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Skin Sensitization Test

Guinea Pig Maximization

Final Report

Verification

Report Number: CSTBB21031167

Article Name: Medical Clean Paper Wiper

Method Standard: ISO 10993-10: 2010

Sponsor

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Abstract

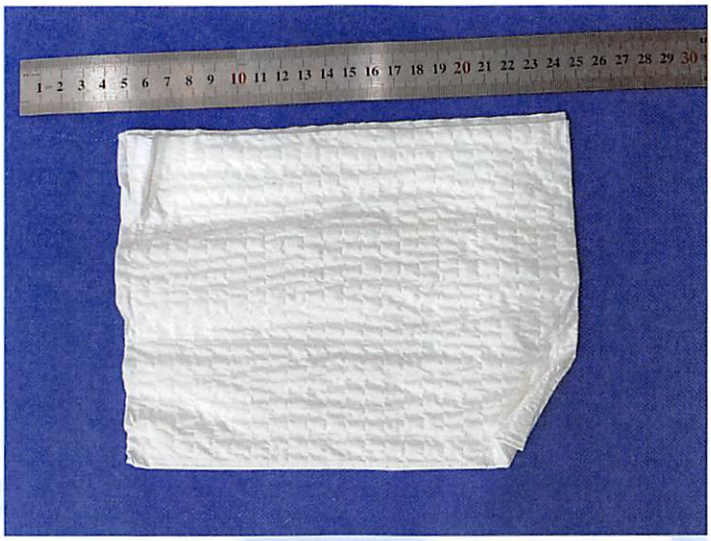
In this study, we took guinea pigs to observe the skin sensitization of the test article according to ISO 10993-10: 2010.

The test article were extracted in Constant Temperature Vibrator at 37 ℃, 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



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Personnel — Betty — 2021-05-17 (Date Completed)

Approved — Study Director — 2021-05-17 (Date Completed)

Supervisory — Test Facility Manager — 2021-05-17 (Date Completed)

CCIC Huatongwei International Inspection (Suzhou) Co., Ltd.

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 2: Animal welfare requirements (ISO 10993-2:2006)

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 materialsy (ISO 10993-12:2021)

3.0 Test and control articles

Groups	Test article	Negative Control Article(Polar)	Negative Control Article(Non-Polar)	Positive Control
Name	Medical Clean Paper Wiper	0.9% Sodium Chloride Injection(SC)	Sesame Oil (SO)	2, 4-Dinitrochlorobenzene (DNCB)
Manufacturer	suzhou Virgil medical Technology Co.,Ltd	Guangxi Yuyuan Pharmaceutical Co., Ltd	Ji'an Lv yuan natural flavor oil refinery, Qingyuan District	TOKYO CHEMICAL INDUSTRY CO., LTD
Size	30*40cm	500 ml	5L	25 g
Model	/	/	/	/
Lot Batch#	20210302	H20120305	20200528	H2UKD-DM
Test Article Material	Paper	/	/	/
Physical State	Solid	Liquid	Liquid	Solid
Color	White	Colorless	Light yellow	Light yellow
Package material	carton	/	/	/
Sterilized or Not	No	/	/	/
Concentration	/	0.9 %	/	Induction Concentration: 0.1 % Challenge Concentration: 0.05 % Dissolved in ethanol

Total Surface/Weight	Not provided	/	/	/
Storage Condition	Room Temp.	Room Temp.	Room Temp.	Room Temp.
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: either sex

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

Shaking water bath (SHB006, calibration data: 2021/03/11), Electronic scale (SHB017, calibration data: 2021/03/11)

6.2 Reagents

Freund’s adjuvant Complete liquid (SIGMA, Lot No: SLCC3348), Sodium dodecyl sulfate (Solarbio, Lot No: 1019Y032)

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
Intradermal induction phase I	Random	2.0 g	0.1 g: 1 ml	SC	20.0 ml	37 ℃	72 h	5.5
		2.0 g		SO	20.0 ml			/
Topical induction phaseII	Random	2.0 g	0.1 g: 1 ml	SC	20.0 ml	37 ℃	72 h	5.5
		2.0 g		SO	20.0 ml			/
Challenge phase	Random	2.0 g	0.1 g: 1 ml	SC	20.0 ml	37 ℃	72 h	5.5
		2.0 g		SO	20.0 ml			/

Both induction and challenge phase extracts were prepared by the number of times. The state of the leaching solution did not change visually after the leaching was advanced.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.The extraction of the test article could be stored at room temperature. for no more than 24 h.

The changes of the leaching solution was observed after extraction. No particulates or color changes were observed in pre- and post-extraction, the color and pH of the extraction solution did not change before and after use,and the pH value was 5.5, the status of the extract was shown in the table below.

Phase	Vehicle	Time Observed	Extracts	Condition of Final Extracts		
				Color	Clear or Not	Particulates
Intradermal induction phase I	Polar	Before Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
		After Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
	Non-Polar	Before Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
		After Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
Topical induction phaseII	Polar	Before Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
		After Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None

	Non-Polar	Before Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
		After Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
Challenge phase	Polar	Before Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
		After Extraction	Test article	Colorless	Clear	None
			Negative Control	Colorless	Clear	None
	Non-Polar	Before Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None
		After Extraction	Test article	Light yellow	Clear	None
			Negative Control	Light yellow	Clear	None

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.

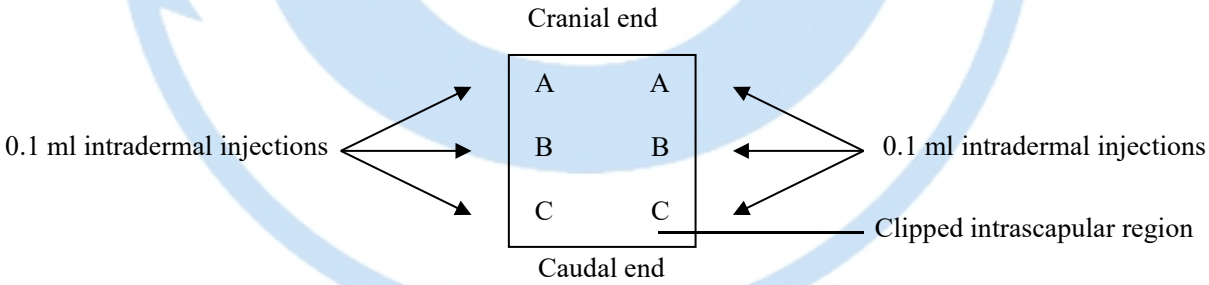

Figure 1 Location of intradermal injection sites

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) h 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. Fourteen days after removal of induction patches, the right and left flank areas of each guinea pig are to be shaved or clipped prior to the test extract for convenience of dermal score.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±2) h and (48±2) h 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 Record

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

13.0 Confidentiality Agreement

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

14.0 Protocol amendment/deviations

There were no amendments or deviations that occurred during the course of this study.

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 article	1	312.2	372.2	0	0	0	0	0%
		2	316.5	374.8	0	0	0	0	
		3	307.4	367.8	0	0	0	0	
		4	309.7	365.0	0	0	0	0	
		5	308.4	363.8	0	0	0	0	
		6	307.7	368.9	0	0	0	0	
		7	313.2	374.8	0	0	0	0	
		8	305.6	362.4	0	0	0	0	
		9	305.5	365.1	0	0	0	0	
		10	306.3	369.7	0	0	0	0	
	Negative Control	11	317.6	376.2	0	0	0	0	0%
		12	318.4	377.5	0	0	0	0	
		13	312.2	374.0	0	0	0	0	
		14	306.3	362.4	0	0	0	0	
		15	302.5	365.5	0	0	0	0	
SO	Test article	16	317.5	377.3	0	0	0	0	0%
		17	313.9	375.0	0	0	0	0	
		18	315.6	376.2	0	0	0	0	
		19	316.7	378.6	0	0	0	0	
		20	303.1	361.5	0	0	0	0	
		21	307.8	366.3	0	0	0	0	
		22	308.9	367.6	0	0	0	0	
		23	318.7	378.3	0	0	0	0	
		24	314.3	375.0	0	0	0	0	
		25	305.4	361.2	0	0	0	0	
	Negative Control	26	312.7	378.3	0	0	0	0	0%
		27	308.2	367.7	0	0	0	0	
		28	309.1	368.3	0	0	0	0	
		29	303.1	364.4	0	0	0	0	
		30	305.0	366.5	0	0	0	0	

Table 3 Positive control

Group	No.	Pretest weigh(g)	Finished weigh(g)	The Challenge patch was removed 24 h later		The Challenge patch was removed 48 h later		Positive rate
				Erythema	Swelling	Erythema	Swelling	
Test	1	307.6	367.8	1	0	1	1	100%
	2	309.6	371.7	1	1	2	1	
	3	311.8	370.6	1	0	1	0	
	4	304.4	362.3	1	1	2	1	
	5	318.3	378.6	1	0	2	1	
	6	306.1	363.0	1	1	1	2	
	7	310.5	369.1	1	1	1	0	
	8	303.2	358.0	2	1	3	2	
	9	305.2	366.9	2	0	1	0	
	10	317.4	382.0	1	0	2	1	
Negative Control	11	316.3	375.1	0	0	0	0	0%
	12	314.8	373.2	0	0	0	0	
	13	307.6	369.7	0	0	0	0	
	14	315.7	378.3	0	0	0	0	
	15	304.6	367.7	0	0	0	0	

Note：The positive control was CSTBB2103001P1 (Finish date：2021-03-26)