NON-GLP
FINAL STUDY REPORT

STUDY TITLE:	**ISO Guinea Pig Maximization Sensitization Test** (Method for Biomaterial Extracts)
TEST CODE NUMBER:	900850
TEST ARTICLE IDENTIFICATION:	PC-ISO (White)
PERFORMING LABORATORY:	WuXi AppTec, Inc. 2540 Executive Drive St. Paul, MN 55120
SPONSOR:	Stratasys, Inc. 7665 Commerce Way Eden Prairie, MN 55344
STUDY NUMBER:	145177
CLIENT MNEMONIC:	STR05
RESULT SUMMARY:	The test article **did not** elicit a sensitization response under the conditions of this assay.

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: 10/14/10
STUDY COMPLETION DATE: 12/07/10

TEST ARTICLE IDENTIFICATION

Test Article Name:	PC-ISO (White)
Lot/Batch #:	Not Applicable
Sterilization Method:	Non-Sterile
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature

TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data. 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.

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.

SAFETY

Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental 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 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.

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 hours 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.

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.

Institutional Animal Care and Use Committee (IACUC)
The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by WuXi AppTec's IACUC prior to the initiation of such procedures.

IACUC Protocol / Approval Date: 98-02E / May, 2009

AMENDMENTS / DEVIATIONS: None.

IDENTIFICATION OF THE TEST SYSTEM

Species/Strain: Albino guinea pig, Hartley strain, specific pathogen free (SPF)

Source: Charles River Laboratories

Sex: Male.

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

Age: Healthy young adults were used.

Animal Identification: The animals were identified per WuXi AppTec SOP: ILS-0112.

HUSBANDRY

Receipt: Animals were received on 10/20/10 according to WuXi AppTec SOP: ILS-0092. Each animal was examined for signs of disease and injury. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were housed in solid bottom cages with contact bedding and up to five guinea pigs per cage. Housing density complied with AAALAC International recommendations. The test and negative control animals were housed separately.

Environment: The environmental conditions in the animal rooms were set to be maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.

Diet: Animals were supplied with certified commercial guinea pig 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.

TEST MATERIAL PREPARATION

The test article appeared to consist of white molded plastic. A representative sample of the test article was cut for extraction, placed into test tubes and prepared at a ratio of 60 cm^2 to 20 mL of extraction vehicle.

TEST ARTICLE EXTRACTION

The extraction mixtures and corresponding control blanks were incubated for 24 ± 2 hours at 70 ± 1 °C. The extracts were manually agitated. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. The test article was observed after all extractions to be intact with no macroscopically observable degradation. After decanting, the extract was not filtered prior to use. The extracts were maintained at room temperature and used within 24 hours of preparation.

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 1. 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.

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 + 0.9% Sterile Saline	1:1
Syringe 2	0.1 mL	Test Extract	NA
Syringe 3	0.1 mL	FCA + 0.9% Sterile Saline (1:1) + Test Extract	1:1
CONTROL GROUP			
Syringe 1	0.1 mL	FCA + 0.9% Sterile Saline	1:1
Syringe 2	0.1 mL	Control Vehicle	NA
Syringe 3	0.1 mL	FCA + 0.9% Sterile Saline (1:1) + Control Vehicle	1:1

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 elastic bandage 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 2. Daily animal health observations were recorded throughout the study period.

TABLE 2: 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.

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

EVALUATION CRITERIA

- 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 using the same animals. The method used shall be as described for the first challenge, with the exception that testing will be performed on a naïve site.

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

ASSAY VALIDITY
Final evaluation of the validity of the assay and test article results was based upon the following criteria and scientific judgment:

A test was considered invalid and will be repeated if:

- less than ten test animals or less than five control animals survived the duration of the study.
- any animals developed frank infection of the injection sites.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: None used.

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE
The care, housing and handling of the animals were in compliance with:

- AAALAC International and NIH 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.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples will be saved as requested by the Sponsor.

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'. None of the test animals challenged with the test article extracts were observed with a sensitization response greater than '0'. A negative sensitization incidence was interpreted for all test animals. See Table 3 for individual animal scores.

TABLE 3: DAILY CHALLENGE OBSERVATIONS

NORMAL SALINE (NS)					
ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
	TEST GROUP				
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
104067	0	0	0	0	-
104068	0	0	0	0	-
104069	0	0	0	0	-
104070	0	0	0	0	-
104071	0	0	0	0	-
104072	0	0	0	0	-
104073	0	0	0	0	-
104074	0	0	0	0	-
104075	0	0	0	0	-
104076	0	0	0	0	-
104077	0	0	0	0	-
Animal #	Negative Control Group				Results (+) or (-)
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
104061	0	0	0	0	-
104062	0	0	0	0	-
104063	0	0	0	0	-
104064	0	0	0	0	-
104065	0	0	0	0	-
104066	0	0	0	0	-

COTTONSEED OIL (CSO)					
ANIMAL #	24 HOURS SCORE		48 HOURS SCORE		RESULTS (+) OR (-)
	TEST GROUP				
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
104084	0	0	0	0	-
104085	0	0	0	0	-
104086	0	0	0	0	-
104087	0	0	0	0	-
104088	0	0	0	0	-
104089	0	0	0	0	-
104090	0	0	0	0	-
104091	0	0	0	0	-
104092	0	0	0	0	-
104093	0	0	0	0	-
104094	0	0	0	0	-
Animal #	Negative Control Group				Results (+) or (-)
	CONTROL VEHICLE	TEST EXTRACT	CONTROL VEHICLE	TEST EXTRACT	
104078	0	0	0	0	-
104079	0	0	0	0	-
104080	0	0	0	0	-
104081	0	0	0	0	-
104082	0	0	0	0	-
104083	0	0	0	0	-

Positive Control: WuXi AppTec completes positive controls every 3 months. A positive control was completed on 08/30/10 (see Table 4 for individual animal scores). The methods for the positive control assay are similar 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 discrete or patchy erythema to moderate and confluent erythema at the challenge sites treated with the 0.15% w/v mixture of DNCB in acetone. All reactions in the positive control test group (score of 1–2 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.

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Table 4: Positive (DNCB) and Negative Control Daily Challenge Observations

Animal #	24 Hours Score		48 Hours Score		Results
Positive Control Group					
	Control Vehicle	DNCB Solution	Control Vehicle	DNCB Solution	Results (+) or (-)
98851	0	1	0	1	+
98852	0	2	0	2	+
98853	0	1	0	1	+
98854	0	1	0	1	+
98855	0	2	0	2	+
98856	0	1	0	1	+
98857	0	1	0	1	+
98858	0	2	0	2	+
98859	0	1	0	1	+
98860	0	1	0	1	+
98861	0	2	0	2	+
Negative Control Group					
	Control Vehicle	DNCB Solution	Control Vehicle	DNCB Solution	Results (+) or (-)
98862	0	0	0	0	-
98863	0	0	0	0	-
98864	0	0	0	0	-
98865	0	0	0	0	-
98866	0	0	0	0	-
98867	0	0	0	0	-

DNCB= dinitrochlorobenzene

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 extracts were observed with a sensitization response greater than '0'.
- The normal saline extract of the test material had a sensitization response of '0' under valid test conditions.
- The cottonseed oil extract of the test material had a sensitization response of '0' under valid test conditions.
- Under the conditions of this protocol, the test article **did not** elicit a sensitization response.

Approval: _____ Date: 12/7/10
Nick Wolner, BA - Study Director

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.

Guidance for Industry (Draft Guidance), 2008: Coronary Drug - Eluting Stents - Nonclinical and Clinical Studies: Companion Document, pg. 12.

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

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0112, Animal Identification

WuXi AppTec SOP: ILS-0155, ISO Guinea Pig Maximization Sensitization Test

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick, Injured, and/or Moribund Animals

WuXi AppTec SOP: ILS-1651, ISO Guinea Pig Positive Control Validation for Maximization Sensitization Test

Material Certifications Disclaimer:

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