Final Report SAM3036-2i

CYTOTOXICITY BY ELUTION TEST

Study Program: SAM3036-2i

Contract n.: E05/0214.1MI

Sponsor: ANDROMEDICAL S.L.
MAR MEDITERRANEO 19
28220 MAJADAHONDA (MADRID)

Test substance: ANDRORING

Study Director..... Date: .....
(Dr. M. Levati)

INDEX

SUMMARY

On the test substance ANDRORING a toxicological study was carried out to evaluate the biocompatibility. The following test was performed:

- cytotoxicity by elution test

A culture of confluent NCTC L929 cells in exponential phase of growth was used in the **cytotoxicity by elution test**. For this purpose, an eluate was prepared by using a culture Medium.
The test sample has been prepared, in static conditions, as described below.
The eluate of the test substance was prepared by mixing the test substance 60 cm^2 into the culture Medium, in order to obtain a surface/volume ratio equal to 3 cm^2/ml.
Then the test sample was incubated at 37°C ±1°C for 72 hours.

2 ml of the extract of the test substance were placed in a CO_2 incubator together with an NCTC L929 cell culture for a period of 48 hours at 37°C ±1°C.
After 24 and 48 hours of incubation, the cell culture was observed with an inverted microscope to evaluate the biological reactivity.

After 24 and 48 hours of contact, in the treated cells with test substance none reaction was observed (reactivity grade 0.00).

On the basis of the results, interpreted according to ISO 10993-5:1999 and USP 28, the test substance ANDRORING can be considered **NON CYTOTOXIC.**

The detailed procedure is reported in Experimental Report SAM3036-2i.

INTRODUCTION

A toxicological study was carried out on behalf of the Sponsor ANDROMEDICAL S.L., in order to provide the necessary data to evaluate any local toxic effects.

The following tests were performed:

- cytotoxicity by elution test

The study was performed at the Assay Center Biolab S.p.A. of Vimodrone (MI) –via B. Buozzi n. 2.

The **cytotoxicity by elution test** started on December 09th, 2005 with eluate preparation and ended on December 14th, 2005 with the last observations.

BIBLIOGRAPHY

- ISO 10993-5:1999
Biological evaluation of medical devices
Tests for in vitro cytotoxicity

- USP 28, 2005

FILING

The study program and all its modifications, raw data and a copy of the final report and all its revisions will be retained in Biolab's archives for a period of 10 years from the issue of the final report.
A retained sample of the test substance has not been archived.
The Sponsor, upon drafting a suitable contract, may request an extension of the conservation of substances (or part of them) for a further period or their restitution.

PROCEDURES

All procedures used during this study are recorded in the Biolab Procedures Manual.

TEST SUBSTANCE

The test substance is a medical device intended to be used as an accessory of the product ANDROPENIS.

Name: ANDRORING

Material of the components: not provided

ANALISED SAMPLES

The sample representative of ANDRORING that has been analysed was a rubber ring.

Batch: 07/04

Expiry date: 06/03/06

Preparation date: not provided

Identification sample No: 05.23310

Receiving No.: R05497.05

Receiving date: December 06th, 2005

The characterisation of the test substance is under Sponsor responsibility.

Experimental Report SAM3036-2i

CYTOTOXICITY BY ELUTION TEST

SENIOR RESEARCHER: M. Levati

EXPERIMENTAL PROCEDURE

1. TEST METHOD

1.1 Characterisation

Mammal fibroblasts ATCC CCL1 NCTC Clone L929.

1.2 Materials and equipment

Colture medium L929

- 500 ml Minimum essential Medium Eagle with Earl's salts (EMEM) with Glutamine (Biowhittaker)
- 50 ml foetal bovine serum (Biowhittaker)
- 5 ml non-essential aminoacids (Biowhittaker)
- Plastic material for cell colture (PBI)
- Inverted Microscope Diavert (Lux octica)
- Laminar flow filtered work area (Flow)
- CO_2 incubator (Flow)
- USP Reference Standard negative control plastic (filaments) (Nova chimica)
- Latex batch 901796060 (Artsana)

2. EXPERIMENTAL DESIGN

The experimental design included 9 plates containing a confluent cell monolayer, subdivided in following groups:

GROUP	PLATE N.1	PLATE N. 2	PLATE N. 3
1 Positive control	2 ml of eluate of positive control	2 ml of eluate of positive control	2 ml of eluate of positive control
2 Negative control	2 ml of eluate of negative control	2 ml of eluate of negative control	2 ml of eluate of negative control
3 Treated	2 ml of eluate of test substance	2 ml of eluate of test substance	2 ml of eluate of test substance

2.1 Sample preparation

The test sample has been prepared, in static conditions, as described below.
The eluate of the test substance was prepared by mixing 60 cm^2 of the test substance (2 pieces) into 20 ml of the culture medium, in order to obtain a surface/volume ratio equal to 3 cm^2/ml.
Then the test sample was incubated at 37°C ±1°C for 72 hours.

2.2 Negative control preparation

The negative control was prepared by immersing 4 g of plastic USP reference standard negative control in 20 ml of culture Medium and then by incubating it for 72 hours at 37°C ±1°C.

2.3 Positive control preparation

The positive control was represented by immersing 4 g of Latex (batch 901796060) in 20 ml of culture Medium in order to obtain a weight/volume ratio equal to 0.2 g/ml and incubated for 72 hours at 37°C ±1°C.

3. TREATMENT

After preparing cell monolayer Petri plates wide 35 mm, the surfactant was removed and replaced with 2 ml of the test substance extract.
The plates were incubated at 37°C ±1°C in a 5% CO_2 atmosphere for 48 hours.
The same process was used for both positive and negative controls.

4. OBSERVATIONS

The cell monolayer was observed with an inverted microscope, after 24 and 48 hours of incubation.
The biological reactivities (cell degeneration and malformations) were evaluated after 48 hours of incubation with a scale ranging from 0 to 4 as shown in the following table:

Grade	Reactivity	Description of the reactivity
0	None	Fair intracytoplasmic granules, no cell lysis
1	Low	Not more than 20% of the cells are rounded and with no intracytoplasmic granules
2	Mild	Not more than 50% of the cells are rounded; intracytoplasmic granules are absent; presence of extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layer contains rounded and/or lysate cells
4	Severe	Clear and complete destruction of the cell layer

INTERPRETATION OF RESULTS

The test substance was classified according with the following key:

0 Non-cytotoxic
1 Slightly cytotoxic
2 Mildly cytotoxic
3 Moderately cytotoxic
4 Severely cytotoxic

According to USP 28, a reactivity grade $\leq$ 2.00 can be considered acceptable for the test substance.

RESULTS

After 24 and 48 hours of contact, in the treated cells with the test substance none reaction was observed (reactivity grade 0.00).

Reactivity grade at 24 hours	:	0.00
Reactivity grade at 48 hours	:	0.00

CONCLUSIONS

On the basis of the results, interpreted according to ISO 10993-5:1999 and USP 28, the test substance ANDRORING can be considered **NON CYTOTOXIC.**

CYTOTOXICITY BY ELUTION TEST

APPENDICES

Appendix n. 1: The cellular reactivity in each plate

TIME OF READING	PLATE N. 1			PLATE N. 2			PLATE N. 3		
	Sample	Cont. -	Cont. +	Sample	Cont. -	Cont. +	Sample	Cont. -	Cont. +
24 hours	0	0	3	0	0	4	0	0	4
48 hours	0	0	4	0	0	4	0	0	4

Grade	Reactivity	Description of the reactivity
0	None	Fair intracytoplasmic granules, no cell lysis
1	Low	Not more than 20% of the cells are rounded and with no intracytoplasmic granules
2	Mild	Not more than 50% of the cells are rounded; intracytoplasmic granules are absent; presence of extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layer contains rounded and/or lysate cells
4	Severe	Clear and complete destruction of the cell layer