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Final Report SAM3036i-1 Rev1

BIOLOGICAL REACTIVITY IN VITRO CYTOTOXICITY - ELUTION TEST

Study Program: SAM3036

Contract n.: E05/0214.1MI

Sponsor: ANDROMEDICAL S.L.
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Test substance: ANDRO-PENIS GOLD

Study Director.....
(Dr. P. Consonni)

Released on: 08/31/06.....

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SUMMARY

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

The cytotoxicity by elution test was performed on samples of different materials which constitute the device and are in contact with the human skin:

The analytical test was accomplished on the

1. Plastic base ring	Plastic
2. Rod (for the articulated screw)	Golden Metal Bar
3. Articulated screw	Golden Metal Bar
4. Adjustable bar screw	Golden Metal Bar
5. Metal bar	Golden Metal Bar
6. Screw	Golden Metal Bar
7. Spring	Golden Metal Bar
8. Screw to ground the spring	Golden Metal Bar
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 cm²/ml for the ALLUMINIUM ALLOY (9-12)

3 cm²/ml for the SILICON (14)

6 cm²/ml for the GOLDEN METAL BAR (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 CO₂ 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 cells treated with the test material none reaction was observed (reactivity grade 0.00).

FOAM (15)

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

ALLUMINUM ALLOY (9-12)

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

SILICON (14)

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

GOLDEN METAL BAR (2-8)

After 24 and 48 hours of contact, in the cells treated with the test material a mild reaction was observed (reactivity grade 2.00)

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 GOLDEN METAL BAR (2-8) can be considered **Mildly CYTOTOXIC**.

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** were performed from September 2nd, 2005 to September 7th, 2005 for the following materials:

- PLASTIC
- FOAM
- ALLUMINUM ALLOY
- SILICON

The **cytotoxicity by elution test** were performed from December 09th, 2005 to December 14th, 2005 for the following materials

- GOLDEN METAL BAR

In this revised report are included also SAM2817-1m citotoxicity results .

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 and raw data 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.

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TEST SUBSTANCE

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 GOLD

ANALYSED SAMPLES

Samples of the following materials:

- PLASTIC
- FOAM
- ALLUMINUM ALLOY
- SILICON

Were obtained from a sample representative of the test substance submitted by the Sponsor, which is identified by the following numbers:

Name: ANDRO-PENIS
ID: 05.16494
Receiving number: R03758.05
Receiving date: August 22th, 2005

Samples of the following materials:

- GOLDEN METAL BAR

Were obtained from a sample representative of the test substance submitted by the Sponsor, which is identified by the following numbers:

Name: ANDRO-PENIS GOLD
ID: 05.23310
Receiving No.: R05497.05
Receiving date: December 06th, 2005
Batch: 07/04
Expiry date: 06/03/06

The characterisation of the test substance is under Sponsor responsibility.

***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)
- CO₂ 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 cm²) of ALLUMINUM ALLOY (9-12) into 59 ml of Medium culture in order to reach a surface/volume ratio of 6 cm²/ml.

1 piece (39 cm²) of SILICON (14) into 13 ml of Medium culture in order to reach a surface/volume ratio of 3 cm²/ml.

2 piece (47.1 cm²) of BRASSED and NICKELED COMPONENTS (2-8) into 7.8 ml of Medium culture in order to reach a surface/volume ratio of 6 cm²/ml.

2.2 Negative control preparation

The negative control was represented by 120 cm² 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 cm² 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% CO₂ 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.

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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 than 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

GOLDEN METAL BAR (2-8)

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

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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 GOLDEN METAL BAR (2-8) can be considered **Mildly CYTOTOXIC**.

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***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 GOLDEN METAL BAR (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	2	0	3	2	0	4	2	0	4
48 hours	2	0	4	2	0	4	2	0	4