Test Report No.: 721629474
Report Date:24 January 2017

ORIGINAL

SUBJECT	**Cytotoxicity test**
TEST LOCATION	**TÜV SÜD China** TÜV SÜD Products Testing (Shanghai) Co., Ltd. B-3/4, No.1999 Du Hui Road, Minhang District Shanghai 201108, P.R. China
CLIENT NAME	**Zhonghong Pulin Medical Products Co., Ltd.**
CLIENT ADDRESS	West Industrial Park, Luannan County, Tangshan City.
TEST PERIOD	08-Dec-2016~04-Jan-2017
TEST REQUEST	Cytotoxicity tested with reference to ISO 10993-5:2009
CONCLUSIONS	According to the standard of ISO 10993-5:2009, the extract of the article showed it had a cytotoxic potential.

Prepared By

(Zhu Yichen)
Customer Service

Authorized By

(Spark Shi)
Technical Manager

Note: (1) General Terms & Conditions as mentioned overleaf. (2) The results relate only to the items tested.(3) The test report shall not be reproduced except in full without the written approval of the laboratory.(4) Without the agreement of the laboratory , the client is not authorized to use the test results for unapproved propaganda.

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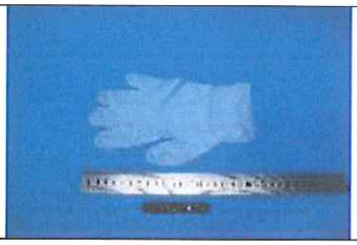
RECEIPT DATE / TEST DATE

08-Dec-2016/08-Dec-2016

THE FOLLOWING SAMPLE(S) WAS/WERE SUBMITTED BY/ ON BEHALF OF THE CLIENTS AS:

Sample Name: Nitrile Glove
Sample Specification: Medium
Batch No./Date: 2016-12-02
Manufacturer: Zhonghong Pulin Medical Products Co., Ltd.

SAMPLE NO.	DESCRIPTION	PHOTOGRAPH
721629474	Blue gloves	

Cytotoxicity test in vitro method

Test requirements

An in vitro cytotoxicity study was conducted to assess the potential for cytotoxicity of the test article, Nail, based on the International Organization for Standardization ISO 10993-5:2009: Biological Evaluation of Medical Devices - Part 5: Tests for in vitro Cytotoxicity; ISO 10993-12:2012: Biological evaluation of medical devices – Part 12: Sample preparation and reference materials.

Four concentration (100%, 75%, 50% and 25%) of the test article extracts, the blank control, the negative control and the positive control were prepared using DMEM supplemented with 10% calf serum. The confluent monolayers of CCL81 (Vero) were incubated with the test extract and other three controls, supplemented with 10% calf serum in a 96-well microplate respectively at 37°C under the condition of 5% CO2. At 24h, the CC KIT-8 reagent was add the plate and read on a microplate reader at 450 and 630nm. The viability of the cells were calculated.

Under the conditions of this study, the viability of 100% extract of the test article was 19.5%, it had a cytotoxic potential.
The test was according to the provisions of the ISO/IEC 17025-2005.

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MATERIALS
The test article provided by the sponsor was identified and handled as follows:

Test Article:	Nitrile Glove
Storage Conditions:	Room temperature
Extraction Vehicle:	GIBCO's DMEM with 10% calf serum
Test Extract Preparation:	Based on the ISO 10993-12, the ratio of 1g test article: 10ml extraction vehicle was used for preparing the test extract at 37℃ for 24 hours.
Reagent Control Preparation:	The extraction vehicle was subjected to the same extraction conditions as described for the test article.
Negative Control Preparation :	High-density polyethylene
Positive Control Preparation:	Extraction vehicle with 5g/L phenol solution, positive control was prepared at 37°C for 24 hours.
Condition of Extracts:	All the extract the test and controls were clear.

TEST METHOD(S)

Test System Management:
Vero, were cultured in DMEM supplemented with 10% calf serum and 1% L-glutamine at 37℃ in a gaseous environment of 5% carbon dioxide (CO_2). The pH was adjusted between 7.2~7.4. Each well was seeded 100μl suspension of 1×10^4 cells per milliliter, and incubated at 37℃ in 5% CO_2 atmosphere for 24h prior to use.

Experimental Procedure:
After incubation, the growth medium was replaced with 100μl four concentraions (100%, 75%, 50% and 25%) of test extract, Blank control, negative control or positive control, supplemented with 10% calf serum respectively. Six replicates were prepared for each group. The microplate were incubated at 37℃ in 5% CO_2 for 24h.
After 24h treatment, 10μl of the CC KIT-8 reagent was added in each well.
Plates with CC KIT-8 reagent were then incubated for 3h at 37℃ in a 5% CO_2 atmosphere. The plate was read on a microplate reader (Labsystems MK3-Thermo) at 450 nm (reference wavlength 630nm). Calculated the viability of the cells according to the formula below:

$$\text{Viability (\%)} = \frac{A_{450nm} - A_{630nm}}{A_{o450nm} - A_{o630nm}} \times 100$$

Where
A is the mean value of the measured optical density of the 100% test extracts of the test sample (the negative control or the positive control);
A_o is the mean value of the measured optical density of the blanks.

Evaluated the grade of cytotoxic reactivity based on the following criteria:
If the viability of the test sample was reduced to <70% of the blank, it had 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.

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TEST RESULT(S)

Group	The optical density (450 nm-630nm)	Viability (%)
100% of the test extract	0.253-0.055	19.5
75% of the test extract	0.667-0.063	59.4
50% of the test extract	0.872-0.066	79.3
25% of the test extract	0.992-0.065	91.1
Negative control	1.074-0.064	99.4
Positive control	0.291-0.064	22.3
Blank control	1.074-0.058	/

Results and conclusions apply only to the article tested.

CONCLUSION

Under the condition of this study, the viability of 100% extract of the test article was 19.5%, it had a cytotoxic potential.

-END OF THE TEST REPORT-

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