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Skin Sensitization Test

Guinea Pig Maximization

Final Report

Verification

Report Number: CSTBB20080830

Article Name: Disposable Medical Protective Gown

Method Standard: ISO 10993-10: 2010

Sponsor

Guangzhou Zhengkang Medical Equipment Co.,Ltd.

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Notices

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Abstract

In this study, we took guinea pigs to observe the skin sensitization of the test article according to ISO 10993-10: 2010.

The test article were extracted in Constant Temperature Vibrator at 50 ℃, 60 rpm for 72 h by 0.9 % Sodium Chloride Injection and Sesame Oil. Mix 50:50 (by volume) stable emulsion of Freund's complete adjuvant with selected solvent. Intradermal induction and topical induction were operated in the clipped intrascapular region of each animal. After the topical induction phase was completed on day 14, all test and control animals were challenged with the test sample. The erythema and edema of the challenge site were observed to test the sensitization response of the test article. According to the Magnusson and Kligman scales, the response to erythema and edema at each application site of the skin was described and scored 24 hours and 48 hours after the challenge phase.

The results showed that the guinea pigs in the negative control group (0.9 % Sodium Chloride Injection, Sesame Oil) retained a normal appearance throughout the test and showed no skin irritants. A severe skin reactions for erythema and oedema were shown in the positive control group (DNCB). While in test article group, the response of skin on testing side did not exceed that on the control side. The skin reactions for erythema and oedema were not observed in test article group. The data of each group met the acceptance criteria, and the results of this test were considered valid.

Based on the above results, it can be concluded that under the experimental conditions, the test article has no potential skin sensitization on guinea pigs in the extraction method.

Study Verification and Signature

Protocol Number	SST2008023003BB
Protocol Effective Date	2020-08-24
Technical Initiation Date	2020-08-28
Technical Completion Date	2020-09-25
Final Report Completion Date	2020-10-13

Personnel Betty 2020-10-13
Date Completed

Approved
Study Director
Date Completed

Supervisory
Test Facility Manager
Date Completed

CCIC Huatongwei international inspection (Suzhou) Co., Ltd.

Quality Assurance Statement and GLP Statement

Quality Assurance Statement

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the HTW's Management.

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase Inspected	Date	Study Director	Management
Experiment	2020-08-28	2020-08-28	2020-08-28
Raw Data	2020-09-25	2020-09-25	2020-09-25
Final Report	2020-10-13	2020-10-13	2020-10-13

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

Hui Tang — 2020-10-13

Quality Assurance — Date

GLP Statement

This study was conducted in compliance with current U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

The sections of the regulations not performed by or under the direction of HTW, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Part 58.105 and 58.113.

Z. — 2020-10-13

Study Director — Date

1.0 Purpose

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

2.0 Reference

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

Biological evaluation of medical devices-Part 12: Sample preparation and reference materials (ISO 10993-12:2012)

Biological evaluation of medical devices-Part 2: Animal welfare requirements (ISO 10993-2:2006)

3.0 Test and control articles

Groups	Test article	Negative Control Article(Polar)	Negative Control Article(Non-Polar)	Positive Control
Name	Disposable Medical Protective Gown	Sodium Chloride Injection (SC)	Sesame Oil (SO)	2, 4-Dinitrochlorobenzene (DNCB)
Manufacture	Guangzhou Zhengkang Medical Equipment Co.,Ltd.	Shijiazhuang No.4 Pharmaceutical	Ji'an Lv yuan natural flavor oil refinery, Qingyuan District	TOKYO CHEMICAL INDUSTRY CO., LTD
Size	M	500 ml	5L	25 g
Model	ZKF-01	/	/	/
Lot Batch#	2020080503	1912121907	20200528	H2UKD-DM
Test Article Material	non-woven fabric	/	/	/
Physical State	Solid	Liquid	Liquid	Solid
Color	White	Colorless	Light yellow	Light yellow
Package material	polybag	/	/	/
Sterilized or Not	No	/	/	/
Concentration	/	0.9 %	/	Induction Concentration: 0.5 % Challenge Concentration: 0.1 % Dissolved in ethanol
Total Surface/Weight	Not provided	/	/	/

Storage Condition	Room Tep.	Room Tep.	Room Tep.	Room Tep.
The information about the test article was supplied by the sponsor wherever applicable.				

4.0 Identification of test system

4.1 Test animal

Species: Hartley Guinea Pig (Cavia Porcellus)

Number: 30 (20 Test +10 Control)

Sex: 15 ♀， 15 ♂

Initial body weight: 300.0~500.0 g

Health status: Healthy, not previously used in other experimental procedures. Female animals were nul liparous and not pregnant.

Animal identification: Ear tag

Cages: Plastic cage

Acclimation Period: 7 days under the same conditions as for the actual test

4.2 Justification of test system

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1 970). The guinea pig is believed to be the most sensitive animal model for this type of study. DNCB is the positive control article recommended in the test instructions. To ensure the sensitivity of the experime ntal system, the positive control article should be verified every three months.

5.0 Animal Management

Animal purchase: Wuxi hengtai experimental animal breeding co. LTD SCXK (SU) 2020-0003

Bedding: Corncob Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd.

Feed: Guinea pigs were fed with full-price pellets Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd.

Water: Drinking water met the Standards for Drinking Water Quality GB 5749-2006

Animal room temperature: 18-26 ℃

Animal room relative humidity: 30 %-70 %

Lights: 12 hours light/dark cycle, full-spectrum lighting

Personnel: Associates involved were appropriately qualified and trained

Selection: Only healthy, previously unused animals were selected

There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

6.0 Equipment and reagents

6.1 Instruments

Constant Temperature Vibrator (SHB007, calibration data: 2020/3/16), Autoclave (SHB026, calibration data: 2020/3/16), Electronic scale (SHB017, calibration data: 2020/3/16)

6.2 Reagents

Freund’s adjuvant Complete liquid (SIGMA, Lot No: SLCD4457), Sodium dodecyl sulfate (SDS SIGMA, Lot No: SLBL2304V)

7.0 Experiment design

7.1 Sample preparation

The extracts of test article will be prepared according to the following steps:

Aseptic Sampling			Extraction in sterile vessels				
Sampling Manner	Actually sampling	Ratio	Reagent		Temperature	Time	pH
Random	120.0 cm^2	6 cm^2: 1 ml	SC	20.0 ml	50 ℃	72 h	5.5
	120.0 cm^2		SO	20.0 ml			/

Both inducements and excitations were prepared by the number of times. The state of the leaching solution did not change visually after the leaching was advanced. After extraction, the samples were stored at room temperature for no more than 24 h. The extraction solution is clear, and the pH value has not been adjusted, filtered, centrifuged, diluted and other processes. The control solution was prepared under the same conditions.

7.2 Test method

7.2.1 Intradermal induction phaseI

A pair of 0.1ml intradermal injections was made for each of the following, into each animal, at the injection sites (A, B and C) as shown in Figure 1 in the clipped intrascapular region.

Site A: A 50:50 (volume ratio) stable emulsion of Freund's complete adjuvant mixed with the chosen solvent.

Site B: The test sample (undiluted extract); the control animals were injected with the solvent alone.

Site C: The test sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50 %); the control animals were injected with an emulsion of the blank liquid with adjuvant.

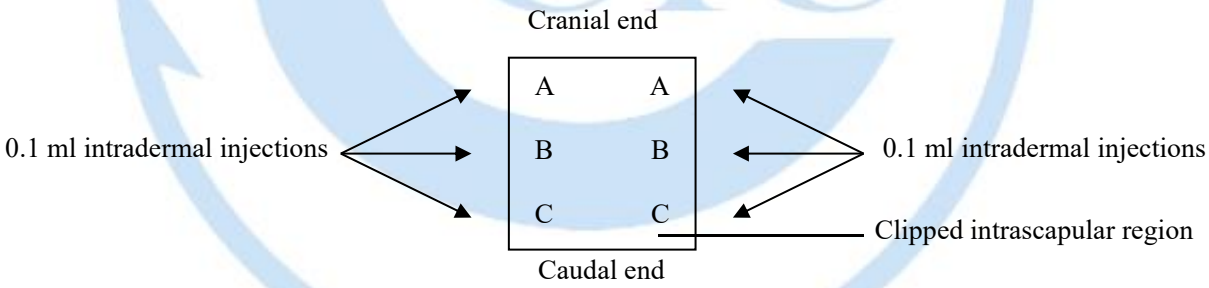

Figure 1 Location of intradermal injection sites

7.2.2 Topical induction phaseII

The maximum concentration that can be achieved in Intradermal induction phase Ⅰ did not produce irritation, animals are pretreated with 10% sodium dodecyl sulfate 24(±2) hours before the topical induction application.

At 7 d after completion of the intradermal induction phase, administer test article extract by topical application to the intrascapular region of each animal, using a patch of area approximately 8 cm^2 (absorbent gauze), so as to cover the intradermal injection sites. Secure the patches with an occlusive dressing. Remove the dressings and patches after (48±2) h.

Treat the control animals similarly, using the blank liquid alone.

7.2.3 Challenge phase

At 14d after completion of the topical induction phase, challenge all test and control animals with the test

sample. Absorbent gauzes (2.5 cmx2.5 cm) were soaked respectively with test article and control article. Apply the test article extract and control article topically to two sites that were not treated during the induction stage. Secure with an occlusive dressing. Remove the dressings and patches after (24±2) h.

8.0 The results observed

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 0.9 % saline 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. If necessary, the fur will be shaved or clipped in advance for the convenience of dermal score.

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

Table 1 Magnusson and Kligman scale

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

9.0 Evaluation criteria

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animals.

If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization.

If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge.

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

10.0 Results of the test

All animals were survived and no abnormal signs were observed during the study.Individual results of dermal scoring for the challenge appear in Table 2.

11.0 Conclusion

The test article showed no evidence of causing delayed dermal contact sensitization in the guinea pig. Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor’s responsibility.

12.0 Compliance

US FDA Good Laboratory Practice Regulations 21 CFR 58, effective June 20, 1979, as amended 52 FR 33780, Sept. 4, 1987, and subsequent amendments

Standard operating procedure of CCIC Huatongwei international inspection (Suzhou) Co., Ltd.

13.0 Record

All raw data pertaining to this study and a copy of the final report are retained in designated Huatongwei archive.

14.0 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

Table 2 Guinea pig Sensitization Dermal Reactions

Group		No.	Pretest weight(g)	Finished weight (g)	The Challenge patch was removed 24h later		The Challenge patch was removed 48h later		Positive rate
					Erythema	Swelling	Erythema	Swelling	
SC	Test	1	313.5	377.1	0	0	0	0	0%
		2	312.1	362.7	0	0	0	0	
		3	304.0	380.6	0	0	0	0	
		4	302.7	351.0	0	0	0	0	
		5	304.4	384.5	0	0	0	0	
		6	310.4	384.6	0	0	0	0	
		7	313.0	354.2	0	0	0	0	
		8	303.6	363.3	0	0	0	0	
		9	302.9	352.1	0	0	0	0	
		10	312.2	367.2	0	0	0	0	
	Control	11	311.7	351.8	0	0	0	0	—
		12	308.1	358.5	0	0	0	0	
		13	309.3	380.7	0	0	0	0	
		14	303.3	378.4	0	0	0	0	
		15	312.4	378.8	0	0	0	0	
SO	Test	16	311.1	372.2	0	0	0	0	0%
		17	309.9	369.4	0	0	0	0	
		18	314.3	384.2	0	0	0	0	
		19	307.9	353.3	0	0	0	0	
		20	317.2	369.1	0	0	0	0	
		21	304.1	363.5	0	0	0	0	
		22	303.3	356.5	0	0	0	0	
		23	309.4	354.2	0	0	0	0	
		24	316.4	363.0	0	0	0	0	
		25	310.3	379.7	0	0	0	0	
	Control	26	317.8	366.1	0	0	0	0	—
		27	302.5	384.6	0	0	0	0	
		28	316.7	379.7	0	0	0	0	
		29	302.4	379.0	0	0	0	0	
		30	313.5	357.8	0	0	0	0	

Table 3 Positive control

Group	No.	Pretest weight(g)	Finished weight (g)	The Challenge patch was removed 24 h later		The Challenge patch was removed 48 h later		Positive rate
				Erythem	Swelling	Erythema	Swelling	
Test	1	309.8	354.0	1	0	1	0	100%
	2	307.2	352.1	2	0	2	0	
	3	306.3	360.2	1	0	1	0	
	4	314.1	382.9	1	0	1	0	
	5	307.1	351.0	1	0	2	0	
	6	318.7	352.9	1	0	2	0	
	7	312.1	374.0	1	0	1	0	
	8	310.4	358.6	1	0	1	0	
	9	303.3	366.1	2	0	2	0	
	10	308.7	354.2	1	0	2	0	
Control	11	312.9	353.0	0	0	0	0	—
	12	307.7	359.0	0	0	0	0	
	13	303.7	353.7	0	0	0	0	
	14	307.9	372.3	0	0	0	0	
	15	310.8	380.9	0	0	0	0	

Note： The positive control was CSTBB20080001P1 (Finish date： 2020-09-11)