

TEST REPORT

NUMBER: 181000805SHA-002

DATE: 16 Mar, 2019

APPLICANT: Hunan Biomaser Technology Co.,LTD.

ADDRESS: No.152, Shenyuan Road, Qingshanpu Town, Changsha, Hunan, China.

SAMPLE DESCRIPTION:

ONE (1) TYPE OF SUBMITTED SAMPLES SAID TO BE:

TEST NAME: Skin Sensitization Test

TEST STANDARD: ISO 10993-10:2010

TEST ARTICLE NAME: PERMANENT MAKEUP NEEDLE

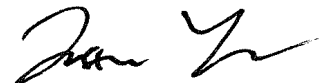
TESTS CONDUCTED:

AS REQUESTED BY THE APPLICANT, THE SUBMITTED SAMPLES WERE
SUBJECTED TO THE SKIN SENSITIZATION TEST
FOR DETAILS REFER TO ATTACHED PAGE(S)

TO BE CONTINUED

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Summary	3
1. Introduction.....	4
2. Material	4
2.1 Sample Identification	4
2.2 Testing Preparation.....	5
3. Test System and Justification	5
3.1 Test System	5
3.2 Justification of Test System	7
3.3 Animal Management	7
4. Method	8
4.1 Induction I	8
4.2 Induction II	9
4.3 Challenge	9
4.4 Observation	10
5. Evaluation and Statistics	10
6. Results.....	10
7. Conclusion	11
8. Quality Assurance	12
9.Proposed Dates.....	12
10. Records.....	12
11. References	12
12.Protocol Changes	12
Appendix 1 Positive Challenge Result.....	13
Attachment 1: Photo of Test Article.....	14

Summary

The test article, Permanent Makeup Needle, Lot: E00280826, was extracted in sodium chloride injection. The extracts were evaluated the potential for delayed dermal contact sensitization based on the requirements of ISO 10993-10:2010 Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.

Each extract was intradermally injected and patched to ten test guinea pigs (per extract) in an attempt to induce sensitization. The vehicle was similarly injected and patched to five control guinea pig (per vehicle). Following a recovery period, the test and control animals received a challenge patch of the appropriate test article extract and reagent control. In addition the test article was applied to the same animals. All sites were scored at 24 and 48 hours after patch removal.

Under the conditions of this study, there is no evidence that the test article extracts would cause delayed dermal contact sensitization on guinea pig.

Authorization for duplication of this report, except in whole, is reserved pending Intertek's written approval.

1. Introduction

Purpose

The test article identified below was extracted and the extracts were evaluated the potential to cause delayed dermal contact sensitization following guinea pig maximization test. This study was conducted based on the requirement of ISO 10993-10: 2010 Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.

Date

The test article was received on August 30, 2018. The extractions were conducted firstly on November 06, 2018. Intradermal induction, topical induction and challenge were done on November 09, 2018, November 17, 2018 and December 01, 2018 respectively. The observations were conducted on December 03 and 04, 2018.

Compliance

The laboratory meets the requirement of international standard ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories. The ANSI-ASQ National Accreditation Board (ANAB) Accreditation Certification No. is AT-1894. The China National Accreditation Service for Conformity Assessment accreditation No. is CNAS L8240.

2. Material

2.1 Sample Identification

The information of test sample was provided by the client, and the test facility is not responsible for its authenticity.

Test Article Name:	Permanent Makeup Needle
Lot:	E00280826
Model:	Not supplied by sponsor
Test Article Identification:	M2018102409
Status:	Packing intact

Stability Testing:	Not supplied by sponsor
Expired Date:	2021/07/08
Strength, Purity and Composition:	Not supplied by sponsor
Physical Description Of The Test Article:	See photo in the attachment
Storage Condition:	Room temperature and avoiding light
Sample Handling:	Disposed by laboratory

2.2 Testing Preparation

Vehicles:	Sodium chloride injection (SC)
Preparation:	Based on a ratio of 0.2 g/mL, 2.5 g of test article was covered with 12.5 mL of the vehicles. The test article was extracted at 37 °C for 72 hours. The extraction vehicles without test article were similarly prepared to serve as control blanks. Extracts were used within 24 h after extraction. The extracts were not processed by filtration, centrifugation or other methods.

Condition of extracts:	<u>SC Test</u>	<u>SC Control</u>
Induction I :	clear	clear
Induction II :	clear	clear
Challenge :	clear	clear
Additional materials:	Freund's Complete Adjuvant (FCA) was mixed with the chosen vehicle and used at induction I and challenge.	
Positive Control:	α -Hexylcinnamaldehyde was selected as positive control.	

3. Test System and Justification

3.1 Test System

The test animal system, equipments and reagent used in this study were identified as following:

Table 1 Table of animal system

Species:	Guinea pig
Breed:	Hartley
Source:	Beijing Jinmuyang Experimental Animal Breeding Co., Ltd.
Sex:	Female, And female animals are nulliparous and non-pregnant.
Body weight range:	310.9 g to 342.5 g at injection
Age:	Young adult
Acclimation period:	Minimum 5 days
Number of animals:	Fifteen
Identification method:	Ear tag

Table 2 Table of Equipments

Equipment	Model number	Identification number	Calibration validity
Pressure steam sterilizer	LDZH-150KBS	SW-YS-383	2019/05/15
Balance	YP50001	SW-YS-186	2019/04/10
Cleaning bench	SJ-CJ-2FD	SW-YS-200	/
Constant temperature vibrator	THZ-92C	SW-YS-091	2019/04/15
Electronic balance	CP1502	SW-YS-056	2019/04/10

Table 3 Table of Reagents

Reagent	Lot	Model	Manufacturer	Storage condition
Sodium chloride injection (SC)	318070602	100 mL:0.9 g	Shandong WEGO Pharmaceutical Co., Ltd.	RT
Freund's Complete Adjuvant (FCA)	SLBQ1109V	F5881-10 mL	SIGMA	4°C
Isopropanol	20150416	500 mL	Tianjin Fuyu Fine Chemical Co., Ltd.	RT

3.2 Justification of Test System

The albino guinea pig has been used historically for sensitization studies. The guinea pig is believed to be the most sensitive animal model for this type of study and specified in the current ISO 10993-10 testing standards.

Pre to OECD 406, the sensitivity and reliability of experimental technique are assessed every three months by use of substances which are known to have mild-to moderate skin sensitization properties. In a properly conducted test, a response of at least 30 % in an adjuvant test is expected for mild/moderate sensitizer. The Positive control selected by Intertek meets the above criteria. The Dermal Reaction of Positive Challenge is summarized in Appendix 1.

3.3 Animal Management

Husbandry: Conditions conformed to Standard Operating Procedures that are based on 'Guide for the Care and Use of Laboratory Animals'.

Food: A commercially available guinea pig feed from Beijing Keao Xieli Feed Co. Ltd. was provided daily.

Water: Potable water which met GB 5749 standards for drinking water quality was provided *ad libitum* through species appropriate water container.

Contaminants: Reasonably expected contaminants in feed or water supplies did not

have the potential to influence the outcome of this test.

Housing: Animals were housed in group in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, animal code and first treatment date.

Environment: The room temperature was monitored daily. the temperature range for the room was within a range of 19-25 °C.

The room humidity was monitored daily. The humidity range for the room was 45-65 %.

The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).

Personnel: Associate involved were appropriately qualified and trained.

Selection: Only healthy, previously unused animals were selected.

4. Method

On the first day of treatment, fifteen guinea pigs per extract (ten test, five control) were weighted and identified. The fur over the dorsoscapular region was removed with an electric clipper.

4.1 Induction I

The test animals were injected with the test article extract and the control animal were injected with the reagent control. Three rows of the intradermal injections (two per row) were given to each animal with an approximate 2 cm × 4 cm boundary of the fur clipped area as illustrated below:



Control animals:

- a. 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle.
- b. 0.1 mL of vehicle.
- c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the vehicle.

Test animal:

- a. 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle.
- b. 0.1 mL of test extract.
- c. 0.1 mL of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the test extract.

To minimize tissue sloughing the 'a' and 'c' injections were slightly deeper than 'b'. Site 'c' was injected slightly more caudal than site 'b'.

4.2 Induction II

The day prior to conducting the Induction II patch, the fur over the dorsoscapular region (same area as used during Induction I) was removed with an electric clipper. The area was left uncovered.

At 7 days after completion of the Induction I injection, 2 cm × 4 cm section of absorbent gauze patch, saturated with approximately 0.4 mL of freshly prepared test article extract, was then topically applied to the previously injected sites of the test animals. The control animals were similarly patched with the appropriate reagent control. Each patch was secured with a nonreactive tape and the trunk of each animal was wrapped with bandage. At 48 hours, the binders and patches were removed.

4.3 Challenge

At 13 days after unwrapping the Induction II wraps, the fur was removed from the upper flank areas with an electric clipper. The 2 cm × 2 cm absorbent gauze patch was saturated with approximately 0.4 mL of the test article extract. Each site was patched for the challenge phase. The control animals were similarly patched with the appropriate reagent control. Each patch was secured to the skin. The trunk of each animal was wrapped with bandage to maintain well-occluded sites. At

24 hours, the wraps and patches were removed and any residue remaining at the sites was removed.

4.4 Observation

Animals were observed daily for general health. Observations for dermal reactions were conducted at 24 and 48 hours after challenge patch removal. Prior to each scoring interval, the sites were wiped with 35% isopropyl alcohol. If necessary, the fur was clipped from each site to facilitate scoring. Scoring was recorded in accordance with the criteria shown below:

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

5. Evaluation and Statistics

Grades of 1 or greater in the test group generally indicated sensitization, provided that grades of less than 1 were observed on the control animals. If grades of 1 or greater were noted on control animals, then the reactions of test animals that exceeded the most severe control reaction were considered to be due to sensitization. The outcome of the test is presented as the frequency of positive challenge results in test and control animals.

If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge. Occasionally, the test group has a greater number of animals showing a response than the control, although the intensity of the reaction is not greater than the exhibited by the control. In these instances, a rechallenge might be necessary to define the response clearly. A rechallenge shall be carried out 1 week to 2 weeks after the first challenge. The method used shall be as described first challenge, using a naive side on the animal.

6. Results

Results of scores for individual animals appear in Table 4. All injection sites appeared normal immediately following injection. The final test sample score for each extract are summarized below:

Table 4 Dermal Reaction of Challenge

Group	SC				Positive rate%
	Animal number	*Weight /g	Score		
			24 hours	48 hours	
Test	1	320.7	0	0	0
	2	331.4	0	0	
	3	328.4	0	0	
	4	318.8	0	0	
	5	310.9	0	0	
	6	317.4	0	0	
	7	328.1	0	0	
	8	328.7	0	0	
	9	317.5	0	0	
	10	328.6	0	0	
Control	21	342.5	0	0	0
	22	317.9	0	0	
	23	315.6	0	0	
	24	334.5	0	0	
	25	312.7	0	0	

Note: * is the weight of the animals at the first test day.

7. Conclusion

Under the conditions of this study, there is no evidence that the test article extracts would cause delayed dermal contact sensitization on guinea pig. The test article would be considered no skin sensitization.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by Intertek. Any extrapolation of these data to other samples is responsibility of the sponsor.

8. Quality Assurance

Inspections were conducted at interval adequate to assure the integrity of the study in conformance with Intertek's procedure.

9. Proposed Dates

The study dates were finalized by the study director following receipt of the sponsor approved protocol and appropriate material for the study. Initiation of the study was date on which the study director signed the protocol. Projected dates for starting the study (extraction) and for the completion of the study (final report release) were provided to the sponsor (or representative of the sponsor).

10. Records

All raw data pertaining to this study and a copy of the final report were retained in designated Intertek archive files.

11. References

- ISO 10993-10:2010 Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.
- ISO 10993-12:2012 Biological evaluation of medical devices – Part 12: Sample preparation and reference materials.

12. Protocol Changes

Any necessary changes to the protocol after sponsor approval or study initiation were documented and approved by the study director as protocol amendments. Copies were distributed to the sponsor, the raw data file and the Intertek quality assurance department.

Appendix 1 Positive Challenge Result

Table 5 Dermal Reaction of Positive Challenge

Group	Animal number	*Weight /g	CO group score		Positive rate%
		Nonpolar	24 hours	48 hours	
Control	26	323.5	0	0	0
	27	350.8	0	0	
	28	328.4	0	0	
	29	334.6	0	0	
	30	340.5	0	0	
Test	11	342.0	0	0	60
	12	352.4	1	1	
	13	330.4	1	0	
	14	336.8	2	1	
	15	340.9	0	0	
	16	321.2	2	1	
	17	330.8	0	0	
	18	320.6	0	0	
	19	324.6	1	0	
	20	328.4	1	0	

Note: * is the weight of the animals at the first test day.

The data of positive control come from Intertek (ID: SQR-MD-DW-4.13-004-A/1-M201804).

Attachment 1: Photo of Test Article



*****End of Report*****