. To prevent rejection, the Sponsor should carefully review the WuXi AppTec final report and notify WuXi AppTec of any perceived deficiencies in these areas before submission of the report to the regulatory agency. WuXi AppTec will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

4.0 TEST ARTICLE CHARACTERIZATION

The Sponsor is responsible for all test article characterization data as specified in the Good Laboratory Practices (GLP) regulations. The identity, strength, stability, purity, and chemical composition of the test article is solely the responsibility of the Sponsor. The Sponsor is responsible for supplying to the testing laboratory results of these determinations and any others that may directly impact the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it is the responsibility of the Sponsor to ensure that the test article submitted for testing is representative of the final product that will be subjected to materials characterization. Any special requirements for handling or storage must be arranged in advance of receipt and the test article must be received in good condition.

5.0 JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

The albino guinea pig has historically been used in skin sensitization tests and is generally accepted as the most appropriate animal model for human allergic contact dermatitis. The guidelines have no alternative (non-animal) methods.

6.0 PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

6.1 Species/Strain: Albino guinea pigs (*Cavia porcellus*), Hartley strain (specific pathogen free)

6.2 Source: A certified commercial vendor will be used as the source of the animals.

6.3 Weight Range: Animals for the main test will be between 300 - 500 g. The preliminary test animals, if needed, may be larger than 500 g, as larger animals are preferable for accommodating the number of applications required.

6.4 Age: Healthy, young adult guinea pigs will be used.

6.5 Number: A minimum of 17 and a maximum of 37 animals will be used for this study, depending on the extracts/vehicles chosen.

6.6 Sex: Either males or females can be used for the study. The specific gender will be recorded in the raw data. If females are used, they will be nulliparous, not pregnant, and housed separately from males for the duration of the test.

6.7 Animal Identification: Cage cards will be labeled and individual guinea pigs will be identified per WuXi AppTec SOP: ILS-0112, Animal Identification (current version).

6.8 IACUC Protocol / Approval Date
98-02D / April, 2006

6.9 Husbandry

6.9.1 Receipt And Acclimation
Receipt will be according to WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals (current version). The animals will be acclimated for a minimum of 5 days under the same conditions as the actual test.

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6.9.2 Housing

Animals will be housed in solid bottom cages with contact bedding and up to five guinea pigs per cage. Housing density complies with the NIH and AAALAC International guidelines for this species.

6.9.3 Environment

The environmental conditions in the animal rooms will be maintained according to WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility (current version). The temperature and photo-period will meet the AAALAC International recommendations for these species. The laboratory and animal rooms will be maintained as limited-access facilities.

6.9.4 Diet

Animals will be supplied with certified commercial feed. There are no known contaminants present in the feed expected to interfere with the test results.

6.9.5 Water

Potable water will be supplied from the St. Paul municipal water supply, *ad libitum.* There are no known contaminants present in the water expected to interfere with the test results.

6.9.6 USDA Animal Welfare Act

In order to satisfy the USDA Animal Welfare Act, the Sponsor agrees that the testing requested for the test article submitted is required in order to satisfy a state or federal regulatory requirement or is scientifically necessary. Further, such testing is not an unnecessary duplication of a previous test submission by the Sponsor. In addition, the duration of test is determined by the cited test references and will not exceed the time limits contained therein. This procedure was reviewed and approved by WuXi AppTec's Institutional Animal Care and Use Committee (IACUC) in compliance with the Animal Welfare Act.

It has been determined that no sedation, analgesia, or anesthesia is necessary in this procedure. In the unlikely event that an animal should become sick or injured, euthanasia or veterinary care will be conducted according to WuXi AppTec SOP: ILS-0233, Proper Handling of Sick and Moribund Animals (current version) and current veterinary medical practices. The objectives of the study will be given full consideration prior to any decisions and the study Sponsor will be advised.

6.10 Testing is performed in strict adherence to WuXi AppTec standard operating procedures (SOPs) which have been constructed to cover all aspects of the work including, but not limited to, receipt, identification, log-in and tracking of test article(s). Additionally, each test is assigned a unique Project Number. This number is used for identification during the course of the test.

7.0 EXPERIMENTAL DESIGN

For evaluation of a test article for sensitization, guinea pigs will be exposed in two induction phases to extracts of the test article or control (vehicle without test article). Following induction phases, the animals will be on a rest period after which the guinea pigs are challenged at a naïve skin site with the test article extracts or control vehicles. The challenge sites of each animal will be observed for evidence of skin sensitization 24 ± 2 and 48 ± 2 hours after removal of the test article extracts or control vehicles.

Test results will be interpreted based upon the percentage of animals exhibiting sensitization. Consideration is given to the overall pattern, intensity, duration, and reactions of the test group as compared with the negative control group. Only when the response to the challenge with the test article extract clearly outweighs the reaction from those of any control is the material judged to have allergic potential. In the event of equivocal results, a rechallenge of all animals may be carried out 7-14 days after the initial challenge [additional fees will apply].

8.0 TEST METHOD

8.1 Selection Of Animals

The animals selected for the study have not been subjected to any previous experimental procedures. Animals are selected from a large pool of animals and will be examined to insure their skin is free from irritation, trauma and disease. Test animals are distributed into the following groups:

1)	Test	(11 animals / extract vehicle)
2)	Irritant Control	(6 animals / extract vehicle)
3)	Preliminary Test (if applicable)	(3 animals)
4)	Positive Control	(11 positive control test / 6 vehicle control)

8.2 Positive Controls

WuXi AppTec performs positive control testing every 3 months per ISO regulations. The methods for the positive control assay are identical to the methods described above in the Experimental Design summary. Guinea pigs utilized for positive control studies are of the Hartley strain and will be supplied by the same vendor as animals used for general testing.

For the induction phases, 0.3% dinitrochlorobenzene (DNCB), a known sensitizer, in ethanol is used. For the challenge phase, 0.15% DNCB in acetone is used. The negative control animals are exposed to only the appropriate vehicle (ethanol is used for Induction I and II and acetone is used for the challenge). Results for the applicable positive control study will appear in the final report for this study.

8.3 Preparation Of Test Article

For test articles having surface dimensions that can be easily measured, the test article size to be extracted will be determined according to ISO guidelines as follows:

Examples of forms of materials	Thickness	Extraction Ratio (surface area or mass/volume) ± 10%
Film, sheet, tubing wall	< 0.5 mm	6 cm^2/mL
Tubing wall, slab, small molded items	0.5 – 1.0 mm	3 cm^2/mL
Larger molded items	> 1.0 mm	3 cm^2/mL
Elastomeric closures	> 1.0 mm	1.25 cm^2/mL
Powder, pellets, foam, non-absorbent molded items	Irregularly shaped solid devices	0.2 g sample/mL
Membranes	Irregularly shaped porous devices (low density materials)	0.1 g/mL
Note: While there are no standardized methods available at present for testing absorbents and hydrocolloids, the following is a suggested protocol: Determine the volume of extraction vehicle that each 0.1 g or 1.0 cm^2 of material absorbs. Then, in performing the material extraction, add this additional volume to each 0.1 g or 1.0 cm^2 in an extraction mixture.		

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When appropriate, the test article will be further cut under aseptic conditions into pieces having approximate dimensions of 5 cm x 0.3 cm.

When the surface area cannot be easily measured, a test article may be extracted using 4 g of test article in 20 mL of extraction vehicle (sample to volume ratio of 1:5). If other test article sizes are used, their preparation and rationale will be described in a protocol amendment and in the test report.

Vehicle controls will be prepared and extracted concurrently with the test article for each phase of the main test.

8.4 Preliminary Tests

The preliminary tests are intended to determine the concentrations of the test material extracts to be used in the main test. Undiluted extracts using the usual solvents need not be subjected to preliminary testing per ISO regulations. For the typical medical device extract, the preliminary test will not be conducted. The decision to run the preliminary test will be based on the discretion of the Study Director and the nature of the test article.

☐ Preliminary Test is applicable.
☒ Preliminary Test NOT applicable.

If applicable, three (3) guinea pigs will be prepared by shaving the flanks with an electric clipper. The guinea pigs will be topically patched with four (4) concentrations (100%, 90%, 75%, and 50%). Each concentration will be applied to a 1 x 1 cm filter paper patch backed by an occlusive tape. If two (2) extracts are selected for this study, the three (3) guinea pigs will be patched with the saline extract concentrations on the right side and with the cottonseed oil concentrations on the left side. At approximately 24 hours after the topical application, the treatment sites are assessed for erythema and edema using the grading scale given in Table 2.

8.4.1 Induction Concentration Selection

If possible, for the topical induction phase, the highest concentration that causes slight erythema but does not otherwise adversely affect the topically patched animals will be selected.

8.4.2 Challenge Concentration Selection

For the challenge phase, the highest concentration that produces no erythema on the topically patched animals will be selected.

8.5 Test Article Administration

8.5.1 Preparation Of The Test Animals

Prior to the test procedures, an area over the shoulder region, approximately 5 x 7 cm, will be prepared by clipping the skin of the test site free of fur with an appropriate blade. Prior to the challenge phase, an area of the right and left flank, approximately 4 x 4 cm, will be prepared by removing the fur with an electric clipper.

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8.5.2 Induction I /Intradermal Induction Phase

Three (3) syringes will be prepared for the test and negative control animals as indicated in Table 1. The prepared syringes will be injected in pairs on each side of the dorsal midline. Injections 1 and 2 will be given in close proximity to each other cranially; injection 3 will be located caudally. The six (6) injection sites will be just within the boundaries of a 2 x 4 cm patch, which will be applied 1 week (± 1 day) following the injections. Injections with Freund's Complete Adjutant (FCA) are made slightly deeper to minimize tissue sloughing.

TABLE 1: FIRST INDUCTION SYRINGE PREPARATION AND DOSE VOLUME

PREPARATION	VOLUME INJECTED PER SITE	SYRINGE CONTENTS	RATIO (V / V)
TEST GROUP			
Syringe 1	0.1 mL	FCA + appropriate vehicle*	1:1
Syringe 2	0.1 mL	Test Extract	NA
Syringe 3	0.1 mL	FCA + Appropriate vehicle* (1:1) + Test extract	1:1
CONTROL GROUP			
Syringe 1	0.1 mL	FCA + appropriate vehicle*	1:1
Syringe 2	0.1 mL	Control Vehicle	NA
Syringe 3	0.1 mL	FCA + Appropriate vehicle* (1:1) + Control vehicle	1:1

*Appropriate vehicle = 0.9% Sterile Saline

8.5.3 Induction II/ Topical Induction Phase

On Day 6, the injection site areas will be treated with 0.5 to 1 g of 10% (w/w) sodium lauryl sulfate (SLS) prepared by mixing powdered SLS with mineral oil. The day following the SLS treatment, remaining SLS residue will be gently wiped from the area with gauze.

Test Group: 24 (± 2) hours after SLS application, the test article extract* will be applied to a 2 x 4 cm piece of filter paper to saturation (0.3 - 0.5 mL). The patch will be secured to the site with non-permeable tape and the trunk wrapped with an expandable wrapping material and secured with tape. This preparation will be left in place for 48 ± 2 hours. Freshly prepared extract will be used for this administration.

* = if necessary, the concentration is determined by the preliminary test.

Control Group: The animals will be exposed to the extract vehicle in the same manner as the test group.

8.5.4 Challenge Phase

Fourteen days (± 1 day) after completion of the topical induction phase, the challenge procedure will be initiated on the 11 test animals and the 6 irritant control animals per extract. A filter paper patch will be saturated with 0.3-0.4 mL of freshly prepared test article extract* and applied to the fur clipped right flank or dorsum of each test animal.

A filter paper patch will be saturated with 0.3-0.4 mL of freshly prepared control vehicle and applied to the fur clipped left flank or dorsum of each test animal.

The negative control animals will be challenged in an identical fashion with similarly prepared patches. The left side of each animal will be patched with a filter paper patch saturated with 0.3-0.4 mL of control vehicle. The right side will be patched with a filter paper patch saturated with 0.3-0.4 mL of the prepared test article applied to the fur clipped flank or dorsum. The trunk of each animal will be wrapped for 24 ± 2 hours with an expandable wrapping material and secured with tape.

* = If necessary, the concentration is determined by the preliminary test.

8.6 Observations

8.6.1 Skin Readings

The day after challenge exposure, the patch will be removed and the area cleaned gently with gauze if necessary. The site will be wiped gently with a 70% isopropyl alcohol soaked gauze sponge prior to each scoring period. The challenge sites will be observed for signs of irritation and sensitization reaction, as indicated by erythema and edema.

Daily challenge observation scores will be recorded 24 ± 2 and 48 ± 2 hours after patch removal in accordance with the following classification system for skin reactions:

TABLE 2: MAGNUSSON AND KLIGMAN 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

Note: Erythema is defined as redness and edema is defined as a swelling at the challenge site. Any other adverse changes at the skin sites shall be recorded and reported.

Background or artifactual reactions from fur clipping, patch edge, or nonspecific FCA effects will not be considered as evidence of sensitization. The treatment with FCA and occlusive dressings may lower the threshold level for skin irritation.

8.6.2 Health Observations

Daily animal health observations will be recorded throughout the study period.

8.7 Termination

After the final observation period and study director approval, the animals will be humanely euthanized by CO_2 gas asphyxiation.

9.0 METHOD FOR CONTROL OF BIAS: Not applicable.

10.0 DATA ANALYSIS: Not applicable.

11.0 STATISTICAL METHODS: None used.

12.0 ASSAY VALIDITY

Final evaluation of the validity of the assay and test article results will be based upon the criteria listed below and scientific judgment.

12.1 A test is considered invalid and will be repeated if less than ten test animals or less than five control animals survive the duration of the study.

12.2 A test is considered invalid and will be repeated if any animals develop frank infection of the injection sites.

13.0 EVALUATION OF RESULTS

Grades of '1' or greater in the test group generally indicate sensitization, provided 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 the test animals which exceed the most severe control reaction are presumed to be due to sensitization.

Occasionally, the test group has a greater number of animals showing a response than the controls, although the intensity of the reaction is not greater than that observed on the controls. In these instances, a rechallenge may be necessary to define the response clearly. A rechallenge shall be carried out 1 - 2 weeks after the first challenge. The method used shall be as described for the first challenge, using the other flank of the animal.

The outcome of the test will be presented as the frequency of positive challenge results in test and control animals.

A test will be repeated in part or in total if a control failure occurs.

14.0 PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revisions and reasons for changes will be documented, signed by the Study Director, dated, maintained with the protocol and reported to the Sponsor. If an event occurs which may have an effect on the validity of the study, the Sponsor will be notified as soon as is practical. If the Study Director is unable to complete the study, an alternate Study Director with full responsibility and authority regarding the study will be assigned.

15.0 FINAL REPORT

The final report will include but will not be limited to: the date of the study initiation and completion, the purpose as stated in the approved protocol, changes in the approved protocol, identification of the test system, applicable positive control results, a description of the methods used and conclusion as it relates to the test.

16.0 RECORD RETENTION

16.1 Study Specific Documents

All of the original raw data developed exclusively for this study shall be retained according to WuXi AppTec, Inc.'s standard operating procedures for archival. These original data include, but are not limited to the following:

16.1.1 All handwritten and equipment generated raw data for control(s) and test article(s).

16.1.2 Any protocol amendments/deviation notifications.

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16.1.3 Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
16.1.4 Original signed protocol.
16.1.5 Certified copy of final study report.
16.1.6 Study-specific SOP deviations made during the study.
16.1.7 Animal health records and daily clinical observations.
16.1.8 QA reports for each QA inspection with comments.

16.2 Facility Specific Documents
The following records shall also be retained according to WuXi AppTec, Inc.'s standard operating procedures for archival. These documents include, but are not limited to, the following:

16.2.1 SOPs which pertain to the study conducted.
16.2.2 Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
16.2.3 Methods which were used or referenced in the study conducted.
16.2.4 Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
16.2.5 Current job descriptions and summary of experience and training for all personnel involved in the study.
16.2.6 Records of analysis of food and water.

17.0 REFERENCES

17.1 ASTM Designation: F720-81 (Reapproved 2002) Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test. Section 13, Volume 13.01, pp. 268-270.

17.2 ISO 10993-10: 2002 Standard and Amendment 1, 2006. Biological Evaluation of Medical Devices, Part 10-Tests for Irritation and Delayed-Type Hypersensitivity. pp. 15-18.

17.3 ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

17.4 Magnusson, B. and Kligman, A.M. "Allergic Contact Dermatitis in the Guinea Pig, Identification of Contact Allergens." Springfield, Ill.: Thomas. 1970.

17.5 Magnusson, B. and Kligman, A.M. "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test." *J. Invest. Dermatol.* 52:268-276. 1969.

17.6 WuXi AppTec Reference Library Contents, Form ALS-4650-1, (current revision).

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18.0 COMPLIANCE

18.1 Animal Husbandry

AAALAC International guidelines as reported in the "Guide for the Care and Use of Laboratory Animals", National Research Council - ILAR, Revised 1996; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986; USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, Animal Welfare, Final Rule 1989.

18.2 GLP Status

If the Sponsor chooses to conduct the study under GLP compliance (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies), the study will be inspected during at least one phase and the final report will be audited by the WuXi AppTec Quality Assurance unit.

19.0 TEST ARTICLE IDENTIFICATION

Test article information to be included in the final report will be provided solely by the Sponsor on the WuXi AppTec test request form attached to this protocol.

20.0 TEST ARTICLE DISPOSITION

It is the responsibility of the Sponsor to retain a sample of the test material. All unused test material will be discarded following study completion unless otherwise requested by Sponsor.

CLIENT PROTOCOL APPROVAL FORM

PLEASE NOTE THAT TESTING CANNOT BE INITIATED UNTIL THIS FORM IS COMPLETED WITH AN AUTHORIZED SIGNATURE AND THE ORIGINAL IS RETURNED TO WUXI APPTEC.

SPONSOR:

Ms. Jennifer Shaw
Nelson Laboratories, Inc.
6280 South Redwood Road
Salt Lake City, UT 84123

NEL05
Phone #: 801-290-7540
Facsimile: 801-963-2630
E-mail: jshaw@nelsonlabs.com

Primary Approval Statement

I have read WuXi AppTec, Inc.'s client protocol, 900850S - ISO Guinea Pig Maximization Sensitization Test (Method for Biomaterial Extracts). I accept the test method described. I understand that my approval will be valid until one or both of the following occur:

1. The protocol is revised and a new version letter is issued.
2. The Primary Approver's position with the Sponsor company is terminated or changes, whichever may occur first.

NAME: Jennifer Shaw TITLE: Subcontracting

SIGNATURE: Jennifer Shaw DATE: 18 Feb 2009

Associate(s) Approval Statement

The Primary Approver (above) has authorized the following Associate(s) to accept the responsibility for submitting samples for testing under this protocol. Each associate understands that their authorization for submission will be valid until one or more of the following has occurred:

1. The protocol has been revised and new version letter has been issued.
2. The primary Approver's position with the Sponsor company is terminated or changes, whichever may occur first.
3. Any of the Associate's positions with the Sponsor company are terminated or change, whichever may occur first.
4. The Primary Approver has removed any Associate's authorization by sending a signed and dated letter to WuXi AppTec, ATTN: Client Services.

☐ I do not wish to have an Associate(s) authorized to initiate testing of samples under this protocol.

☒ I do wish to have the following Associate(s) authorized to initiate testing of samples under this protocol.

Thor Rollins
Name of Associate (please print)

—
Name of Associate (please print)

Tarika Onishi
Name of Associate (please print)

—
Name of Associate (please print)

WUXI APPTEC, INC.:

NAME: Nick Wolner
Study Director

SIGNATURE: Nick Wolner DATE: 3/12/09
Study Director