检 验 报 告

Test Article	Flame retardant Medical Grade PVC		
Model / Type	/	**Trade Mark**	/
Test Type	Commission Test		
Sponsor	Shenzhen YONGQIANFU Industrial CO., Ltd		
Applicant Address	2Bldg, NO.2 Industrial park, Xinwei Village, Dalang, LongHua Town, Shenzhen City		
Manufacturer	Shenzhen LiHengTong Plastic, Co., Ltd.		
Lot No. / Identification No.	/	**Date of Manufacturing**	2012-12-23
Application Date	Jan. 5, 2013	**Accepting Date**	Jan. 8, 2013
Test Items	*In vitro* Cytotoxicity Test		
Test in Accordance with	ISO 10993-5:2009<Biological evaluation of medical devices- Tests for *in vitro* cytotoxicity>		
Summary	The test article, Flame retardant Medical Grade PVC, was extracted by MEM supplemented with 10% fetal calf serum. The resulting extract was evaluated for *in vitro* cytotoxicity test by MTT cytotoxicity test to determine the potential cytotoxicity in accordance with the requirements of ISO 10993-5:2009 Biological evaluation of medical devices, Part 5: Tests for *in vitro* cytotoxicity. L-929 cells were seeded in the 96-well assay microtiter plate. After the incubation of cells for 24h±2h at 37°C±1°C and 5% CO_2, blank control, negative control, positive control and four different concentrations of the test article extract were added to the microtiter plate. After incubation for 24h±2h, the medium was removed from the plates. 50μL of the MTT solution was then added to each test well and the plates are further incubated for 2h. MTT solution was removed and 100μL of isopropanol was added to each well. Absorbance was measured at test wavelength of 570nm and reference wavelength of 650nm. The viability rate compared to the blank was calculated. Under the conditions of this study, the Viab.% of the positive control group and negative control group were 14% and 99%; the Viab.% of 100 %, 50%, 25% and 12.5% extract were 106%, 107%, 107% and 109%.		
Authorized Signatory		**Date completed**	Apr. 1, 2013

INTRODUCTION

The test article identified below was extracted, and the extract was subjected to an *in vitro* cytotoxicity test by MTT cytotoxicity test for biocompatibility evaluation in accordance with the Annex C of the ISO 10993-5:2009 Biological evaluation of medical devices- Tests for *in vitro* cytotoxicity. The test article was accepted on Jan. 8, 2013. The extraction was applied from Jan. 14, 2013 to Jan. 15, 2013, and the observations were concluded on Jan. 16, 2013.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	Flame retardant Medical Grade PVC
Identification No.:	/
Storage Conditions:	Room temperature
Cell line:	Cultures of L-929 cells, mouse fibroblast cells (ATCC CCL1, NCTC Clone 929, Clone of Strain L), was purchased from China Center for Type Culture Collection. Cultures were grown and used as monolayers in disposable tissue culture labware at 37±1℃ in a humidified atmosphere of 5±1% CO_2 in air.
MEM:	Gibco, With Earle's salts and L-Glutamine, Batch No.: 1128321
MEM:	Gibco, With Earle's salts, without L-Glutamine and Phenol Red, Batch No.: 862548
Foetal calf serum:	Gibco; Batch No.: 8122818
MTT:	Sigma; Batch No.: MKBF7873V MTT is soluted fresh in MEM without supplements and without phenol red at a concentration of 1mg/mL. Solution is sterilized by sterile filtration using syringe filters (pore size≤0.22μm). The solution should be used the same day.
DMSO:	Sigma; Batch No.: SZBA040S
Extraction vehicle(1×MEM):	MEM supplemented 10% foetal calf serum and antibiotics (100U/ml penicillin, 100ug/ml streptomycin).
Test article preparation:	Based on a ratio of 0.2g/mL, 2.04g of test article (as show in Fig.1) was covered with 10.2mL of the extraction vehicle and extracted at 37℃±1℃ for 24h±2h. The extract of test article is transparent with no presence of particulates. The extract was used immediately. Prior to use, the extracts was sterilized by membrane filtration (0.22μm).The filtration extracts of test article was diluted by MEM supplemented 10% foetal calf serum. The 100 %, 50%, 25% and 12.5% extract were tested.
Negative control preparation:	Based on a ratio of 0.2g/mL, 2.00g of the high-density polyethylene (The first Pharmaceutical Factory of Beijing, K4912R) was extracted in the extraction vehicle at 37℃±1℃

for 24h±2h. The 100 % extract was tested.

Positive control preparation: 1×MEM supplemented with 10% DMSO

Blank control preparation: 1×MEM

METHODS

Experimental Procedure:

1. L-929 cell suspension preparation
   a. L-929 cell monolayer confluence was cultured with 1×MEM for 48h~72h and removed from culture flasks by enzymatic digestion (trypsin/EDTA).
   b. The cell suspension is mechanical mixed before counting under microscope.
   c. The cell suspension was adjusted to the acquired density of 1×10^5 cells/ml by a proper addition of cell culture fluid.

2. MTT assay
   a. Using a multichannel pipette, dispensed 100µl culture medium only into the peripheral wells of a 96-well tissue culture microtitre plate. In the remaining wells, dispense 100µl of a cell suspension of 1×10^5 cells/ml (= 1×10^4 cells/well).
   b. Incubated cell cultures for 24h±2h at 37°C±1°C in a humidified atmosphere of 5% CO_2 in air, and then aspirated culture medium from the wells.
   c. The extract of test article was diluted by MEM for four different concentrations.
   d. Per well, added 100µl of treatment medium containing either the appropriate concentration of sample extract, or the negative control, or the positive control, or nothing but blank.
   e. Incubated cells for 24h±2h (5 % CO_2, 37℃±1℃, > 90 % humidity).
   f. After 24h±2h treatment, examined each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record changes in the morphology of the cells due to cytotoxic effects of the test sample extract.
   g. After the examination of the plates, carefully removed the culture medium from the plates. Pipet 50µL of MTT solution into each well, and incubate the plate for 2h at 37℃±1℃. Then the MTT solution is discanted and 100µl of isopropanol were added in each well. Swayed this plate and subsequently transferred it to a microplate reader equipped with a 570 nm filter to read the absorbance (reference wavelength 650 nm).
   h. A decrease in number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlates to the amount of blue-violet formazan formed, as monitored by the optical density at 570nm. To calculate the reduction of viability compared to the blank Equation is used:

$$Viab.\% = \frac{100 \times OD_{570e}}{OD_{570b}}$$

where

OD570e is the mean value of the measured optical density of the 100% extracts of the test sample;

OD570b is the mean value of the measured optical density of the blanks.

i. Evaluation Criteria

If 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.

A test meets the acceptance criteria if the mean OD570 of blanks is ≥0.2.

A test meets acceptance criteria if the left and the right mean of the blanks do not differ by more than 15 % from the mean of all blanks.

RESULTS

The results of optical density show as Table.1:

Table.1

Groups	Blank Control		Positive Control	Negative Control	100% Extract	50% Extract	25% Extract	12.5% Extract
	Left	Right						
Well 1	0.155	0.112	0.019	0.115	0.114	0.123	0.157	0.118
Well 2	0.098	0.104	0.018	0.132	0.132	0.117	0.127	0.122
Well 3	0.105	0.135	0.012	0.109	0.100	0.156	0.135	0.115
Well 4	0.149	0.153	0.018	0.102	0.131	0.131	0.124	0.197
Well 5	0.132	0.117	0.017	0.136	0.162	0.116	0.125	0.109
Well 6	0.080	0.097	0.016	0.115	0.124	0.124	0.101	0.125
Mean OD	0.120	0.120	0.167	0.118	0.127	0.128	0.128	0.131
	0.120							
Viab.%	/		14%	99%	106%	107%	107%	109%

CONCLUSION

Under the conditions of this study, the Viab.% of the positive control group and negative control group were 14% and 99%; the Viab.% of 100 %, 50%, 25% and 12.5% extract were 106%, 107%, 107% and 109%.The negative controls, positive controls and blank controls performed as anticipated.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by our testing center. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with ISO 17025.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated archive files in our testing center.

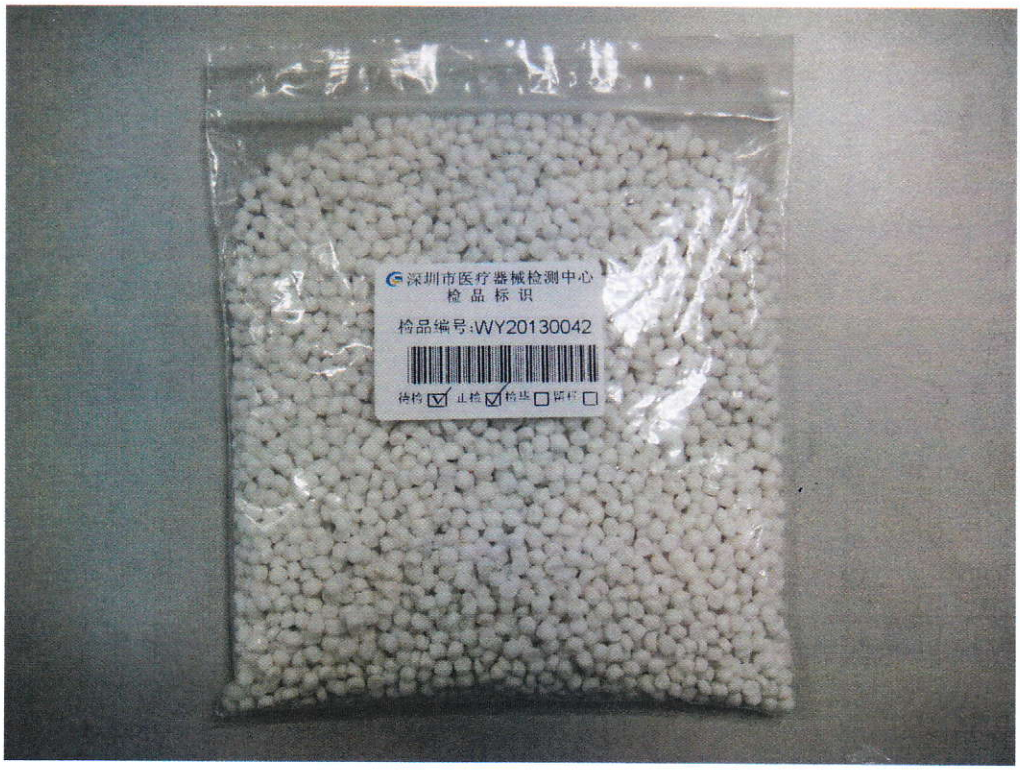

Fig.1 Test Article
（Blank Below）

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Shenzhen Testing Center of Medical Devices

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