Final report of the study

CYTOTOXICITY STUDY PERFORMED ON THE PRODUCT: DLYTE DRY ELECTOPOLISHING DRYLYTE-DLYTE-01.

Sponsor: **GPA INNOVA**

Approved by

Area	Name / Signature	Date
Biolab S.L. Study Director. Colmenar Viejo, Madrid.	Sonsoles Mª Cuesta Sonsoles	16. Dec. 19

Table of contents

1 Title

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GPA INNOVA
Calle Caracas, 13-15 Nau 6.
08030 Barcelona.

3 Research center

Laboratorio BIOLAB S.L.
Avda. de los Reyes. Nave 59.
Polígono Industrial "La Mina".
28770 Colmenar Viejo, Madrid.

4 Personnel

Study Director: Sonsoles Mª Cuesta Sierra, PhD. in Pharmacy.
Contact details: Avda. Europa 13, Pozuelo de Alarcón, Madrid (Spain).

Principal analyst: Irene Amorós PhD in Pharmacy.

5 References

- UNE-EN ISO 10993-5:2009. Evaluación biológica de productos sanitarios. Parte 5: Ensayos de citotoxicidad in vitro. (ISO 10993-5:2009. Biological evaluation of medical devices. Part 5: Test for in vitro cytotoxicity).
- UNE-EN ISO 10993-12:2013. Evaluación biológica de productos sanitarios. Parte 12: Preparación de muestras y materiales de referencia. (ISO 10993-12:2012. Biological evaluation of medical devices. Sample preparation and reference materials).
- Internal procedures. Biolab S.L.

6 Product

The product of the assay was the following:

Product	**DLYTE DRY ELECTOPOLISHING DRYLYTE-01**
Batch	169
Material	Chrome-cobalt.
Description	The product is a material that has gone through a polishing process.
Reference	NOBILSTAR
Sample sent	12 pieces.
Manufacturer	GPA Innova
Storage and conservation	Room temperature

7 Performance dates

Date schedule of the different stages:

Approval protocol date:	28-Oct-19
Starting date of the test:	19-Nov-19
Final date of the test:	21-Nov-19
Final report date:	16-Dec-19

8 Cytotoxicity study

The objective of this study has been to establish the biocompatibility of the tested product by the evaluation of "in vitro" cytotoxicity, on mammalian cells. The cell viability is quantitatively determined through a vital staining.

The test is based on metabolic reduction of MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] by mitochondrial succinate dehydrogenase enzyme in a colored solution (formazan). This allows to determine the mitochondrial function of the treated cells. The number of living cells is proportional to the amount of formazan produced.

The study has been made according to the specifications of standard UNE-EN-ISO 10993-5:2009.

8.1 Test sample preparation

The sample was received, registered and stored following the established indications in the procedure LMPR-002-7 and it has been kept at room temperature.

The solid material to be tested was prepared as an extract following the specifications given in the standard UNE-EN ISO 10993-12: 2013, developed in the internal procedure LMPR-007-4, knowing that the relationship between sample surface / extraction vehicle volume (RPMI medium with serum at the concentration of 5x), must be 3 cm^2/ml for articles with a thickness >1 mm:

Total surface of 1 unit ≈ 4.8 cm^2

The extract was prepared using the necessary number of product units using RPMI medium with serum at the concentration of 5x as extraction vehicle. The set was subjected to heating at 37°C ± 1°C for 24 hours.

The sample extract obtained was diluted in sterile purified water/RPMI to obtain the finals concentrations of 100%, 75%, 50% and 25%.

As positive control, phenol solutions at 0.2%, 0.1%, 0.02% and 0.01% prepared at double concentration were used. These solutions were incorporated in the test by mixing them in equal parts with RPMI medium 2x.

As negative control, sterile purified water was used. This diluent was incorporated in the test by mixing it in equal parts with RPMI medium 2x.

As a blank control, RPMI 5x was used. The blank control was diluted to obtain RPMI 1x with sterile purified water.

The solutions obtained were used on the same day of collection.

8.2 Materials, reagents and equipment

Materials:

- Tissue culture plates 96 wells.
- Glass bottles.

- Centrifuge tubes.
- Sterile Pasteur tips and pipettes.
- Cell culture bottles T-25 and T-75.

Equipment:

- Digital balance.
- Centrifuge. Brand: Orto Alresa. Inv. No. 187.
- Inverted microscope. Brand: Lan Optics. Inv. No. 053.
- CO_2 Incubator. Brand: Nuaire. Range: 35-37°C. Inv. No. 054. Last calibration date: 11-2018.
- Incubator. Brand: Selecta. Range: 35-37°C. Inv. No. 043. Last calibration date: 11-2019.
- Spectrophotometer plate reader. Brand: Thermofisher. Inv. No. 161. Last calibration date: 01-2019.
- Laminar flow cabinet Telstar Bio-II-A/P. Inv. No. 055. Last qualification date: 05-2019.

Solutions and reagents:

- Cell line CCL 81 "Vero" (mammalian fibroblasts).
- Fetal bovine serum. Brand: Sigma. Batch: BCBS1251V. Expiry date: 03-2021.
- Sterile RPMI medium. Brand: Sigma. Batch: SLBW4454. Expiry date: 10-2020.
- PBS sterile solution. Batch: 2850. Expiry date: 01-2020.
- Tripan blue. Brand: Sigma. Batch: RNBF7486. Expiry date: 04-2022.
- MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] 0.5 mg/ml. Brand: Sigma. Batch: MKCF0652. Expiry date: 09-2022.
- 2-propanol. Brand: Sigma. Batch: STBH8142. Expiry date: 03-2024.
- Phenol. Batch: BCBZ3802. Expiry date: Feb-2024.
- Sterile purified water.

Instruments and equipment are under a calibration program that ensures the reliability of the obtained results. The temperature of the incubators has been monitored during all time of the study.

8.3 Experimental test procedure

Cells were cultured according to the procedure LTPB-005-3, to obtain enough quantity for the test. The test was performed with Nuclon plates of 96 wells. 10.000 viable cells were added per well leaving wells without cells as blank control of reagents.

1. 100 µl RPMI medium were added to all wells. Plates were incubated at 37°C with 5% CO_2 for 18-24 hours.

2. The next day, the medium was removed from the wells and it was added 100 µl of new medium. In this step, mixed with the new medium, it was added the test product, the diluent control and the positive and negative controls. The plates were incubated for 24 hours at 37°C in atmosphere with 5% CO_2.

3. Test product and controls used in the assay:

    a. Problem: extract in RPMI 5x was dissolved in sterile purified water/RPMI:

- 100%: 4 ml ext. in RPMI 5x + 16 ml sterile purified water.
- 75%: 3 parts ext. 100% + 1 part RPMI 1x.
- 50%: 2 parts ext. 100% + 2 parts RPMI 1x.
- 25%: 1 part ext. 100% + 3 parts RPMI 1x.

b. Negative control: 1 part of sterile purified water + 1 part RPMI 2x.

c. Positive control: Phenol solutions prepared at the concentration of 0.4%; 0.2%; 0.04% and 0.02% and mixed in equal parts with RPMI 2x for obtain the final concentrations of 0.2%, 0.1%, 0.02% and 0.01%.

d. Blank control: RPMI 5x was diluted to obtain RPMI 1x with and without cells.

4. After incubation of the sample and controls, the medium was removed and in each well 50 µl of MTT solution was added.

5. In order to allow the formation of formazan crystals, the plates were incubated for 2 hour at 37°C with 5% of CO_2.

6. MTT solution was removed and 100µl ml of isopropanol was added in each well to dissolve the formazan crystals.

7. The plate was mixed, was left 2-3 minutes at room temperature, was re-mixed and the absorbances of wells were read.

8 replicas of each concentration of the test product and 8 replicas of each one of the controls used have been performed.

Results reading

The results were obtained by spectrophotometric reading. The absorbance of each well was read by spectrophotometer at 570 nm. The average of the sample and controls absorbance obtained in the replicas was calculated.

The percentage of viability has been determined by the formula:

OD average of problem x 100 / OD average of blank control = Viability %

8.4 Results

The results obtained in test are attached as an annex of calculations.

8.5 Conclusions

Based on results, the product meets the acceptance criteria of study, i.e.:

- The viability has been superior than 70% in all concentrations tested compared to the blank control.
- The extract at 50% of the test sample has shown greater or equal viability than the extract at 100%.
- The mean value of blanks has not differed by more than 15% of the average value of all blanks.

The product can be considered non-cytotoxic.

9 Documentation and archive

Quality Assurance Unit (QAU) has inspected the complete study. It has conducted a protocol inspection established and approved, the raw data and the final report as well as processes and facilities to ensure that they have met the GLP standards in all points.

There has been no abnormal findings or any amendment to the protocol.

Biolab S.L. sent to the Sponsor one copy of the study approved by both sides.

In the Biolab S.L. archive are stored the original of the study protocol signed and approved by both sides, the original of the final report, all data and documents submitted by the Sponsor about the sample that have a confidential character, all the original raw and intermediate data generated during the test and the originals of the certificates of materials and equipment calibrations.

Final report of the study

CYTOTOXICITY STUDY PERFORMED ON THE PRODUCT: DLYTE DRY ELECTOPOLISHING DRYLYTE-DLYTE-MIX MSA-S.

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6 Product

The product of the assay was the following:

Product	DLYTE DRY ELECTOPOLISHING DRYLYTE-MIX MSA-S
Batch	2840
Material	Titanium.
Description	The product is a material that has gone through a polishing process.
Reference	TRITAN
Sample sent	4 pieces.
Manufacturer	GPA Innova
Storage and conservation	Room temperature

7 Performance dates

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