Final Report SAM3036-1i

CYTOTOXICITY BY ELUTION TEST

Study Program: SAM3036-1i

Contract n.: E05/0214.1MI

Sponsor: ANDROMEDICAL S.L.
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28220 MAJADAHONDA (MADRID)

Test substance: ANDROPENIS GOLD (Metal Bar)

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

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SUMMARY

On the test substance ANDROPENIS GOLD a toxicological study was carried out to evaluate the cytotoxicity of the metal bar.

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 37.2 cm^2 into the culture Medium, in order to obtain a surface/volume ratio equal to 6 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 cells treated with the test substance a mild reaction was observed (reactivity grade 2.00).

On the basis of the results, interpreted according to ISO 10993-5:1999 and USP 28, the test substance metal bar of ANDROPENIS GOLD can be considered **MILDLY CYTOTOXIC.**

The detailed procedure is reported in Experimental Report SAM3036-1i.

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.

Name: ANDROPENIS GOLD

Material of the components: not provided

ANALYSED SAMPLES

The sample representative of ANDROPENIS GOLD that has been analysed was the metal bar.

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 SAM3036-1i

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 37.2 cm^2 of the test substance into 6.2 ml of the culture medium, in order to obtain a surface/volume ratio equal to 6 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 reactivity (cell degeneration and malformations) was 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 cells treated with the test substance a mild reaction was observed (reactivity grade 2.00).

Reactivity grade at 24 hours	:	2.00
Reactivity grade at 48 hours	:	2.00

CONCLUSIONS

On the basis of the results, interpreted according to ISO 10993-5:1999 and USP 28, the metal bar of ANDROPENIS GOLD can be considered **MILDLY CYTOTOXIC** and complies with USP 28 requirements for cytotoxicity of medical devices.

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	2	0	3	2	0	4	2	0	4
48 hours	2	0	4	2	0	4	2	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

Final Report SAM2817i-1

BIOLOGICAL REACTIVITY IN VITRO CYTOTOXICITY - ELUTION TEST

Study Program: SAM2817

Contract n.: E05/0137.4MI

Sponsor: ANDROMEDICAL S.L.
Mar Mediterràneo, 19
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MADRID – (ES)

Test substance: ANDRO-PENIS

Study Director..... Released on: .....
(Dr. P. Consonni)

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SUMMARY

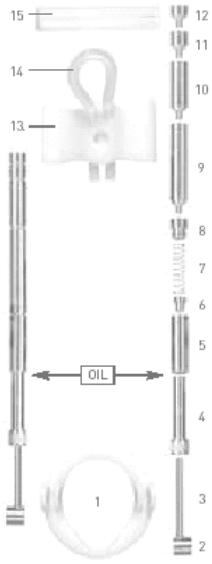
A toxicological study was performed according to ISO 10993-5:1999 on the test substance ANDRO-PENIS to determine the biocompatibility.

The following test was performed:

- cytotoxicity by elution

The analytical test was accomplished on the different materials which constitute the device and are in contact with the human skin:

1.	Plastic base ring	Plastic
2.	Rod (for the articulated screw)	Brass and Nickel
3.	Articulated screw	Brass and Nickel
4.	Adjustable bar screw	Brass and Nickel
5.	Metal bar	Brass and Nickel
6.	Screw	Brass and Nickel
7.	Spring	Brass and Nickel
8.	Screw to ground the spring	Brass and Nickel
9.	Large 4 cm axis	Aluminium alloy
10.	Medium 2 cm axis	Aluminium alloy
11.	Small 0.5 cm axis	Aluminium alloy
12.	Minimum 0.3 cm axis	Aluminium alloy
13.	Superior plastic support	Plastic
14.	silicone band	Silicon
15.	Andro-Top	Foam



The **cytotoxicity by elution test** was performed using a NCTC L929 cell culture in exponential phase of growth.
The eluates of the test material were performed in static conditions by immersing test material into Medium culture in order to reach a weight/volume ratio of:
0.2 g/ml for the PLASTIC (1 and 13)
0.2 g/ml for the FOAM (15)
in order to reach a surface/volume ratio of:
6 cm^2/ml for the ALLUMINUM ALLOY (9-12)
3 cm^2/ml for the SILICON (14)
6 cm^2/ml for the BRASSED and NICKELED COMPONENTS (2-8)

The test sample was then incubated at 37°C ±1°C for 24 hours.

2 ml of eluate of each test material were applied to the monolayer of NCTC L929 and it was incubated at 37°C ±1°C in CO_2 atmosphere for 48 hours.
After 24 and 48 hours of incubation the cells culture were observed to evaluate the biological reaction.

PLASTIC (1 and 13)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed (reactivity grade 0.00).

FOAM (15)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed (reactivity grade 0.00).

ALLUMINUM ALLOY (9-12)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed (reactivity grade 0.00).

SILICON (14)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed (reactivity grade 0.00).

BRASSED and NICKELED COMPONENTS (2-8)
After 24 of contact, in the treated cells with test material not more than 70% of the cell layers contain rounded cells and are lysed.
(reactivity grade 3.00).

After 48 hours of contact, in the treated cells with test material complete destruction of the cell layers was observed (reactivity grade 4.00).

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material PLASTIC (1 and 13)can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material FOAM (15)can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material ALLUMINUM ALLOY (9-12)can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material SILICON (14)can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material BRASSED and NICKELED COMPONENTS (2-8) can be considered **Severely CYTOTOXIC.**

The detailed procedure is reported in Experimental Report SAM2817i-1.A1.

INTRODUCTION

This study has been carried out on behalf on the Sponsor ANDROMEDICAL S.L. to evaluate the capacity of the test material and/or its eluable components, to induce local effects.
The following test was performed:

- cytotoxicity by elution

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

The **cytotoxicity by elution test** started on September 2nd, 2005 with eluate preparation and ended on September 7th, 2005.

BIBLIOGRAPHY

1. ISO 10993-5: 1999
   Biological evaluation of medical devices
   Test for in vitro cytotoxicity.

2. USP28 – NF 23
   <87> Biological Reactivity test, in vitro
   Elution test

RECORD FILING

The study program and all its modifications and amendments, 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 has not been kept.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the substances for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

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

TEST SUBSTANCE DESCRIPTION

The test substance is a device consisting of different parts made of plastic and metallic materials intended to human use in contact with the skin.

Name: ANDRO-PENIS

ANALYSED SAMPLE

The analysed sample, representative of the test substance, is identified by the following numbers:

Name: ANDRO-PENIS

Acceptance number: 05.16494

Receiving number: R03758.05

Receiving date: August 22th, 2005

Experimental Report SAM2817i.A1

BIOLOGICAL REACTIVITY IN VITRO CYTOTOXICITY - ELUTION TEST

SENIOR RESEARCHER: P. Consonni

EXPERIMENTAL PROCEDURE

1. TEST METHOD

1.1 Characterisation

Mammal fibroblasts ATCC CCL1 NCTC Clone L929.

1.2 Substances and equipment

Culture 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 substance for cell culture (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)
Natural rubber (positive control)

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 plastic	2 ml of eluate of positive plastic	2 ml of eluate of positive plastic
2 Negative control	2 ml of eluate of negative plastic	2 ml of eluate of negative plastic	2 ml of eluate of negative plastic
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 eluate of the test substance was prepared in static conditions by immersing:
1 piece (11.8 g) of PLASTIC (1 and 13) into 59 ml of Medium culture in order to reach a weight/volume ratio of 0.2 g/ml.
4 g of FOAM (15) into 20 ml of Medium culture in order to reach a weight/volume ratio of 0.2 g/ml.
6 pieces (75 cm^2) of ALLUMINUM ALLOY (9-12) into 59 ml of Medium culture in order to reach a surface/volume ratio of 6 cm^2/ml.
1 piece (39 cm^2) of SILICON (14) into 13 ml of Medium culture in order to reach a surface/volume ratio of 3 cm^2/ml.
2 piece (47.1 cm^2) of BRASSED and NICKELED COMPONENTS (2-8) into 7.8 ml of Medium culture in order to reach a surface/volume ratio of 6 cm^2/ml.

2.2 Negative control preparation

The negative control was represented by 120 cm^2 of plastic USP Reference Standard negative control put in 20 ml of culture Medium and incubated for 72 hours 37° ±1°C.

2.3 Positive control preparation

The positive control was represented by 120 cm^2 of natural rubber, put in 20 ml of culture medium and incubated for 72 hours at 37°C ±1°C.

3. TREATMENT

2 ml of cell suspension were pipetted from a confluent culture to 35 mm plates.
The plates were incubated at 37°C ±1°C in a 5% CO_2 atmosphere, allowing cell sedimentation and the constitution of a confluent monolayer.
From each plate the surfactant was removed and substituted with 2 ml of extract as reported in experimental design.
The same procedure was used both for the positive and the negative control.

4. OBSERVATIONS

After 24 and 48 hours of incubation the plates were observed with an inverted microscope.
The biological reactivity (cellular degeneration and malformation) is described and rated on a scale of 0 to 4 as reported in the following table:

GRADE	REACTIVITY	DESCRIPTION OF REACTIVITY ZONE
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells and/or are lysed
4	Severe	Nearly complete destruction of the cell layers

INTERPRETATION OF RESULTS

When the cellular cultures treated with the test substance showed more then one reactivity 0, the test should be repeated with several quantitative dilution of the extract.

The test substance was classified according with the following scale:

0 Non cytotoxic
1 Slightly cytotoxic
2 Moderately cytotoxic
3 Severely cytotoxic

RESULTS

PLASTIC (1 and 13)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed.

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

FOAM (15)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed.

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

ALLUMINUM ALLOY (9-12)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed.

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

SILICON (14)
After 24 and 48 hours of contact, in the treated cells with test material none reaction was observed.

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

BRASSED and NICKELED COMPONENTS (2-8)
After 24 of contact, in the treated cells with test material not more than 70% of the cell layers contain rounded cells and are lysed.

After 48 hours of contact, in the treated cells with test material complete destruction of the cell layers was observed.

Reactivity grade at 24 hours : 3.00
Reactivity grade at 48 hours : 4.00

CONCLUSIONS

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material PLASTIC (1 and 13) can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material FOAM (15) can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material ALLUMINUM ALLOY (9-12) can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material SILICON (14) can be considered **NON CYTOTOXIC.**

On the basis of the results, interpreted according to ISO 10993-5:1999, the test material BRASSED and NICKELED COMPONENTS (2-8) can be considered **Severely CYTOTOXIC.**

BIOLOGICAL REACTIVITY IN VITRO CYTOTOXICITY - ELUTION TEST

APPENDICES

Appendix n. 1:

Cellular reactivity in each plate for SUPERIOR PLASTIC SUPPORT (13)

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	3	0	0	3
48 hours	0	0	4	0	0	4	0	0	4

Cellular reactivity in each plate for Andro-Top (15)

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	3	0	0	3
48 hours	0	0	4	0	0	4	0	0	4

Cellular reactivity in each plate for AXIS (9-12)

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	3	0	0	3
48 hours	0	0	4	0	0	4	0	0	4

Cellular reactivity in each plate for SILICON BAND (14)

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	3	0	0	3
48 hours	0	0	4	0	0	4	0	0	4

Cellular reactivity in each plate for "BRASSED and NICKELED COMPONENTS" (2-8)

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

BIOLOGICAL REACTIVITY IN VITRO CYTOTOXICITY - ELUTION TEST

ADDENDUM

(4 Pages)