**Final Report 2008/910-1 SAMi**

# *CYTOTOXICITY ELUTION TEST*

Study Program: 2008/910 SAM

Contract n.: E08/0432.1MI

Sponsor: ANDROMEDICAL S.L.
EDIFICIO -AMERICA II
28023 C/PROCION,N°7-NUCLEO 4
OFICINALIS I-L
MADRID– (ES)

Test product: ANDROPENIS GOLD - METAL BAR -

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Study Director: Paolo Pescio ..... Issued on: ..... Nov 24th 2008
(Paolo Pescio)

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## ***SUMMARY***

On test product "ANDROPENIS GOLD - METAL BAR -" was carried out a toxicological study based on EN ISO 10993-5:1999 aimed to evaluate any cytotoxicity effects.

The following test was performed:

- cytotoxicity by elution test

The **cytotoxicity by elution test** was performed using a NCTC L929 cell culture in exponential phase of growth. An extract using culture medium was prepared.
In dynamic condition the extract of the test product was performed by immersing the test product into culture medium in order to reach a ratio of 0,2g/ml.
Then the test sample was incubated for 72 hours at temperature of 37°C ±1°C.

2 ml of the extract were applied to the monolayer of NCTC L929 and 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.

After 24 and 48 hours of contact, in the treated cells it was found discrete intracytoplasmic granules, no cell lysis (reactivity grade 0).

On the basis of the results, interpreted according to ISO 10993-5:1999 the test product can be considered **NON CYTOTOXIC** and interpreted according to USP 31 – NF26 <87> the test product meets the requirements of the test (since the response is not greater than grade 2).

# ***INTRODUCTION***

This study has been carried out on behalf of the Sponsor ANDROPENIS S.L. on the product "ANDROPENIS GOLD - METAL BAR -".

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

The **cytotoxicity** test started on October 31st, 2008 and was completed on November 05th, 2008.

## BIBLIOGRAPHY

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

USP31 – NF26
<<87>> Biological reactivity in vitro test
- Elution test

## RECORD FILING

The study program and all 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.

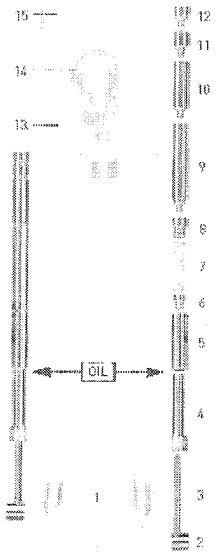
## PROCEDURES

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

## ***TEST SUBSTANCE***

The test product consists of a metal bar of the medical device Andropenis Gold.

Name: ANDROPENIS GOLD - METAL BAR -




metal bar (ID 5)

## ***ANALYSED SAMPLE***

The specimen analysed, representative of the test product consists of a gold metal bar.

Batch: 07/08

Preparation date: July 2008

Sample identification No: 08.10855-S

Receiving No: R05226.08

Receiving date: October 20th, 2008

*The characterisation of the test product is under Sponsor responsibility*

# *CYTOTOXICITY - ELUTION TEST*

SENIOR RESEARCHER: V. Freli

# *EXPERIMENTAL PROCEDURE*

## 1. TEST METHOD

### *1.1 Characterisation*

Mammal fibroblasts ATCC CCL1 NCTC Clone L929.

### *1.2 Materials 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 material 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)
Latex from glove

## 2. EXPERIMENTAL DESIGN

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

| GROUP | REPLAY N.1 | REPLAY N. 2 | REPLAY N. 3 |
|---|---|---|---|
| 1 Positive control | 2 ml of extract of positive plastic | 2 ml of extract of positive plastic | 2 ml of extract of positive plastic |
| 2 Negative control | 2 ml of extract of negative plastic | 2 ml of extract of negative plastic | 2 ml of extract of negative plastic |
| 3 Treated | 2 ml of extract of test product | 2 ml of extract of test product | 2 ml of extract of test product |

### 2.1 Sample preparation

In dynamic condition the extract of the test product was performed by immersing the test product into culture medium in order to reach a ratio of 0,2g/ml.

Then the test sample was incubated for 72 hours at temperature of 37°C ±1°C.

### 2.2 Negative control preparation

The negative control was represented by plastic USP Reference Standard negative control put into culture Medium and incubated for 72 hours at temperature of 37° ±1°C.

### 2.3 Positive control preparation

The positive control was represented by lattice, put into culture medium and incubated for 72 hours at temperature of 37°C ±1°C.

## 3. TREATMENT

From a confluent culture in 35 mm plates Medium was substituted with 1 ml, 0,5 ml and 0,2 ml of test material extract in physiologic solution and respectively 1 ml, 1,5 ml and 1,8 ml of culture Medium.
The plates were incubated at 37°C ±1°C in a 5% $CO_2$ atmosphere for 48 hours.
For negative control was used the same protocol using physiologic solution instead test material extract, For positive control Medium was replaced with 2 ml of latex extract.

## 4. OBSERVATIONS

After 24 and 28 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, according to USP as reported in following table:

| Grade | Reactivity | Reactivity description |
|---|---|---|
| 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; occasional lysed cells are present |
| 2 | Mild | Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells |
| 3 | Moderate | Not more than 70% of the cell layers contain rounded cells or are lysed |
| 4 | Severe | Nearly complete destruction of the cell layers |

## INTERPRETATION OF RESULTS

The test product is classified, according to ISO 10993-5 with following valuation:

| | |
|---|---|
| From 0 to 1 | Not cytotoxic |
| From 1 to 2 | Slightly cytotoxic |
| From 2 to 3 | Moderately cytotoxic |
| From 3 to 4 | Seriously cytotoxic |

## RESULTS

After 24 and 48 hours of contact, in the treated cells it was found discrete intracytoplasmic granules, no cell lysis.

Reactivity grade at 24 hours: 0
Reactivity grade at 48 hours: 0

Completely results are reported in Appendix 1.

Positive and negative controls meet the assay reliability.

## DEVIATION

No deviation has been recorded from study program.

## CONCLUSIONS

On the basis of the results, interpreted according to EN ISO 10993-5:1999, the test product "ANDROPENIS GOLD - METAL BAR -" must be considered **NOT CYTOTOXIC.**

## ***APPENDIX n°1:Cellular reactivity results***

| | TIME OF READING | 24 hours | 48 hours |
|---|---|---|---|
| Replay 1 | Positive Control | 3 | 4 |
| | Negative Control | 0 | 0 |
| | Sample | 0 | 0 |
| Replay 2 | Positive Control | 3 | 4 |
| | Negative Control | 0 | 0 |
| | Sample | 0 | 0 |
| Replay 3 | Positive Control | 3 | 4 |
| | Negative Control | 0 | 0 |
| | Sample | 0 | 0 |

| Grade | Reactivity | Reactivity desription |
|---|---|---|
| 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; occasional lysed cells are present |
| 2 | Mild | Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells |
| 3 | Moderate | Not more than 70% of the cell layers contain rounded cells or are lysed |
| 4 | Severe | Nearly complete destruction of the cell layers |