

TEST REPORT

NUMBER: 181000805SHA-001

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: In Vitro Cytotoxicity Test

TEST STANDARD: ISO 10993-5:2009

TEST ARTICLE NAME: PERMANENT MAKEUP NEEDLE

TESTS CONDUCTED:

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

TO BE CONTINUED

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Summary

The test article, Permanent Makeup Needle, Lot: E00280826, was extracted by sodium chloride injection. The extract was evaluated for cytotoxicity based on the requirement of ISO 10993-5:2009 Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity.

L929 cells were seeded into 96-well plates and maintained in culture for 24 h to form a semi-confluent monolayer. They were then exposed to the test compound over a range of concentrations. After 24 h exposure, the formazan formation was determined for each treatment concentration and compared to that determined in control cultures. For each treatment the percentage inhibition of growth is calculated.

Under the conditions of this study, the test extracts would be considered no cytotoxic potential. The negative controls, blank controls, and the positive controls performed as anticipated.

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 whether leachables extracted from the material would cause cytotoxicity. This study was conducted based on the requirement of ISO 10993-5:2009 Biological evaluation of medical device – Part 5: Tests for *in vitro* cytotoxicity.

Date

The test article was received on August 30, 2018. The test article was first exposed to the extract on November 08, 2018 and observations were concluded on November 10, 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
Test Article Identification:	M2018102409
Manufacture:	Intertek Testing Services Shanghai Limited.
Batch Number:	E00280826
Sterilization Lot Number:	Not supplied by the sponsor
Model:	Not supplied by the sponsor
Stability Testing:	Not supplied by the sponsor
Expired Date:	2021/07/08
Strength, Purity and Composition:	Sponsor selects not to provide this information to Intertek and takes full responsibility for this data and can supply this information if requested to do so.

Status:	Packing intact
Physical Description Of The Test Article:	Solid, 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)
Test Article	Based on a ratio of 0.2 g/mL, 2.46 g of the article was covered with 12.3 mL
Preparation:	of SC. The sample was extracted at 37°C for 24 hours. Serial dilutions were prepared (80%, 50%, 25% and 10% extracts). Extracts were used immediately after extraction. The extracts were not processed by filtration, centrifugation or other methods. The pH of extract was approximately 6.4.
Positive Control	The current positive control material, phenol (0.25%) was used to determine a cytotoxic. The positive control sample was subjected to the same extraction condition as described for the test article.
Negative Control	The current negative control material, high density polyethylene, was used as the negative control. Based on the USP ratio of 3 cm ² /mL, a 15 cm ² portion of the control material was covered with 5 mL of MEM. The negative control sample was subjected to the same extraction condition as described for the test article.
Blank Control	MEM without test material was subjected to the same extraction condition as described for the test article.

Condition of	<u>Test</u>	<u>Negative</u>	<u>Positive</u>	<u>Blank</u>
Extracts	Extract	clear	clear	clear

3. Test System and Justification

3.1 Test System Management

L-929 mouse fibroblast cells (NCTC, Clone 929, Generation of 15 from Zhongqiao Xinzhou) were propagated at 37°C in a gaseous environment of 5% carbon dioxide (CO₂) in a culture bottle containing MEM.

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

Table 1 Table of Equipments

Equipment	Model Number	Identification Number	Calibration validity
CO ₂ Incubator	T190	SW-YS-081	2019/04/17
Centrifuge	L-600	SW-YS-204	/
Pressure Steam Sterilizer	MSL.N	SW-YS-369	2019/01/10
Inverted Microscope	CKX-41	SW-YS-026	/
Water bath	HH-2	SW-YS-010	2019/04/15
Microplate Reader	Versa Max	SW-YS-306	2019/05/03
Water-bathing Constant Temperature Vibrator	SHA-C	SW-YS-396	2019/04/15
Electronic Balance	YP502	SW-YS-458	2019/04/10
Pipette	(20-200 µL)	SW-YS-410	2019/10/21
	(100-1000 µL)	SW-YS-404	2019/10/21
Clean Bench	SW-CJ-2F	SW-YS-919	/
Cell Counter	Scepter 2.0	SW-YS-017	/

Table 2 Table of Reagents

Reagent	Lot	Model	Manufacturer	Storage condition
MTT	WXBB2488V	V900888-1G	SIGMA	4°C
Foetal Calf Serum	18060501	100 mL	Tianhang Biotechnology	-20°C

Trypsin	20130323	25 g	Amresco	4°C
Isopropanol	20150401	AR500 mL	Tianjin Fuyu	RT
Phenol	20130113	AR500 mL	Tianjin Ruijinte	RT
MEM	1967518	11095-080 500mL	Gibco	4°C
High Density Polyethylene	KOM357	/	USP	RT
MEM NEAA(100×)	1896361	11140-050 100mL	Gibco	4°C
Penicillin-Streptomycin	1981207	15140-122 100 mL	Gibco	-20°C
DMEM (powder)	20171012001	250 g	Hangzhou Baisi	Cool Dry
NaCl	20171109	500 g	Yantai Shuangshuang	RT
KCl	20170825	500 g	Tianjin Yongda	RT
Na ₂ HPO ₄ ·12H ₂ O	20170110	500 g	Tianjin Kermel	RT
KH ₂ PO ₄	2015060166	500 g	Tianjin Zhiyuan	RT
Sodium Pyruvate	1951212	11360-070 100mL	Gibco	4°C
Sodium Bicarbonate	1855024	25080-094 100mL	Gibco	RT
Minimum Essential Medium (MEM)	1819694	41500-067 1L	Gibco	4°C
Sodium chloride injection (SC)	318041403	500ml:4.5g	Shandong WEGO Pharmaceutical	RT

3.2 Justification of Test System

The L-929 cell is specified in the current ISO 10993-5 testing standards and has been used historically to evaluate biomaterial extracts.

4. Method

After thawing from stock, the cells were passaged two to three times before using in the test. Cell cultures were removed from culture bottles by enzymatic digestion and the cell suspension was centrifuged at 200 G for 3 min. The cells were then resuspended in culture medium and the cell suspension was adjusted at a density of 1×10^5 cells/mL. Using a multichannel pipette, dispense 100µL culture medium only (blank) into the peripheral wells of a 96-well tissue culture microtiter plate. In the remaining wells, 100 µL of a cell suspension of 1×10^5 cells/mL were dispensed. The

cells were incubated for 24 hours (5% CO₂, 37°C, >90% humidity) so that cells form a half confluent monolayer. The plate was examined under microscope to ensure that cell growth was relatively even across the microtiter plate.

After 24 h incubation, the culture medium was aspirated from the cells. Per well, 100 µL of treatment medium (diluted by 5×MEM) containing either the appropriate concentration (80%, 50%, 25%, 10%) of sample extract or the negatives control, or the positive control, or blank control were added. The cells then were incubated for 24 h (5% CO₂, 37°C, >90% humidity).

After 24 h treatment, the plate was examined under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. After the examination of the plates, the culture medium was carefully removed from the plates. Subsequently, 50 µL of 1 mg/mL MTT solution was added to each test well and the plate was further incubated for 2 h in the incubator at 37°C. Then the MTT solution was discarded and 100 µL of isopropanol was added in each well. This plate was swayed for 10 min and subsequently transferred to a microplate reader equipped with a 570 nm filter to read the absorbance (reference wavelength 650 nm).

5. Evaluation and Statistics

A decrease in number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlated to the amount of blue-violet formazan formed, as monitored by the optical density at 570 nm. To calculate the reduction of viability compared to the blank. Equation was used:

$$\text{Viab. \%} = \frac{\text{OD}_{570\text{e}}}{\text{OD}_{570\text{b}}} \times 100\%$$

Where

OD_{570e} is the mean value of the measured optical density of the 100% extracts of the test sample;

OD_{570b} is the mean value of the measured optical density of the blanks.

If the viability is reduced to <70% of the blank, it has a cytotoxic potential. The 50% extract of the test sample should have at least the same or a higher viability than the 100% extract; otherwise the test should be repeated.

6. Results

The observations and absorbance of extracts of test article, positive control, negative control and blank appear in Table 3.

Table 3 The cytotoxicity results

		B1	B2	PC	NC	TS I	TS II	TSIII	TSIV
Microscopic Observation	24 h	I	I	I	I	I	I	I	I
	48 h	I	I	IV	I	I	I	I	I
OD value	1	0.3548	0.4091	0.0113	0.2951	0.3465	0.3474	0.4055	0.3766
	2	0.3702	0.3499	0.0122	0.3337	0.3601	0.3688	0.3757	0.3600
	3	0.3878	0.3652	0.0105	0.3512	0.3766	0.3705	0.3796	0.3746
	4	0.3995	0.3606	0.0115	0.3495	0.3893	0.3591	0.3705	0.4082
	5	0.3815	0.3684	0.0124	0.3749	0.3629	0.3993	0.3812	0.3964
	6	0.3658	0.4627	0.0076	0.3942	0.3335	0.3786	0.3701	0.3559
	mean	0.3766	0.3860	0.0109	0.3498	0.3615	0.3706	0.3804	0.3786
		0.3813							
Viab. %		98.77	101.23	2.86	91.73	94.80	97.20	99.77	99.30

Note: B- Blank; PC- Positive Control; NC-Negative Control; TS-Test Sample; TS1, TS2, TS3 and TS4 is the 80% extract, 50% extract, 25% extract and 10% extract individually.

Cell morphology description of microscopic observation:

I : Discrete intracytoplasmic granules; no cell lysis.

II : Some cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present.

III: Visible cells are round, cell lysis and empty areas between cells.

IV: Nearly complete destruction of the cell layers.

The 50% extract of the test sample showed higher viability compared with 100% extract. The mean OD₅₇₀ of blanks were ≥ 0.2 . The mean of the blanks did not differ by more than 15% from the mean of all blanks. The results met all criteria of quality control. The positive control extract had cytotoxicity. While the negative control and test sample extracts did not show cytotoxic potential.

7. Conclusion

Under the conditions of this study, the result was 94.80% > 70%, showed no evidence of causing viability reduction. The test extracts were not cytotoxic potential. The negative controls, reagent controls and positive control performed as anticipated.

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 10993-5:2009 Biological evaluation of medical device – Part 5: Tests for *in vitro* cytotoxicity.
- 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.

Attachment 1: Photo of Test Article



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