Test Report

TEST ITEMS

Test for genotoxicity
(*In vitro* chromosomal aberration study in mammalian cells)

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.
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SUMMARY

The test article, Non-absorbable Surgical Suture (model:PTFE) was extracted in Minimum Essential Medium and DMSO. A chromosomal aberration study was conducted to determine whether the extracts would cause genotoxicity in Chinese Hamster Lung cells 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.

A monolayer of Chinese Hamster Lung (CHL) cells was exposed to the test article extracts in triplicate cultures and in the presence and absence of S9 metabolic activation. Parallel testing was also conducted with negative and positive controls. Culture medium was used as the negative control. Mitomycin C (MMC) served as the positive control in the absence of S9 and cyclophosphamide (CP) served as the positive control in the presence of S9.

Under the conditions of this assay, the extracts of test article were not considered genotoxic to Chinese Hamster Lung cells in the presence and absence of S9 metabolic activation. The negative and positive controls performed as expected.

Study and Supervisory Personnel: JI Lina
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Study Director: Sun Jiao — Nov 23, 2015

SUN Jiao, Ph.D. Date Completed

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INTRODUCTION

A chromosomal aberration study was conducted to determine whether the Minimum Essential Medium and DMSO extracts of Non-absorbable Surgical Suture (model:PTFE) would cause clastogenic changes in Chinese Hamster Lung (CHL) cells. The test was performed in the presence and absence of S9 metabolic activation. The chromosomal aberration genotoxicity test employs CHL cells to detect chromosome structural changes. The detection of the aberrations was accomplished by observing chromosomes in metaphase which had been strained with Giemsa. The test article received on Jun.16, 2015. The test began on Sep.14, 2015, and cell counts concluded on Oct.28, 2015.

This study was completed in the Lab of Shanghai Biomaterials Research & Test Center (SBRTC). SBRTC 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
Storage Conditions:	Room temperature
Extraction Vehicle:	Minimum Essential Medium < Lot №.K1652143> 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 Minimum Essential Medium 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
Negative Control:	The extraction vehicle without any materials was similarly prepared at 37℃ for 72h.
Positive Control::	Mitomycin C (MMC) was prepared in the absence of S9. Cyclophosphamide (CP) was prepared in the presence of S9.
Condition of Extracts:	All the extracts of the test articles and controls were clear.

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METHODS

Test System Management:

CHL cells, cloned with a chromosome number of 25, were obtained from the cell bank of Shanghai Institutes for Biological Sciences. The cell line had an average generation time of 12-13h. The cells were propagated at 37℃ in sealed flasks containing sterile Minimum Essential Medium supplemented with 10% bovine serum. Culture flasks which contained a confluent (30-60%) cell monolayer were selected. Aseptic procedures were used in the handling of the cell cultures.

Metabolic Activation:

The S9 homogenate was purchased from School of Public Health, Fudan University. Aliquots of S9 were thawed immediately before use and added to the other components to form the activation system described as follow.

Negative Control:

The negative control was tested to determine the spontaneous aberration frequency rate. This data represented a base rate to which the number of chromosomal aberrations per cell was compared to determine whether the test article extract had significant clastogenic properties.

Positive Control:

A known direct acting genotoxic compound, Mitomycin C (MMC), was used as a positive control to demonstrate that CHL cells were sensitive to mutagens in the absence of metabolic activation. The positive control was prepared by adding MMC to yield a final concentration of 0.25μg/ml, an indirect acting genotoxic compound, was used as the positive control in order to demonstrate that CHL cells were sensitive to mutagens in the presence of metabolic activator. The positive control was prepared by adding CP to yield a final concentration of 20μg/ml.

Experimental Procedure:

For the assays conducted with and without metabolic activation (S9) for 4 hours, the growth medium in each culture flask was replaced with 10ml of the test extract. In a similar manner, the growth medium in negative control flask was replaced with 10ml of the negative control blank (Minimum Essential Medium). Each of the positive control flask received MMC at a final concentration of 0.25μg/ml.After 17h of incubating at 37℃ in the presence of CO_2, the medium was decanted and the cultures were rinsed twice with phosphate buffered saline (PBS). Following incubation, a 10ml portion of Minimum Essential Medium supplemented with 1μg/ml of colcemid was added to each test and control flask. The flasks were incubated for an additional 3h.

For the assay conducted without S9 for 24 hours, the test extract, negative control, and positive controls were supplemented without the mixture of S9. After 21h of incubation, the medium was decanted from the flasks and the cell monolayer was rinsed twice with PBS. Following incubation, a

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10ml portion of Minimum Essential Medium supplemented with 1μg/ml of colcemid was added to each test and control flask. The flasks were incubated for an additional 3h.
After harvesting, slides of the cells were prepared, stained with Giemsa, and examined microscopically for chromosomal aberrations at 100X magnification.

Treatment of results:
The percentage of cells with structural chromosome aberrations was calculated. The test article was evaluated only for structural aberrations and not for numerical aberrations. Aberrations include breaks (B), fragments (F), gaps (G), triadials(TR), quadriradial(QR), rings(R) and other category.

Evaluation of results:
There are several criteria for determining a positive result, such as a concentration-related, or a reproducible increase in the number of cells with chromosome aberrations. Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results. Statistical significant should not be the only determining factor for a positive response.
A test article, for which the results do not meet the above criteria is considered non-mutagenic in this system.

RESULTS

The average percentages of cells with aberrations are summarized in Table Ⅰ and Table Ⅱ. The positive and negative control performed as anticipated and met the criteria for a valid assay. There were no significantly statistical differences in the percentage of aberrant cells between the test extract groups and the negative control.

TABLE Ⅰ

AVERAGE PERCENTAGES OF CELLS WITH ABERRATIONS AND P VALUE

Groups(MEM)	Percent Cells with Aberrations
With Metabolic Activation（4h）	
Negative Control	0%
CP Positive Control	14.5% (P<0.01)*
Test A	0% (P> 0.05)
Test B	0% (P> 0.05)
Test C	0% (P> 0.05)
Without Metabolic Activation（4h）	
Negative Control	0%
MMC Positive Control	16% (P<0.01)*
Test A	0% (P> 0.05)
Test B	0% (P> 0.05)

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Test C	0% (P> 0.05)
Without Metabolic Activation（24h）	
Negative Control	0%
MMC Positive Control	15% (P<0.01)*
Test A	0% (P> 0.05)
Test B	0% (P> 0.05)
Test C	0% (P> 0.05)

*=Significantly different from the negative control

TABLE II

AVERAGE PERCENTAGES OF CELLS WITH ABERRATIONS AND P VALUE

Groups(DMSO)	Percent Cells with Aberrations
With Metabolic Activation（4h）	
Negative Control	0%
CP Positive Control	14.5% (P<0.01)*
Test A	0% (P> 0.05)
Test B	0% (P> 0.05)
Test C	0% (P> 0.05)
Without Metabolic Activation（4h）	
Negative Control	0%
MMC Positive Control	16% (P<0.01)*
Test A	0% (P> 0.05)
Test B	0% (P> 0.05)
Test C	0% (P> 0.05)
Without Metabolic Activation（24h）	
Negative Control	0%
MMC Positive Control	15% (P<0.01)*
Test A	0% (P> 0.05)
Test B	0% (P> 0.05)
Test C	0% (P> 0.05)

*=Significantly different from the negative control

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by any extrapolation of these data to other samples is the responsibility of the sponsor.

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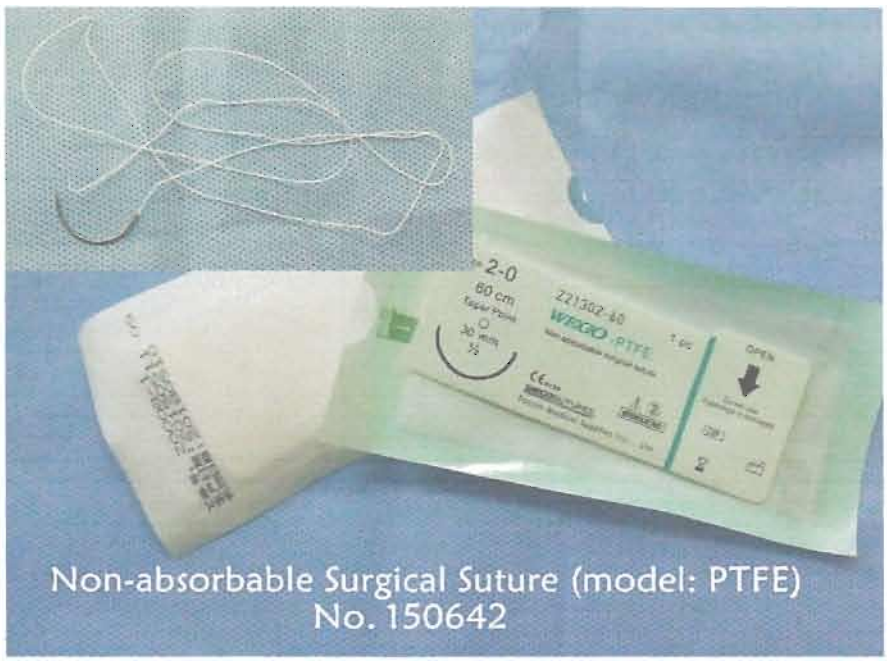
CONCLUSION

Under the conditions of this assay, the extracts of test article were not considered genotoxic to CHL cells in the presence or absence of S9 metabolic activation.

RECORD STORAGE

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

PHOTOGRAPH OF THE TEST ARTICLE



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