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Non-GLP
FINAL STUDY REPORT

STUDY TITLE:	**ISO Intracutaneous Reactivity Test**
TEST CODE:	9107000
TEST ARTICLE IDENTIFICATION:	PC-ISO (White) Lot # NA
PERFORMING LABORATORY:	WuXi AppTec, Inc. 2540 Executive Drive St. Paul, MN 55120
SPONSOR:	Stratasys, Inc. 7665 Commerce Way Eden Prairie, MN 55344
STUDY NUMBER:	145178
CLIENT MNEMONIC:	STR05
RESULT SUMMARY:	Under the test conditions of this protocol, the test article would be considered a **non-irritant.**

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PURPOSE
The purpose of this test was to determine if any chemicals that may leach or be extracted from the test article were capable of causing local irritation in the dermal tissues of rabbits.

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

DATE SAMPLE RECEIVED: 10/14/10
STUDY COMPLETION DATE: 11/11/10

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

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.

EXPERIMENTAL DESIGN

Experimental Summary
The purpose of this test was to determine if chemicals that may leach or be extracted from the test material are capable of causing local irritation in the dermal tissues of the rabbit. The study was conducted in accordance with ISO 10993-10: 2002 Standard, Amendment 1, 2006, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-type Hypersensitivity, pages 23 – 25.

Each rabbit received five sequential 0.2 mL intracutaneous injections along either side of the dorsal mid-line with the test article extract on one side and the concurrent vehicle control on the other. The vehicles used were 0.9% normal saline and cottonseed oil.

The irritation reaction of the test extracts were compared to vehicle controls and recorded over a 72-hour period according to the standard ISO irritation scoring system. According to ISO 10993:10 test criteria, if the difference between the average scores for the extract of the test article and the vehicle control is less than or equal to 1.0, the test article is considered non-irritating.

Justification For Selection Of The Test System
This test method and species have historically been used to assess the potential of the material under test to produce intradermal irritation to help determine biocompatibility of materials used in medical devices. The animal species, number and route of test article administration will be as recommended in ISO 10993-10.

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: 07-122B / June 2010

AMENDMENTS/DEVIATIONS: None.

TEST SYSTEM

Species/Strain: Albino rabbits (*Oryctolagus cuniculus*) / New Zealand White strain

Source: Bakkom Rabbitry, Viroqua, WI

Sex: Female. Females used were nulliparous and non-pregnant.

Weight Range: Each rabbit weighed at least 2.0 kg.

Age: The rabbits were young adults. No particular age was required, however vendor practice assures consistency in the age of the animals.

Number: A total of two animals per extract or pair of extracts were used for this study.

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

HUSBANDRY

Receipt: Animals were received on 10/26/10 according to WuXi AppTec SOP: ILS-0092. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were individually housed in stainless steel caging. Housing dimensions complied with NIH and AAALAC International guidelines for this species.

Environment: The environmental conditions in the animal rooms were 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 feed, *ad libitum*. There were no known contaminants present in the feed expected to interfere with the test results. Feed analysis results are available and archived by WuXi AppTec.

Water: Potable water was supplied from the St. Paul municipal water supply, *ad libitum.* There were no known contaminants present in the water expected to interfere with the test results. Periodic analysis of the water is conducted and the results are archived by WuXi AppTec.

TEST AND CONTROL MATERIAL PREPARATION
The test article appeared to consist of filament. 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 ± 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. The test article was observed after extraction to be intact with no macroscopically observable degradation. 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 placed on study as available. Any animals considered unsuitable due to poor health, abraded skin or outlying body weight were excluded from the study.

ANIMAL PREPARATION
Each animal was weighed and the weight recorded prior to test injection. The fur of the animals was clipped on both sides of the spinal column to expose a sufficient sized area for injection.

TEST ARTICLE ADMINISTRATION
The two test article extracts and the two vehicle controls were each injected into two rabbits. Each rabbit received five sequential 0.2 mL intracutaneous injections of the test article extract on the right side of the vertebral column and similarly the control vehicle on the left side. The second test and control extract injections were parallel and distal to the first injection sites.

OBSERVATIONS AND SCORING
The animals were observed daily for abnormal clinical signs. The appearance of each injection site was noted at 24 ± 2, 48 ± 2 and 72 ± 2 hours post injection. The tissue reactions were rated for gross evidence of erythema and edema. The skin was lightly swabbed with dilute alcohol to enhance the appearance of any erythema or edema. The intradermal injection of CSO frequently elicits an inflammatory response. CSO erythema scores ≤ 2 are considered normal.

TERMINATION
All animals were euthanized according to WuXi AppTec SOP: ILS-0230 by lethal injection with a sodium pentobarbital based solution after the 72-hour observations were recorded.

EVALUATION CRITERIA
According to ISO 10993-10, the requirements of the test are met if the difference between the test article and the control mean score is 1.0 or less.

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

METHOD FOR CONTROL OF BIAS: Not applicable.

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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 returned as requested by the Sponsor.

RESULTS

Clinical Observations
None of the animals on study showed abnormal clinical signs during the 72-hour test period.

Dermal Observations
A hematoma was reported on test site 5 of animal 17666 throughout the scoring duration. Additionally animal 17623 was reported with hematomas present on test sites 1 and 3 throughout the scoring duration. There were no other significant dermal reactions observed at the 0.9% normal saline and cottonseed oil injected test and control sites on the rabbits at the 24, 48, and 72 hour observation periods.

TABLE 1: DERMAL OBSERVATIONS – 0.9% NORMAL SALINE

RABBIT	CONTROL SCORES			TEST SCORES		
17666	0	0	0	3	3	2
17623	0	0	0	0	0	0
Total	0			8		
Average (Total/12)	0/12 = 0			8/12 = 0.7		
Comparative Results	0.7					

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TABLE 2: DERMAL OBSERVATIONS – COTTONSEED OIL

RABBIT	CONTROL SCORES			TEST SCORES		
17666	5	5	5	5	5	5
17623	5	5	5	8	7	5
Total	30			35		
Average (Total/12)	30/12 = 2.5			35/12 = 2.9		
Comparative Results	0.4					

ER=Erythema ED=Edema

CALCULATIONS

The erythema and edema scores were determined for each test sample and control vehicle. Each total score was divided by 12 (2 animals *x* 3 observation periods *x* 2 scoring categories) to determine the overall mean score for each test extract versus each corresponding control. The results are presented in Tables 1 and 2.

ANALYSIS AND CONCLUSION

- The test was considered valid based upon scientific judgment.
- The differences in the mean test and control scores of the 0.9% normal saline and cottonseed oil extract dermal observations were less than 1.0, indicating that the requirements of the ISO Intracutaneous Reactivity Test **have been met** by the test article.
- Under the test conditions of this protocol, the test article would be considered a **non-irritant.**

Approval: _____ Date: 11/11/10
Kent Grove, BS, MS, HT –Study Director

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REFERENCES

ISO 10993-10: 2002 Standard, Amendment 1, 2006, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-type Hypersensitivity, Pages 23 – 25.

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

U.S. Pharmacopeia, Section 88, current revision.

WuXi AppTec SOP: ALS-0260, Sample Extraction Procedures

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-0230, Euthanasia Procedures

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

WuXi AppTec SOP: TRG-0300, Preparation of Biomaterials for Extraction

Material Certifications Disclaimer:

The Stratasys ABS M30i and PC-ISO filament has been independently tested and certified as a medical grade plastic according to ISO guidelines. This certification notwithstanding, *neither* Stratasys nor the raw material manufacturer can approve resins/plastics for particular medical, pharmaceutical, food or other applications. It is the responsibility of the FDM system end-user to make a determination of the part's suitability for end-user's intended purpose through appropriate testing and analysis of all the component parts and materials to be used in the finished products.

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