Final Report

Study Title:	**ISO Primary Skin Irritation Test (GLP)**
Sponsor:	**Klarity Medical Products, LLC** **1987 Coffman Road** **Newark, OH 43055** **US**
Sponsor Account Number:	**4003497**
Performing Laboratory:	WuXi AppTec 2540 Executive Drive St. Paul, MN 55120 US
Folder Number:	D00009282
Test Code:	910699.1
Report Number:	16055
Sample Number:	D00009282001
Test Article Name:	Klarity AccuCushion R550-M
Test Article Lot#:	50506Z

QUALITY ASSURANCE UNIT SUMMARY

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.

Critical Phase	Date	Study Director	Management
Dosing	06/18/15	06/19/15	06/26/15
Final Report	06/26/15	06/26/15	06/26/15

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

Quality Assurance Auditor: Lenay Gallagher Date: 6/29/15
Lenay Gallagher

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 are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Kara Somers Date: 7/1/15
Kara Somers

Professional Personnel Involved:

Teri Tanquist, BS	Vice President of Operations
Roxanne Miller, AA, CVT	Sr. Director of Operations
Sarah Steinmetz, BA	Director of Study Operations
Lynn Devine-McDonald, BA	Director of In-Life Operations
Kara Somers, BS	Associate Study Director

PURPOSE: This test was designed to determine the dermal irritation potential of the test article on the shaved skin of the rabbit as required by regulation of medical device biocompatibility.

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

DATE SAMPLE RECEIVED: 06/08/15
STUDY INITIATION DATE: 06/10/15
STUDY COMPLETION DATE: 07/01/15

TEST ARTICLE IDENTIFICATION :

Test Article Name:	Klarity AccuCushion R550-M
Lot #:	50506Z
Sterilization Method:	Non-Sterile
Physical State:	Insoluble Material
Expiration Date:	05/06/18
Storage Conditions:	Room Temperature
Intended Use/Application:	Patient stabilization for Radiation therapy treatments.
Physical Description:	According to the Sponsor, the test article consisted of a moldable pillow with nylon fabric surface.

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.

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 a 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
The objective of this test was to determine the dermal irritation potential of the test article on the shaved skin of the rabbit as required by regulation of medical device biocompatibility. This study was conducted in accordance with the International Organization of Standardization: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

The Sponsor-supplied test article and negative control patches were wet with tap water and applied to the shaved skin of three adult albino rabbits. After a minimum four hour exposure period, the patches were removed. The observations for skin irritation were conducted at 60 ± 6 minutes after unwrapping, and at 24 ± 2, 48 ± 2, and 72 ± 2 hours. A comparative control or control vehicle was run concurrently with the test article or test extract. The test was evaluated by an overall collection and interpretation of the data, using the dermal scores as reference marks, and based on the nature and reversibility of the skin lesions.

Justification for Selection of the Test System
The New Zealand albino rabbit is the standard laboratory animal used for skin irritant evaluation. It is used because it is readily available and because of the existing historical data base for comparative evaluation.

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 the WuXi AppTec IACUC prior to the initiation of such procedures.

IACUC Protocol/ Effective Date: 07-123C / June, 2013

PROTOCOL AMENDMENTS/DEVIATIONS
There were no amendments or deviations that occurred during the course of this study.

IDENTIFICATION OF THE TEST SYSTEM

Species / Strain: All of the animals used in this study were albino rabbits (*Oryctolagus cuniculus*), New Zealand White strain.

Source: The animals were obtained from Charles River Laboratories, a previously approved vendor of commercial laboratory animals.

Sex: All of the animals used were female, nulliparous and non-pregnant.

Age: All animals were approximately 16 weeks old at the start of the study.

Number: Three animals were used for this study.

Table 1: Preliminary Animal Data

Animal Number	Beginning Body Weight (kg)
38777	3.4
38778	3.2
38779	3.1

Body Weights: All animals had body weights greater than the protocol minimum of 2.0 kg at the start of the study.

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

HUSBANDRY

Receipt: Animals were received on 05/27/15. Each animal was examined for signs of disease and injury prior to entry into the research area. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were individually housed in suspended stainless steel caging. Cage dimensions were in compliance with NIH and AAALAC International guidelines.

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 this species. The laboratory and animal rooms were maintained as limited-access facilities.

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

Water: Animals were given potable water from the municipal water supply. No known contaminants present in the water were expected to interfere with the test results.

TEST MATERIAL PREPARATION
A representative sample of the test article was cut into approximately 2.5 x 2.5 cm patches. The plastic packaging (bag) was removed and only the nylon fabric was tested.

NEGATIVE CONTROL SAMPLE PREPARATION
Six patches of approximately 2.5 x 2.5 cm were cut from absorbent gauze.

SELECTION OF ANIMALS
Animals were randomly placed in cages upon receipt and placed on study as available. Animals considered unsuitable due to poor health or unacceptable skin conditions were excluded from the study.

ANIMAL PREPARATION
Each rabbit was weighed and the weight recorded prior to test patch application. The fur of the animals was clipped on both sides of the spinal column to expose a sufficient sized area for patch application. Loose fur was removed from the skin.

TEST ARTICLE ADMINISTRATION
The test articles and control patches were wet with tap water and applied to the shaved backs of three rabbits (one test article on each side of the vertebral column). The patches were held in place by wrapping the trunk of the animals with an elastic bandage and securing with hypoallergenic tape.

After a minimum four hour exposure period, the animals were unwrapped and the locations of the patches were marked on the skin to aid in scoring.

EVALUATION CRITERIA
The primary irritation index (PII) was calculated, using the dermal scores. For any animal with a dermal response, the maximum irritation response was determined as well as the time of the initial response and time of maximum response.

Only the 24 ± 2, 48 ± 2, and 72 ± 2 hour observation periods will be used for the calculations. After the 72 ± 2 hour grading, all erythema grades plus edema grades 24 ± 2, 48 ± 2, and 72 ± 2 hour are totaled separately for each test sample and control for each animal. The primary irritation score for an animal is calculated by dividing the sum of all the scores by 6 (2 test/observation sites x 3 time points.) The primary irritation index is obtained for the test sample by the addition of all the primary irritation scores of the individual animals and by dividing by the number of animals.

The primary irritation scores for the control sites were subtracted from the total primary irritation scores of the test sites and this value was divided by the total number of animals, yielding the primary irritation index. This number was used to categorize the test article response with Table 2.

Table 2: Primary Irritation Response Categories in the Rabbit

Response Category	Comparative Mean Score (PII)
Negligible	>0 to 0.4
Slight	0.5 to 1.9
Moderate	2 to 4.9
Severe	5 to 8

Note- The Primary Irritation Index (PII) was determined by subtracting the Total Primary Irritation Score for the control sites from the Total Primary Irritation Score of the test sites and dividing that value by the total number of animals used in the study.

ASSAY VALIDITY
Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment. The study was considered valid since all test subjects survived the test period and all patches remained in place for the duration of the wrapped period.

OBSERVATIONS AND SCORING
The animals were observed daily for abnormal clinical signs. Detailed dermal observations were recorded at 60 ± 6 minutes post unwrapping, and at 24 ± 2, 48 ± 2 and 72 ± 2 hours according to Table 3. The tissue reactions were rated for gross evidence of erythema and edema.

Table 3: Dermal Observation Scoring

Erythema		Edema	
0 =	No erythema	0 =	No edema
1 =	Very slight erythema (barely perceptible)	1 =	Very slight edema (barely perceptible)
2 =	Well defined erythema	2 =	Well-defined edema (edges of area well-defined by definite raising)
3 =	Moderate to severe erythema	3 =	Moderate edema (raised ~ 1 mm)
4 =	Severe erythema (beet redness) to slight eschar formation preventing grading of erythema	4 =	Severe edema (raised > 1 mm and extending beyond exposure area)

TERMINATION
The rabbits were sacrificed by lethal injection with a sodium pentobarbital based solution (Euthasol) after the final observation period.

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 at WuXi AppTec, 2540 Executive Drive, St. Paul, MN 55120. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE

Animal Welfare
WuXi AppTec maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of the study also conformed to the following regulations and guidelines regarding animal care and welfare:

- NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- (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.
- WuXi AppTec Policy on Humane Care.

International Standard
ISO 10993-10: 2010, "Biological Evaluation of Medical Devices, Part 10-Tests for Irritation and Skin Sensitization" pp. 7-11, 27, 28.

ISO 10993-12: 2012 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

POSITIVE CONTROL

WuXi AppTec completes positive control validations semi-annually. A positive control was completed on 01/23/15 (see Tables 4 and 5 for individual animal scores). The methods utilized for the positive control assay were similar to the methods described in the Experimental Method Summary. Rabbits utilized for positive control studies were of the New Zealand White strain and were supplied by similar vendors as animals used for general testing. 2.5% sodium lauryl sulfate (SLS) in 0.9% NaCl and 5.0% sodium lauryl sulfate (SLS) in 0.9% NaCl were used as the positive control test articles.

Table 4: Calculation Of The Primary Irritation Index And Final Result for 2.5% SLS

Rabbit #	Primary Irritation Scores		Irritation Response Category
	Test (2.5% SLS)	Control	
37345	1.8	0.0	2 to 4.9-------- **Moderate**
37342	2.5	0.0	
37346	2.2	0.0	
Total	6.5	0.0	
Primary Irritation Index (PII) (Total Test – Total Control) / 3	2.2		

Table 5: Calculation Of The Primary Irritation Index And Final Result for 5.0% SLS

Rabbit #	Primary Irritation Scores		Irritation Response Category
	Test (5% SLS)	Control	
37345	2.0	0.0	2 to 4.9-------- **Moderate**
37342	3.2	0.0	
37346	3.3	0.0	
Total	8.5	0.0	
Primary Irritation Index (PII) (Total Test – Total Control) / 3	2.8		

RESULTS

Clinical Observations: None of the animals on study showed abnormal clinical signs during the 60 minute, 24, 48 and 72 hour observation periods.

Dermal Observations: There were no significant dermal reactions observed at the test sites on the rabbits at the 60 minute, 24, 48 and 72 hour observation periods.

Calculations: The sum of the erythema and edema scores for the test article and control sites were calculated for only the 24, 48 and 72 hour observation periods for each rabbit. The total scores were divided by 6 (2 observation sites *x* 3 observation periods) to determine the primary irritation score.

The primary irritation score for the control sites of each rabbit were then totaled and subtracted from the totaled primary irritation score of the test site. This value was then divided by the total number of animals to yield the primary irritation index. The results are presented in Tables 6 and 7.

Table 6: Test And Control Totals And Calculation Of The Primary Irritation Score

Rabbit # 38777	60 Minutes				24 Hour				48 Hour				72 Hour				Total Scores	Primary Irritation Score
	Left		Right		Left		Right		Left		Right		Left		Right			
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED		
TEST Scores	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CONTROL Scores	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Rabbit # 38778	60 Minutes				24 Hour				48 Hour				72 Hour				Total Scores	Primary Irritation Score
	Left		Right		Left		Right		Left		Right		Left		Right			
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED		
TEST Scores	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CONTROL Scores	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Rabbit # 38779	60 Minutes				24 Hour				48 Hour				72 Hour				Total Scores	Primary Irritation Score
	Left		Right		Left		Right		Left		Right		Left		Right			
	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED		
TEST Scores	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CONTROL Scores	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 7: Calculation of the Primary Irritation Index and Final Result

Rabbit #	Primary Irritation Scores		Irritation Response Category
	Test	Control	
38777	0	0	0 to 0.4 -------- **Negligible**
38778	0	0	
38779	0	0	
Total	0	0	
Primary Irritation Index (PII) (Total Test – Total Control) / 3	0		

ANALYSIS AND CONCLUSION

The test was considered valid as the test article remained in contact with skin for a minimum four hour exposure. Based on the PII for the test article, the irritation response was negligible. Under the conditions of this protocol, the test article is considered a **negligible irritant**.

REFERENCES

Maibach, H.I., Zhai, H., Dermatoxicology 6th Edition, pp. 183-189, CRC Press LLC, Boca Raton, FL, 2004.

US EPA – Office of Prevention, Pesticides, and Toxic Substances, (OPPTS), Health Effects Guideline, OPPTS 870.200 Acute Dermal Toxicity.

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, Placing Animal Orders and Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0112, Animal Identification

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

WuXi AppTec SOP: ILS-0456, Positive Control of the Primary Skin Irritation Test

WuXi AppTec SOP: ILS-0490, Primary Skin Irritation

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

Copy of Original

Test Request Form, and Protocol

WuXi AppTec

Biocompatibility
TEST REQUEST FORM

FOR WUXI APPTEC USE ONLY: D9282-1

P.O. NUMBER: KP520424

QUOTE NUMBER (QUO - ##### - ###### - #): QUO - 15997 - SIL8W6 - 0

Complete this form and include it with your test article shipment. Ship to:
WuXi AppTec • 2540 Executive Drive • St. Paul, MN 55120
(1) 651.675.2000 TOLL FREE 888.794.0077

CLIENT INFORMATION

ACCOUNT NUMBER (Required): 4003497

COMPANY NAME • STREET ADDRESS • CITY / STATE / ZIP • COUNTRY	CONTACT NAME	
Klarity Medical Products, LLC 1987 Coffman Road Newark Ohio 43055	Peter Larson	BBJ 6-8-15
	PHONE 740-788-8107 ext 111	EMAIL peter@klaritymedica.com ② medical

REQUESTED TESTING

TEST CODE	TEST NAME
140150.1	ISO Agarose Overlay Using L-929 Mouse Fibroblast Cells (GLP)

Protocol Version #: 18 • Effective Date: 12/17/2013

TEST CODE	TEST NAME
900899.1	Buehler Dermal Sensitization - Repeated Patch (GLP)

Protocol Version #: 17 • Effective Date: 06/20/2013

TEST CODE	TEST NAME
910699.1	ISO Primary Skin Irritation (GLP) – 4 hours ①

Protocol Version #: 18 • Effective Date: 07/15/2013

☐ *Check here if more space is needed to list requested testing. An additional page – with space for continued test listings – will be added.*

Form ALO-0000-1.15 WUXI-3177063916 • Page 1 of 4 Effective Date: 06.01.15

① per sponsor JN 6/10/15
② SP tes 6/8/15

TEST ARTICLE INFORMATION	QUANTITY OF TEST ARTICLES SUBMITTED:	CLICK HERE for Sample Requirements Guide

TEST ARTICLE NAME (As to be described on the final report)
Klarity AccuCushion R550-M

LOT NUMBER: SD1062	EXPIRATION DATE:
☐ Various (LIST ATTACHED) ☐ N.A. *Limit of 25 characters for lot number. To provide unique identifiers or other information that needs to be specified in test article information for the final report, enter in "Test Article Name" space above.*	5/6/18

PHYSICAL DESCRIPTION (As to be described on the final report)
Moldable pillow with nylon fabric surface

INTENDED USE / APPLICATION
Patient stabilization for Radiation therapy treatments

PHYSICAL STATE	SAFETY PRECAUTIONS	CONTROLLED STORAGE CONDITIONS
Insoluble	None/Unknown	Room Temperature

STERILITY			
Select one of the three options shown.	☐ Test article submitted sterile. Indicate sterilization method: Not Applicable	☐ Test article is not sterile. WuXi AppTec to expose to: Not Applicable *Additional fees apply.*	☑ Test article is not sterile. To be tested non-sterile.

For GLP studies (required by US FDA)
TEST ARTICLE CHARACTERIZATION

(1) ☒ Sponsor affirms test article has been characterized or that characterization testing is planned.

Sponsor is solely responsible for all test article characterization data as required in Good Laboratory Practices (GLP) regulations (21CFR58) – identity, strength, stability, purity, and chemical composition. Sponsor is also responsible for ensuring that test article here submitted is representative of the final product that will be subjected to materials characterization.

TEST ARTICLE DISPOSITION	Courier and account # for shipping: (Required for return)
(NOTE: Additional fee may apply for return.)	

(1) per SPONSOR SN 6/10/15

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TEST ARTICLE HANDLING & PREPARATON

Preparation Requirements / Instructions ☐ N.A.	☐ Do NOT cut test article.	Is test article absorbent?
NOTE: Entire article will be tested unless information is provided here as to material/components to be included/excluded for testing.	*Unless box is checked, test article may be cut into sizes needed for testing*	Unknown
remove plastic packaging (bag) before testing. Surface to be tested is the nylon fabric which will come into contact with patients skin during use.	*NOTE: Hemocompatibility testing typically requires the test article to be cut*	

EXTRACTION PARAMETERS ☐ N.A.

If requested tests require extraction, this entire section must be completed by Sponsor. For assistance, contact your Project Manager.

NS = Normal Saline SO = Sesame Oil AE = Alcohol Saline PEG = Polyethylene Glycol PBS = Phosphate Buffered Saline DMSO = Dimethyl Sulfoxide

TYPE OF EXTRACT	EXTRACTION RATIO	EXTRACTION CONDITIONS
Cytotoxicity: Culture Media Sensitization • Irritation • Acute Systemic Toxicity • Mouse Micronucleus: Not Applicable LLNA • Ames • Mouse Lymphoma • Chromosomal Aberration: Not Applicable *Select "NS & PEG" for materials incompatible with DMSO. Select "NS & DMSO or NS & PEG" if DMSO compatibility unknown. WuXi AppTec to determine extract.* Subacute/Subchronic Toxicity: Not Applicable	*Per ISO 10993-12, a surface area ratio should be used whenever possible rather than a weight ratio. The "surface area" includes the combined areas of all sides of the test article and excludes indeterminate surface irregularities.*	Cytotoxicity: 37°C / 24 hrs *All other extraction testing:* ***Conditions recommended for most devices: 50°C / 72 hrs*** Not Applicable *Extraction conditions are based on an exaggeration of product use. For insoluble materials, use highest temperature possible without causing degradation of material.*

FOR HEMOCOMPATIBILITY TESTS *(incl. Hemolysis, PTT, P&L Counts, IVT Hemo, PT and Complement Activation)* ☐ N.A.

☐ Test blood-contacting portions ONLY (per ISO 10993-4) *NOTE: Entire article will be tested unless box is checked.* **Specify blood-contacting components/materials to be tested:**	PTT (Partial Thromboplastin Time): **RATIO: 4cm² / 1mL** Platelet & Leukocyte Count and IVT Hemocompatibility: **RATIO: 12cm² / 1mL** Complement Activation and PT (Prothrombin Time): **RATIO:** Not Applicable	ASTM Hemolysis **TYPE OF EXTRACT: PBS** **RATIO:** Not Applicable **CONDITIONS** Direct Contact: 37°C / 3 hrs Extract Method: Not Applicable

FOR MHLW (Japan) TESTS ☐ N.A.

Extraction parameters for MHLW tests are the same as for ISO/ASTM except for the specific tests described here.

MHLW Genotoxicity and Sensitization *These tests are performed using exhaustive extraction method in methanol/acetone with terminal evaporation.*	MHLW Cytotoxicity RATIO: Not Applicable	MHLW Hemolysis CONDITIONS: Not Applicable

☐ Check here if comparison/control article is being submitted. *(If checked, you will see an additional section that must be completed.)*

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COMMENTS

May be disposed of with normal household waste.

Sponsor signature is required before testing will be initiated.
Services requested in this form will be governed in accordance with WuXi AppTec's "Standard Terms and Conditions." To the extent WuXi AppTec's Standard Terms and Conditions are in conflict with an applicable agreement (Agreement) between Customer listed in this form and WuXi AppTec, such Agreement will govern.

Print / Save as PDF

To save an electronic copy of this completed form, select Adobe PDF Writer or Microsoft XPS Document Writer in your print dialog.

Always print a hard copy to ship with your samples.

TESTING AUTHORIZATION & PROTOCOL APPROVAL

2015.06.02 09:40:08 -05'00'	Peter M. Larson	6/1/15
SIGNATURE	PRINT NAME	DATE

For any test being conducted GLP:
By signing above, you acknowledge that you have reviewed the most current version of the protocol(s) listed on this form and your signature constitutes approval of the protocol(s). If you would like to review any or all of the protocols, click here to email WuXi AppTec and indicate the protocol(s) you want to review.

Initiated in Starlims by Study Director on 6/1/15. KES 6/1/15

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(For Laboratory Use Only)

WuXi AppTec Study # D9282-1

WuXi AppTec

PROTOCOL TITLE: **Primary Skin Irritation**

TEST CODE: 910699

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

EFFECTIVE DATE: 15 July 2013

GLP PROTOCOL: 910699-18

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

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1.0 PURPOSE

This test is designed to determine the dermal irritation potential of the test article on the shaved skin of the rabbit as required by regulation of medical device biocompatibility.

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

3.0 SCHEDULING AND DISCLAIMER

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 3 - 7 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 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.

3.3 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.

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.

4.0 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.

5.0 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 are 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.

6.0 SAFETY

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

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7.0 EXPERIMENTAL DESIGN

7.1 Experimental Overview

The objective of this test is to determine the dermal irritation potential of the test article on the shaved skin of the rabbit as required by regulation of medical device biocompatibility. This study will be conducted in accordance with the International Organization of Standardization: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitization.

The Sponsor-supplied test article will be applied to the shaved skin of three adult albino rabbits. After the predetermined exposure period, the patches will be removed. The observations for skin irritation will be conducted at 60 ± 6 minutes after unwrapping, and at 24 ± 2, 48 ± 2, and 72 ± 2 hours. A comparative control or control vehicle will be run concurrently with the test article or test extract. The test will be evaluated by an overall collection and interpretation of the data, using the dermal scores as reference marks, and based on the nature and reversibility of the skin lesions.

The sensitivity of the assay will be demonstrated semi-annually through positive control validation using the primary skin irritation test method. A suitable positive control will be utilized, such as sodium laurel sulfate (SLS).

7.2 Justification For Selection Of The Test System

The New Zealand albino rabbit is the standard laboratory animal used for skin irritant evaluation. Rabbits are used as they are readily available and because of the existing historical data base for comparative evaluation.

7.3 Institutional Animal Care and Use Committee (IACUC)

This protocol was reviewed and approved by WuXi AppTec's IACUC in compliance with the Animal Welfare Act.

IACUC Protocol / Effective Date: 07-123C / June, 2013

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 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.

7.4 Amendments/Deviations

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.

8.0 IDENTIFICATION OF THE TEST SYSTEM

8.1 Species/Strain: All of the animals to be used in this testing will be albino rabbits *(Oryctolagus cuniculus)*, New Zealand White strain.

8.2 Source: Animals will be obtained from a previously approved vendor of commercial laboratory animals.

8.3 Sex: Either males or females may be used for this study. Any females used will be nulliparous and non-pregnant.

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8.4 **Weight Range:** Each selected rabbit will weigh at least 2.0 kilograms at the start of the study.

8.5 **Age:** No specific age is required for this study.

8.6 **Number:** Three animals will be used for this study.

8.7 **Animal Identification:** Individual animals will be identified per WuXi AppTec SOP: ILS-0112.

8.8 **Husbandry**

8.8.1 **Receipt And Acclimation**
Receipt will be according to WuXi AppTec SOP: ILS-0092. The animals will be acclimated for a minimum of 5 days, under the same conditions as the actual test.

8.8.2 **Housing**
Rabbits will be housed individually in suspended stainless steel cages. Housing dimensions will comply with NIH and AAALAC International guidelines for this species.

8.8.3 **Environment**
The environmental conditions in the animal rooms will be set to be maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photoperiod will meet the AAALAC International recommendations for this species. The laboratory and animal rooms will be maintained as limited-access facilities.

8.8.4 **Feed**
Animals will be supplied with certified commercial feed. There are no known contaminants present in the feed expected to interfere with the test results. Analytical results of the feed will be archived at WuXi AppTec.

8.8.5 **Water**
Animals will be supplied with potable water from the local municipal water supply, *ad libitum*. There are no known contaminants present in the water expected to interfere with the test results. Periodic analysis of the water is conducted and the results will be archived at WuXi AppTec.

9.0 TEST METHOD

9.1 **Selection Of Animals**
Animals will be selected at random from a larger pool of animals. Selection criteria will be based on the required weight range of this study and the condition and suitability of the animal skin, as observed after shaving. Each animal is observed for any signs of clinical disease prior to introduction into the study.

9.2 **Positive Controls**
WuXi AppTec performs positive control testing a minimum of every six months. The methods for the positive control assay are identical to the methods described above in the experimental design summary. Rabbits utilized for positive control studies are of the New Zealand White strain and will be supplied by the same vendor as animals used for general testing. Sodium laurel sulfate, a well-known irritant, will be used as the positive control substance. Results for the applicable positive control study will appear in the final report for this study.

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9.3 Test Article Preparation

9.3.1 Test Article Prepared As A Liquid, Gel, Or Powder

The liquid test article will be applied directly to the skin of the rabbit. Appropriate liquid controls will be run concurrently with the test article. The gel or powder test article will be applied directly to the skin of the rabbit. Powders may be moistened with an appropriate solvent prior to application if the Sponsor requests.

Controls using the same solvent will be run concurrently with the moistened powders. If not specified, tap water will be used as the control. Gel and non-moistened powder test articles will be moistened with tap water or an appropriate solvent prior to placement to ensure good contact with the skin of the animal.

9.3.2 Test Article Prepared As A Solid

Solid materials, which have appropriate physical states, such as sheets or films, shall be tested without modification. The test patches will be prepared by cutting the sample into 2.5 cm x 2.5 cm squares. Suitable negative control samples will be prepared in the same way. Absorbent gauze may be used as a substitute if a more suitable control was not identified.

The test and control article will be placed directly onto a non-occlusive dressing, such as hypoallergenic tape, for application to the skin. The test and control articles will be moistened with tap water prior application to ensure good contact with the skin of the animal.

9.3.3 Test Article Prepared As An Extract

A solid may also be tested by preparing an extract from the solid. The Sponsor supplied test article will be prepared and extracted as indicated on the WuXi AppTec test request form attached to this protocol and according to WuXi AppTec SOPs: TRG-0300 and ALS-0260 (see Table 1).

Table 1: Standard Surface Areas and Extract Liquid Volumes

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 to 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/mL
Membranes, textiles	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, a suggested protocol is as follows: - 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.		

The extractions will be stored at room temperature and used within 24 hours of preparation. A blank control sample, using the extracting solvent, will be run concurrently with the test extract.

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9.3.4 pH Measurement Prior To Dosing

If applicable, the pH of the test article will be measured prior to testing. Test articles with a pH of less than or equal to '2' or greater than or equal to '11.5' will not be tested.

In exceptional cases, where further risk characterization / assessment is needed, it might be necessary to test materials which are either an irritant or have a pH outside the range mentioned above. These cases will be justified and documented.

9.4 Test Article Administration

9.4.1 Test Animal Preparation

The fur from the back of each test rabbit will be removed by clipping with an electric shaver and an appropriate blade, 4 – 24 hours before the test. Abrading the skin will be avoided when shaving and only animals with healthy intact skin will be used for testing.

9.4.2 Patch Application And Removal

a. After the animals have been properly restrained, two test patches, wet with tap water if applicable (see Section 9.3 for details), will be placed on the shaved back of each animal on each side of the vertebral column.

a.1 If the test article is a liquid, powder or gel, 0.5 g or 0.5 mL will be applied directly to the skin and covered with 2.5 cm x 2.5 cm non-occlusive dressing, such as absorbent gauze and backed with hypoallergenic tape. The test article may also be applied to absorbent gauze and applied directly to the skin and backed with hypoallergenic tape.

a.2 If the test article is prepared as an extract, 0.5 mL will be placed onto a 2.5 cm x 2.5 cm non-occlusive dressing, such as absorbent gauze, and backed with hypoallergenic tape. See Table 2 for additional instructions for differing physical states.

Table 2: Preparation Instructions

Physical State	Handling	Preparation Instruction
Liquid	Neat or Dilute	measure 0.5 mL for each dose
Aerosol	Neat or Dilute	collect in a container and measure 0.5 mL for each dose
Gel - Semi-solid	Neat or Dilute	measure 0.5 mL or 0.5 g for each dose as applicable
Powder	Neat or Dilute	measure 0.5 g for each dose moisten with water or suitable vehicle
Moldable Solid	Neat or Extract	measure 0.5 mL of extract or 0.5 g or 6 cm^2 for each dose moisten with water or suitable vehicle
Formed Solid	Neat or Extract	measure 0.5 mL of extract or 5 g or 6 cm^2 for each dose moisten with water or suitable vehicle
Other	Other	provide written instructions attached to this protocol

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b. A suitable negative control will be used. If a negative control is not supplied, a 2.5 cm x 2.5 cm square of 2-ply absorbent gauze backed with hypoallergenic tape will be used. The negative control will be wet with tap water if applicable (see section 9.3 for details).

c. The test and control patches will be placed on the clipped dorsal regions of skin using the schematic below. The test/control sites will be marked with a non-toxic permanent ink marker.

Cranial End			KEY
1	|	2	1. Test Sites
2	|	1	2. Control Sites
Caudal End			

Figure 1: Sample Placement Schematic

d. The trunk of each animal will be wrapped with an elastic bandage and the wrap secured with hypoallergenic tape. The access to the patch by the animal and resulting ingestion or inhalation of the test material will be prevented.

e. After a minimum 4 hour exposure, the bandages will be removed and the residual test material wiped from the rabbits skin. The test site will be marked with a non-toxic permanent ink marker.

f. Should the test material require a longer exposure duration please indicate the duration on the WuXi AppTec test request form attached to this protocol.

9.5 Observations

9.5.1 Animals will be observed daily for clinical health and well being.

9.5.2 Detailed dermal observations will be recorded at 60 ± 6 minutes post-unwrapping, and at 24 ± 2, 48 ± 2 and 72 ± 2 hours according to Table 3. If significant observations continue up to 72 ± 2 hours, an extended evaluation period may be conducted to observe the reversibility or irreversibility of the dermal effects. This should last no longer than 14 days.

9.5.3 In addition to the observation of irritation, any lesions and other toxic effects will be fully documented.

Table 3: Dermal Observation Scoring

Erythema	Edema
0 = No erythema 1 = Very slight erythema (barely perceptible) 2 = Well defined erythema 3 = Moderate to severe erythema 4 = Severe erythema (beet redness) to slight eschar formation preventing grading of erythema	0 = No edema 1 = Very slight edema (barely perceptible) 2 = Well-defined edema (edges of area well-defined by definite raising) 3 = Moderate edema (raised approximately 1 mm) 4 = Severe edema (raised > 1 mm and extending beyond exposure area)

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9.6 **Termination**
All animals will be euthanized by lethal injection of a sodium pentobarbital based solution after the final observation period.

10.0 **TEST EVALUATION**
The primary irritation index (PII) will be calculated, using the dermal scores. For any animal with a dermal response, the maximum irritation response will be determined as well as the time of the initial response and time of maximum response.

Only the 24 ± 2, 48 ± 2, and 72 ± 2 hour observation periods will be used for the calculations. After the 72 ± 2 hour grading, all erythema grades plus edema grades 24 ± 2, 48 ± 2, and 72 ± 2 hour are totaled separately for each test sample and control for each animal. The primary irritation score for an animal is calculated by dividing the sum of all the scores by 6 (2 test/observation sites x 3 time points). The primary irritation index is obtained for the test sample by the addition of the all the primary irritation scores of the individual animals and by dividing by the number of animals.

The primary irritation scores for the control sites will be subtracted from the total primary irritation scores of the test sites and this value will be divided by the total number of animals., yielding the primary irritation index. This number is used to categorize the test article response with Table 4.

Table 4: Primary Irritation Response Categories in the Rabbit

Response Category	Primary Irritation Index (PII)
Negligible	> 0 to 0.4
Slight	0.5 to 1.9
Moderate	2 to 4.9
Severe	5 to 8

Note- The Primary Irritation Index (PII) is determined by subtracting the Total Primary Irritation Score of the control sites from the Total Primary Irritation Score of the test sites and dividing that value by the total number of animals used in the study.

A test will be repeated in part or in total if a control failure occurs. A control failure occurs if there are erythema or edema scores greater than 2.

11.0 **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. The study will be considered valid if all test subjects survive the test period and all patches remain in place for the duration of the wrapped period.

12.0 **METHOD FOR CONTROL OF BIAS:** Not applicable.

13.0 **DATA ANALYSIS:** Not applicable.

14.0 **STATISTICAL METHODS:** None used.

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, a description of the methods used and conclusion as it relates to the test.

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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.
- **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** 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.

17.0 COMPLIANCE

17.1 Animal Welfare

WuXi AppTec, Inc. maintains the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accreditation. All applicable portions of the study will also conform to the following regulations and guidelines regarding animal care and welfare:

- **17.1.1** NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council of the National Academies, eighth edition, 2011;
- **17.1.2** (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;
- **17.1.3** USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, *Animal Welfare*, Final Rule 1989; and
- **17.1.4** WuXi AppTec Policy on Humane Care.

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

17.3 GLP Status
The study will be conducted 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 AppTec Quality Assurance unit.

17.4 International Standards

17.4.1 ISO 10993-10: 2010 Biological Evaluation of Medical Devices, Part 10-Tests for Irritation and Skin Sensitization. pp. 7-11, 27, 28.

17.4.2 ISO 10993-12:2012 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

18.0 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 the Sponsor.

19.0 REFERENCES

19.1 Maibach, H.I., Zhai, H., Dermatoxicology 6th Edition, pp. 183-189, CRC Press LLC, Boca Raton, FL, 2004.

19.2 US EPA – Office of Prevention, Pesticides, and Toxic Substances, (OPPTS), Health Effects Guideline, OPPTS 870.200 Acute Dermal Toxicity.

19.3 WuXi AppTec SOP: ALS-0260, Sample Extraction Procedures

19.4 WuXi AppTec Reference Library Contents, Form ALS-4650-1

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

19.6 WuXi AppTec SOP: ILS-0092, Placing Animal Orders and Receiving Shipments of Animals

19.7 WuXi AppTec SOP: ILS-0112, Animal Identification

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

19.9 WuXi AppTec SOP: ILS-0456, Positive Control of the Primary Skin Irritation Test

19.10 WuXi AppTec SOP: ILS-0490, Primary Skin Irritation

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

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20.0 VERSION CHANGE SUMMARY – FROM Version 910699-17 to 910699-18

20.1 Section 9.4 was updated to reflect the current test article application procedure.

20.2 Section 19.9 was added to reference WuXi AppTec's Primary Skin Positive Control SOP.

20.3 Section 9.2 added to reference the Positive Controls for the Primary Skin Irritation Test.

20.4 Section 9.3.3 was updated.

20.5 Table 3 referenced correctly in Sections 9.3.2, 9.3.2 b, and 9.4.2

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