Test Report

TEST ITEMS

Test for genotoxicity (Mouse Lymphoma Mutagenesis Assay)

TEST ARTICLE

Non-absorbable Surgical Suture (model: PTFE)
<Specification: Z21302-60 (USP2-0/EP2.5)>

IDENTIFICATION №

150642

MANUFACTURE

Foosin Medical Supplies Inc., Ltd.

SPONSOR

TÜV SÜD Certification and Testing (China) Co., Ltd.
(Address: M Building, No.7 Wangjing Zhonghuan Nanlu, Chaoyang District, Beijing 100102, P.R. China)

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SUMMARY

The test article, Non-absorbable Surgical Suture(model:PTFE), was extracted in RPMI-1640 medium and DMSO. The extracts were tested by using the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis assay (96 micro-well method). This study was conducted to satisfy, in part, the genotoxicity requirement of the International Organization for Standardization ISO 10993-3: 2014: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity; ISO 10993-12:2012: Biological evaluation of medical devices, Part 12: Sample preparation and reference materials.

The L5178Y/TK $^{+/-}$ cells were grown as suspension cultures in 10% horse serum medium (R10) in culture flasks. Afterwards, they were exposed to the test article extracts in the presence and absence of S_9 metabolic activation. Parallel testing was also conducted with a negative and positive control. Culture medium was used as the negative control. Both methylmethansulfonate (MMS) in the absence of S_9 and cyclophosphamide (CP) in the presence of S_9 served as the positive controls.

Under the conditions of this assay, the extracts of test article were concluded to be negative in the L5178Y/TK $^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the presence and absence of S_9 metabolic activation. The negative and positive controls performed as expected.

Study and Supervisory
Personnel: JI Lina
LU Hua

Study Director: Sun Jiao — Nov 23. 2015

SUN Jiao, Ph.D. — Date Completed

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INTRODUCTION

The purpose of this study was to evaluate the mutagenic potential of test article extracts based on quantitation of forward mutations at the thymidine kinase locus of L5178Y/TK$^{+/-}$ Mouse Lymphoma cells in the presence and absence of a metabolic activation system. The test article was received on Jun.16, 2015. The test began on Sep.28, 2015, and ended on Nov.2, 2015.

This study was completed in the Lab of Shanghai Biomaterials Research & Test Center (SBRTC) and was conducted in accordance with the provisions of the ISO/IEC 17025-2005.

MATERIALS

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

Test Article:	Non-absorbable Surgical Suture(model:PTFE) < Specification: Z21302-60(USP2-0/EP2.5) >
Identification №:	150642
Package Delineation:	STERILE
Storage Conditions:	Room temperature
Extraction Vehicles:	RPMI-1640 medium < Lot №.NAF1408> DMSO< Lot №.20141118>
Test Article Extract:	Based the ISO, 6cm^2/ml [Surface area of the test article to volume of extraction vehicle],367.3cm^2 test article was covered with 61.2ml RPMI-1640 and 56.5cm^2 test article was covered with 9.4ml DMSO respectively under sterile conditions for preparing the test article extracts at 37℃ for 72h . The test extracts were then serially diluted with the same vehicle and resulted in the following concentrations: Test A: 25% diluent of the test extraction Test B: 50% diluent of the test extraction Test C: 100% test extraction according to the requirement The extracts were used immediately after extraction.
Negative Control:	The extraction vehicle without any test article was subjected to conditions identical to which the test article was subjected during its extraction.
Positive control:	Methylmethansulfonate (MMS) in the absence of S9 (Sigma, USA)

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Cyclophosphamide (CP) in the presence of S9 (Sigma, USA)

Condition of Extracts: All the extract of the test and controls were clear.

Source of Cell Line:

L5178Y cells, clone 3.7.2C was obtained from the cell bank of Shanghai Institutes for Biological Sciences, Shanghai, China.

Justification of Test System:

Historically, L5178Y cells have been used to evaluate the mutagenic potential of the test article extracts because they demonstrate sensitivity to extractable mutagenic articles.

METHODS

Test System:

Prior to use in the assay, L5178Y cells were cleaned of spontaneous TK cells by culturing in a restrictive medium.

Metabolic Activation System:

The S_9 homogenate was purchased from Shanghai BioPlus Biotech Co.,Ltd. Aliquot of S_9 was thawed immediately before use and was added to the other components to form the activation system.

Mutagenesis Assay:

The mutagenesis assay was used to evaluate the mutagenic potential of the test article extracts. The cultures of L5178Y mouse lymphoma cells were exposed to each test article extract, the negative controls and positive controls in both the presence and absence of S_9 mix.

Treatment of the Target Cells:

The mutagenesis assay was performed according to OECD 476, using the 96 micro-well method. At least three concentrations were used. The L5178Y/TK $^{+/-}$ cells was grown as suspension cultures in R10 in culture flasks. Treatment was carried out in conical tubes by combining 10ml 1×10^6 L5178Y/TK$^{+/-}$ cells, 1mL KCL or 1mL S_9 activation mixture and 9mL test article extract or negative control in a total volume of 20 mL (Final concentration was no more than 1% for the DMSO extraction) .The positive controls were treated with 200μL MMS without S_9 mix (at final concentration of 10μg /mL in treatment medium) and 200μL CP with S_9 mix (at final concentration of 3μg /mL in treatment medium). Treatment tubes were incubating at 37℃ in the presence of CO_2.The MMS and CP were freshly prepared. The cells were incubated at 37℃ for 4h with and without S_9 mix and 24h without S_9 mix.

After the treatment period, the cells were washed once with RPMI-1640 (R0). Then, the cells were suspended in 20ml RPMI-1640 with 10% horse serum (R10) and adjusted to the concentration of approximately 2×10^5 cells/ml. Part of the cells in each group were diluted to 8 cells /ml with RPMI-1640 with 20% horse serum medium （R20）.And 200μL were placed into each well of the plates. The plates were incubated at 37℃ in a humidified incubator with 5% CO_2 for 12 days. Afterwards the plating efficiency was calculated as PE_0. The rest of the cells were continued to be

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cultured for 2 days.

Expression of the Mutant Phenotype:

During these 2 days, the cultures were counted and adjusted to 2×10^5 cells/ml daily for expression of the mutant phenotype. Cultures with less than 2×10^5 cells/ml were not adjusted.

After 2 days, the cultures were adjusted to the concentration of 1×10^4 cells/mL by using R20. Part of the cells were prepared for TFT resistance. First, TFT was added to cultures to a final concentration of 3μg /mL in 50 mL of the negative control, 50 mL of the test article and positive controls, respectively. Each TFT-treated culture was dispensed at 200μL per well onto 96 well-micro-well plates.

The rest of the cells were diluted to 8 cells /mL. 200μL was placed into each well of the plates. The plates were incubated at 37℃ in a humidified incubator with 5% CO_2 in air for 12 days. Afterwards the plating efficiency was calculated as PE_2.

Colony Counting

After the incubation period, 96 well micro-well plates were counted for the total number of colonies per plate. The number of wells containing colonies was counted by naked eye. The colonies are scored of small and large colonies. The number of negative wells per 96 well micro-well plates will be counted for the survival (PE_0), viability (PE_2), and mutation (TFT) plates, and then the plating efficiency in both the survival/ viability plates and mutant selection plates is calculated and the relative total growth (RTG) and the mutant frequency (MF) will be determined.

Criteria for a Valid Test

The following criteria must be met for the mutagenesis assay to be considered valid:

Negative and Positive Controls:

The range of plating efficiency for the negative control has to be from 60% to 120% for survival (PE_0) andfor viability (PE_2) plates. The average spontaneous mutant frequency of negative controls with and without S_9 mix should ideally be within the range of $35\text{-}140\times10^{-6}$. For the positive control, it must exhibit mutant frequencies $\geq100\times10^{-6}$ mutants over the negative control for the 4 h exposure, or $\geq 300\times10^{-6}$ mutants over the negative control with 40 % small colonies for 24 h exposure. When the MF of the positive controls increases twofold or more over the negative controls, the experimental sensitivity is acceptable. A statistically significant dose-related increase in MF is required for a positive response.

RESULTS

According to ISO 10993-3 requirement, the range of plating efficiency was relatively constant to be considered valid. The frequency ranges expected from the laboratory's historical control data. There was no concentration-related increase in mutant frequency. The results of the mutagenesis assay were presented in TABLE Ⅰ－Ⅱ. Cloning data and total toxicity data for L5178Y/TK $^{+/-}$mouse lymphoma cells treated with extracts of the test article were shown as follows.

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TABLE I

MUTAGENESIS ASSAY RESULTS

RPMI-1640		EW_0	PE_0	EW_2	PE_2	RTG	EW_{SC}	EW	S-MF ($\times10^{-6}$)	MF ($\times10^{-6}$)
$-S_9$ (4h)	Negative Control	51	83%	56	77%	100%	180	156	41.9	134.8
	Test A	47	88%	49	85%	69%	177	156	47.7	121.6
	Test B	55	78%	54	79%	76%	180	165	40.7	95.6
	Test C	52	82%	56	77%	68%	169	150	82.8	160.3
	Positive Control	77	57%	80	55%	57%	138	89	301.8	702.6
$+S_9$ (4h)	Negative Control	49	85%	57	76%	100%	176	160	57.3	120.1
	Test A	56	77%	50	84%	72%	171	148	68.9	154.8
	Test B	52	82%	56	77%	75%	179	162	45.5	110.3
	Test C	49	85%	51	83%	76%	175	153	55.9	137.0
	Positive Control	92	46%	84	52%	49%	128	82	392.4	823.3
$-S_9$ (24h)	Negative Control	52	82%	59	74%	100%	184	172	28.9	74.6
	Test A	50	84%	52	82%	125%	170	151	74.5	147.1
	Test B	46	89%	56	77%	99%	174	159	63.9	122.4
	Test C	59	74%	49	85%	120%	176	154	51.0	129.2
	Positive Control	81	54%	85	51%	57%	132	90	367.9	743.9

EW: Empty well; PE: plating efficiency; RTG: Relative Total Growth; S-MF: Small Colony Mutation Frequency; MF: Total Mutation Frequency

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TABLE II

MUTAGENESIS ASSAY RESULTS

DMSO		EW_0	PE_0	EW_2	PE_2	RTG	EW_{SC}	EW	S-MF ($\times10^{-6}$)	MF ($\times10^{-6}$)
-S_9 (4h)	Negative Control	46	89%	58	75%	100%	175	163	62.0	109.4
	Test A	52	82%	55	78%	78%	170	152	77.9	149.5
	Test B	46	89%	60	73%	73%	177	165	55.9	104.2
	Test C	56	77%	53	80%	70%	173	149	64.8	157.6
	Positive Control	77	57%	80	55%	59%	138	89	301.8	702.6
+S_9 (4h)	Negative Control	51	83%	60	73%	100%	173	158	71.7	134.0
	Test A	46	89%	55	78%	71%	179	163	44.9	104.8
	Test B	55	78%	58	75%	74%	172	157	73.5	134.5
	Test C	62	71%	50	84%	68%	177	157	48.4	119.7
	Positive Control	92	46%	84	52%	51%	128	82	392.4	823.3
-S_9 (24h)	Negative Control	44	92%	62	71%	100%	179	165	49.6	107.3
	Test A	49	85%	54	79%	95%	169	150	80.5	155.7
	Test B	57	76%	52	82%	119%	170	152	74.5	143.1
	Test C	63	70%	59	74%	111%	173	159	51.3	127.9
	Positive Control	81	54%	85	51%	59%	132	90	367.9	743.9

EW: Empty well; PE: plating efficiency; RTG: Relative Total Growth; S-MF: Small Colony Mutation Frequency; MF: Total Mutation Frequency

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by Shanghai Biomaterials Research & Test Center.

CONCLUSION

All criteria for a valid study were met as expected. Under the conditions of this study, the results of the L5178Y/TK $^{+/-}$ Mouse Lymphoma Mutagenesis Assay indicated that extracts of the test article did not cause a positive response in the non-activated and S_9-activated systems and were concluded to be negative.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be stored in the designated archive files at Shanghai Biomaterials Research & Test Center.

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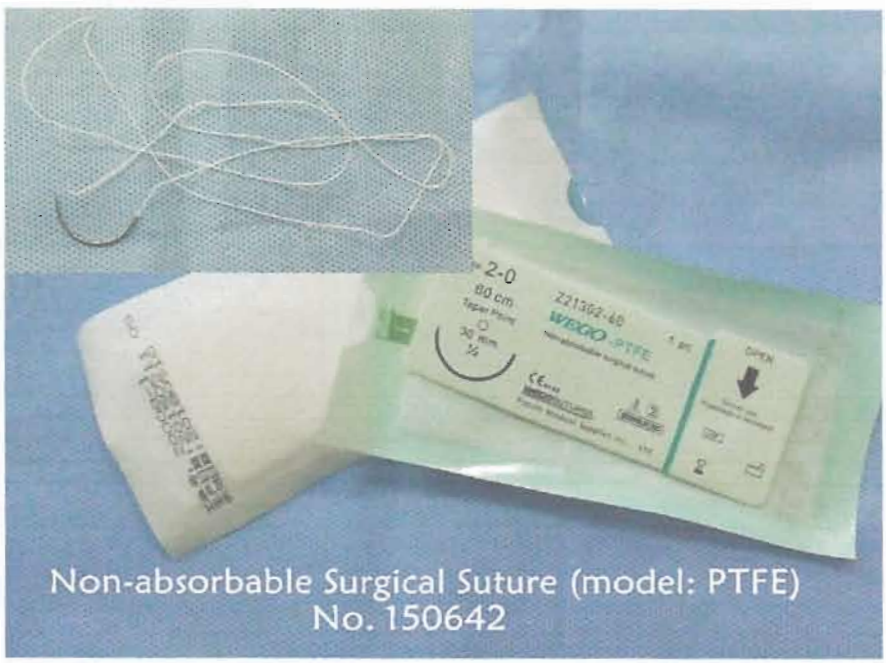
PHOTOGRAPH OF THE TEST ARTICLE



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