Test Report

TEST ITEMS

Test for genotoxicity (Bacterial reverse mutation study)

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

A Salmonella typhimurium reverse mutation standard plate incorporation study was conducted to determine whether the saline and DMSO extracts of Non-absorbable Surgical Suture (model:PTFE) would cause mutagenic changes in the average number of revertants for histidine-dependent Salmonella typhimurium strains TA_{97},TA_{98},TA_{100} ,TA_{102} and TA_{1535} in the presence and absence of S9 metabolic activation. 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 test article was extracted in 0.9% sodium chloride injection (SC) and DMSO respectively and then diluted into four concentrations by two-fold serial dilutions. Individual tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution were inoculated with 0.1 ml of culture of tester strain and 0.1 ml of the test article extract. A 0.5 ml aliquot of 10% S9-mix (rat liver metabolizing system) was added when necessary. The mixture was poured on to Vogel-Bonner E agar plates in triplicate. Parallel testing was also conducted with negative controls and six positive controls. The mean number of revertants of the triplicate test plates was compared to those of the negative control plates for each of the five tester strains employed.

There was no diminution of the background lawn in the four concentrations of the test extracts for tester strains TA_{97}, TA_{98}, TA_{100}, TA_{102} and TA_{1535}. The strains yield spontaneous revertant colony plate counts within the frequency ranges expected from the laboratory 's historical control data. In no case was there a twofold or greater increase in the mean number of revertants in the presence of the test article extracts.

Under the conditions of this assay, the test article extracts were considered to be nonmutagenic to Salmonella typhimurium tester strains TA_{97}, TA_{98}, TA_{100}, TA_{102} and TA_{1535}. The negative and positive controls performed as anticipated.

Study and Supervisory
Personnel: JI Lina
LU Hua

Study Director: Sun Jiao, Ph.D. Nov 23. 2015 Date Completed

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INTRODUCTION

The purpose of the bacterial reverse mutation study was to evaluate whether the SC and DMSO test article extracts would cause mutagenic changes in the average number of revertants for Salmonella typhimurium tester strains TA_{97}, TA_{98}, TA_{100}, TA_{102} and TA_{1535}.The test was performed in the presence and absence of S9 metabolic activation. Bacterial reverse mutation tests have been widely used as a rapid screening procedure for the determination of mutagenic and potential carcinogenic hazards. The test article was received on Jun.16, 2015. The preliminary toxicity screen began on Oct.8, 2015, and the testing ended on Oct.30, 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:	0.9% sodium chloride injection (SC) < Lot №.K141028E> DMSO< Lot №.20141118>
Test Article Extract:	Based the ISO, $6cm^2/ml$ [Surface area of the test article to volume of extraction vehicle],$84.8cm^2$ of the test article was respectively immersed in 14.1ml of SC and DMSO under sterile conditions for preparing the SC and DMSO test extracts at 37℃ for 72h.The test extracts were then serially diluted with the same vehicle and resulted in the following concentrations: Test A: 12.5% diluent of the test extraction Test B: 25% diluent of the test extraction Test C: 50% diluent of the test extraction Test D: 100% test extraction according to the requirement The extracts were used immediately after extraction.

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Negative Control:	0.9% SC or DMSO without test material was similarly subjected to the extraction conditions
Positive control:	Without S9: TA100 and TA102 ——MMS TA97—— atebrin TA98—— P-Nitroquinoline TA1535—— Sodium azide With S9: TA97,TA98, TA100 and TA1535——2-aminofluorene TA102——1，8-dihydroxyanthraquinone
Condition of Extracts:	All the extracts of the test and controls were clear.

Identification of Test System:

The test system were Salmonella typhimurium tester strains TA_{97}, TA_{98}, TA_{100} , TA_{102} and TA_{1535}. The tester strains were obtained from School of Public Health, FuDan University, China.
Historically, Salmonella typhimurium tester strains have been used for test for genotoxicity (Bacterial reverse mutation study) because they demonstrate sensitivity to mutagenic articles.

METHODS

Test System:

Each Salmonella Typhimurium tester strain contains a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. These genetically altered strains (TA_{97}, TA_{98}, TA_{100} , TA_{102} and TA_{1535}) cannot grow in the absence of histidine respectively. When they are placed in a histidine-free medium, only those bacterials which mutate spontaneously back to their wild type state (histidine independent by manufacturing their own histidine) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased. The amino-acid requirement for growth, the presence or absence of R-factor plasmids and other phenotypic characteristics of salmonella typhimurium strains TA_{97}, TA_{98}, TA_{100} ,TA_{102} and TA_{1535} were checked for another study in Jul.8, 2015.

Tester Strain	Mutations
S.typhimurium TA_{97}	his^-, rfa, ΔuvrB, R-factor
S.typhimurium TA_{98}	his^-, rfa, ΔuvrB, R-factor
S.typhimurium TA_{100}	his^-, rfa, ΔuvrB, R-factor
S.typhimurium TA_{102}	his^-, rfa, R-factor, pAQ1
S.typhimurium TA_{1535}	his^-, rfa, ΔuvrB

his^- = histidine-free
rfa = cause partial loss of the lipopolysaccharide wall which increases

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permeability of the cell to large molecules (i.e crystal violet inhibition)

Δ uvrB = deficient DNA excision –repair system (i.e. ultraviolet sensitivity)

R-factor = contain plasmid PKM101 (i.e. ampicillin resistance)

pAQ1 = contain plasmid pAQ1 (i.e. tetracycline resistance)

Metabolic Activation:

The S9 homogenate was purchased from School of Public Health, FuDan University, China.

Just prior to use, the S9 homogenate was mixed with a buffer containing 0.4M $MgCl_2$/1.65M KCl, 1.0M Glucose-6-phosphate, 0.1M NADP, 0.2M sodium phosphate buffer, and sterile purified water.

Preparation of Tester Strains:

Cultures of Salmonella Typhimurium, TA_{97}, TA_{98}, TA_{100}, TA_{102} and TA_{1535} were inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculate broth cultures were incubated at 37℃ in an incubator shaker operating at 120 rpm for 10 hours.

Negative Controls:

Negative Controls were tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of revertant colonies that developed in each test plate was compared to determine whether the test article had significant mutagenic properties.

Positive Controls:

Positive controls were used to demonstrate that the tester strains were sensitive to mutation to wild type state.

Standard Plate Incorporation Assay:

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the Salmonella Typhimurium were inoculated with 0.1ml of culture for each of the five tester strains and 0.1ml of the test article extract. A 0.5ml of 10% S9 mix, simulating metabolic activation, was added when necessary. The mixture was poured on to Vogel-Bonner E agar plates in triplicate. Parallel testing was also conducted with a negative control and six positive controls.

The plates were incubated at 37℃ for 48h. Following incubation period, the revertant colonies on each plate were recorded. The mean number of revertants from triplicate testing was determined for the negative controls, the test article extracts and the positive controls. The mean number of revertants of the test plates was compared to the mean number of revertants of the negative control for each of the five tester strains employed.

Evaluation and Statistics:

For the test article extract to be evaluated as a test failure or "potential mutagen" there must have been a two-fold or greater increase in the number of mean revertants over the means obtained from the negative controls for any or all five tester strains. The mean revertant number of each positive control must have exhibited at least a 3-fold increase over the respective negative control mean of the Salmonella tester strain employed, the negative control results of each tester strain exhibited a characteristic number of spontaneous revertants based on historical data collected at SBRTC.

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RESULTS

Test System:

According to ISO 10993-3 requirement, the spontaneous mutation rate (or reversion rate) for any one strain is relatively constant. The stains yield spontaneous revertant colony plate counts within the frequency ranges expected from the laboratory's historical control data. The phenotypic characteristics of salmonella typhimurium strains TA_{97}, TA_{98}, TA_{100}, TA_{102} and TA_{1535} were checked for another study in Jul.8, 2015. Complete data is traceable in laboratory records (see TABLE Ⅰ).

Standard Plate Incorporation Assay:

The results were summarized in TABLE Ⅱ-Ⅲ.There was no concentration-related increase over the range tested, or a reproducible increase at one concentration in the number of revertant colonies. In no case was there a two fold or greater increase in the mean number of revertants of tester strains TA_{97}, TA_{98}, TA_{100}, TA_{102} and TA_{1535} in the presence of the test article extracts. The mean revertant number of each positive control exhibited at least a 3-fold increase over the respective mean of the Salmonella typhimurium strain employed.

TABLE Ⅰ
STRAIN CHARACTERISTICS AND STANDARD PLATE COUNTS

	Tester Strains				
Characteristics (expected)	TA97	TA98	TA100	TA102	TA_{1535}
Histidine Requirement: (Growth)	G	G	G	G	G
Rfa Mutation: CV(sensitive)	S	S	S	S	S
Ampicillin: (Resistant) (Sensitive)	R	R	R	R	S
Tetracycline Resistant (Growth)	NG	NG	NG	G	G
UVrb (No Growth)	NG	NG	NG	G	NG
Total Plate Count	125	30	140	300	10

R= Resistant S= Sensitive NG=No Growth G=Growth

TABLE Ⅱ

		Salmonella typhimurium Tester Strains									
SC		TA_{97}		TA_{98}		TA_{100}		TA_{102}		TA_{1535}	
		- S9	+ S9	- S9	+ S9	- S9	+S9	- S9	+ S9	- S9	+ S9
12.5% extract	$\bar{x}$ ±SD	115±12	118±16	30±5	33±5	140±15	139±17	291±21	302±23	7±2	10±2
25% extract	$\bar{x}$ ±SD	105±9	119±5	23±3	29±6	134±14	135±16	296±26	324±23	10±3	11±3
50% extract	$\bar{x}$ ±SD	111±13	115±10	21±3	25±3	148±21	154±16	289±26	319±22	7±2	11±1

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100% extract	$\bar{x}$ ±SD	111±7	113±14	22±6	28±2	136±11	146±12	302±18	309±23	11±2	12±4
Negative control	$\bar{x}$ ±SD	106±8	115±11	22±2	26±3	118±16	135±6	292±25	310±30	7±3	9±3
Positive control	$\bar{x}$ ±SD	1123±118	1187±79	412±36	779±59	1259±100	1282±145	1382±105	1479±142	449±63	540±67

STANDARD PLATE INCORPORATION ASSAY RESULTS

$\bar{x}$ ±SD: Mean of triplicate plates ± Standard deviation

TABLE III

STANDARD PLATE INCORPORATION ASSAY RESULTS

Salmonella typhimurium Tester Strains											
DMSO		TA_{97}		TA_{98}		TA_{100}		TA_{102}		TA_{1535}	
		- S9	+ S9	- S9	+ S9	- S9	+S9	- S9	+ S9	- S9	+ S9
12.5% extract	$\bar{x}$ ±SD	107±8	114±11	19±3	27±3	123±8	141±15	286±21	290±27	7±3	10±3
25% extract	$\bar{x}$ ±SD	111±13	120±8	22±3	25±5	142±17	146±15	298±23	321±27	7±2	8±3
50% extract	$\bar{x}$ ±SD	114±13	118±17	28±5	30±4	128±13	123±14	297±29	293±23	9±4	11±4
100% extract	$\bar{x}$ ±SD	115±9	119±13	20±5	26±3	112±11	125±17	285±26	301±27	7±3	8±2
Negative control	$\bar{x}$ ±SD	104±12	118±11	17±3	25±2	119±13	121±15	276±21	296±26	6±3	10±3
Positive control	$\bar{x}$ ±SD	1123±118	1187±79	412±36	779±59	1259±100	1282±145	1382±105	1479±142	449±63	540±67

$\bar{x}$ ±SD: Mean of triplicate plates ± Standard deviation

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

Under the conditions of this assay, the test article extract was considered to be nonmutagenic to Salmonella typhimurium tester strains TA_{97}, TA_{98}, TA_{100}, TA_{102} and TA_{1535}. The negative and positive controls performed as anticipated.

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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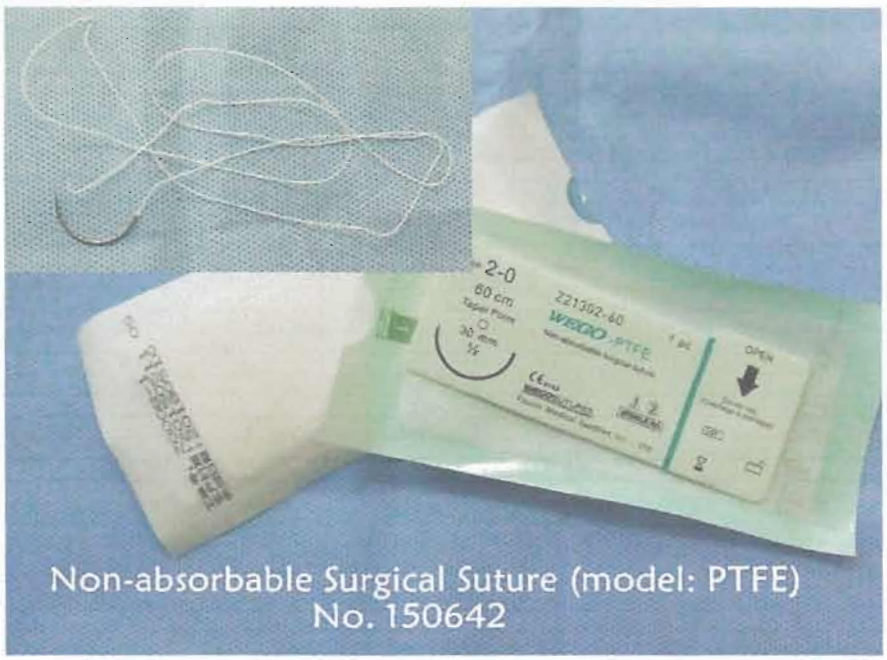
PHOTOGRAPH OF THE TEST ARTICLE



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