# Test Report

# Qualitative in vitro cytotoxicity MEM elution assay of “Walletmor Payment Implant with Vivokey biopolymer”

Study code: 21-45-1

Report Index: 21-45-1-SP.01

Date of issue: 24.05.2021





## 1. TYPE AND PURPOSE OF THE TEST

The scope of the study included testing of the cytotoxic properties of the test item carried out in accordance with the principles of ISO 10993-5:2009(E) and ISO 10993-12:2021(E) standards.

The aim of the study was to determine the cytotoxic properties of the test item provided by the Customer.

## 2. CONSENT OF THE LOCAL ETHICS COMMITEE

N/A

## 3. DATES

Experimental starting date: 17.05.2021

Experimental completion date: 21.05.2021

## 4. ADDRESSES

**Customer**

Name: Walletmor LTD

Address: 85 Great Portland St, W1W 7LT London, United Kingdom

**Test Facility**

Name: Konmex sp. z o.o.

Address: ul. Nałkowskiej 5, 05-410 Józefów, Poland

## 5. PERSONNEL

**Study Director**

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**Study Personnel**

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## 6. TEST ITEM

### a. Test Item

**Table 1. Information on test item.**

| | | |
|---|---|---|
| **Name:** Walletmor Payment Implant with Vivokey biopolymer | | |
| **REF:** N/A | **LOT:** N/A | **Item code:** 21-45-A |
| **Mfg date:** 01.01.2021 | **Exp date:** 01.01.2029 | **Sterile:** EO |
| **Storage conditions:** Temperature: normal, Humidity: N/A | | |
| **Intended use:** N/A | | |

Information above were submitted by the Customer. Responsibility for assuring proper conditions during transportation of the test items lied with the Customer. Results apply to the samples as received.

**Receiving date:** 2021-05-06

**Storage conditions:** Test item was stored at temperature 18-25ºC

### b. Reference material (if applicable)

N/A

### c. Reliability check

**Table 2. Information on reliability check.**

| | |
|---|---|
| Negative (K-) | HDPE |
| Positive (K+) | Latex |
| Blank (B) | Supplemented MEM |

### d. Extraction

**Table 3. Information on extracts.**

| No | Extract | Vehicle | Ratio | Time [h] | Temperature [ºC] |
|---|---|---|---|---|---|
| 1. | 7 x 21-45-A | Supplemented medium | 6 $cm^2$/mL | 72±2 | 37±1 |
| 2. | K+ | Supplemented medium | 6 $cm^2$/mL | 72±2 | 37±1 |
| 3. | K- | Supplemented medium | 0,2 g/mL | 72±2 | 37±1 |



## 7. TEST SYSTEM

**Description of the test system**

Cell line: L929
Passage no.: 26
Culturing: 37±1°C, 5±0,1%,

Medium:MEM supplemented with 10% FBS, 4 mM Glutamax, 100 IU/ml penicillin, 100 µg/ml streptomycin

Before the study, cells were passaged not less than twice after thawing.

**Justification of assay system**

The evaluation of the cytotoxic potential using MEM elution assay is described by the International Organization for Standardization - ISO: 10993-5:2009 (E), part 5.

**Animal identification (if applicable)**

N/A

**Quarantine (if applicable)**

N/A

## 8. SAMPLE PREPARATION

Before the study, the test item undergone no preparations. According to ISO 10993-12:2021(E), an extract of the test item in culture medium was prepared by immersing the device in the medium and extracted as indicated in Table 3. After extraction the extracts of the controls and test item were clear, with no observable particulates. Extracts were not filtered, centrifuged or otherwise altered and were used immediately after preparation.

## 9. DESCRIPTION OF TEST METHOD

Triplicate monolayers of L929 cells were dosed with 600 µL of extracts and incubated in the presence of 5±0,1 % $CO_2$ according to Table 3. Staining solution was prepared, just before use, by mixing Trypan Blue solution with supplemented MEM in 1:1 ratio. Following the incubation, 100 µL of prepared staining solution were dispended in each well. Afterwards, cytotoxicity was assessed by microscopic observations.



## 10. EQUIPMENT

**Table 4. Equipment used in the study.**

| No | Name | Serial No | Calibration Certificate No |
|---|---|---|---|
| 1. | Balance | 3536 | 5128/1446/20 |
| 2. | Laminar flow hood | 332 | 085 |
| 3. | Incubator | 15-17793 | 02/JK |
| 4. | Water bath | 17177 | WL54.2020.02 |
| 5. | Cell counter | 508BR08886 | WL46.2021.01 |
| 6. | Microscope | 1619957-1 | N/A |
| 7. | Centrifuge | 10251108815 | 03/JK |
| 8. | Microscope | 419098 | N/A |
| 9. | Pipetor | N/A | N/A |
| 10. | Pipette 10 µl | LE11810 | 21-03-02/04651 |
| 11. | Pipette 200 µl | MH11356 | SW/0101/2021 |
| 12. | Pipette 1000 µl | MD10937 | SW/0097/2021 |

## 11. MATERIALS

**Table 5. Materials used in the study.**

| No. | Name | Manufacturer | REF | LOT |
|---|---|---|---|---|
| 1. | MEM | Gibco | 51200-046 | 2162330 |
| 2. | Pen/Strep | Sigma Aldrich | P4458-100ML | 0000088375 |
| 3. | Glutamax | Gibco | 35050-061 | 2238969 |
| 4. | FBS | Gibco | 10270-106 | 2232584 |
| 5. | PBS | Gibco | 18912-014 | 2165328 |
| 6. | TrypLE Express | Gibco | 12604-021 | 2192947 |
| 7. | Trypan Blue Dye | BioRad | 1450013 | 64274014 |
| 8. | Trypan Blue | Sigma Aldrich | T8154-100ML | RNBJ4500 |
| 9. | HDPE | LG Chem | SM800F7 | 20141 |
| 10. | Latex | Mercator Medical | RC10003085 | 1906026B185 |
| 11. | Line Antybacteria 70 | Linegal Chemicals | LL-0001.2 | 100/W/2020 |



## 12. RESULTS

Cytotoxic potential was assessed based on ISO 10993-5:2009(E), Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity, Table 1 - "Qualitative morphological grading of cytotoxicity of extracts".

The achievement of a numerical grade 3 or greater is considered as cytotoxic effect.

Acceptance criteria: blank grade < 3

positive control grade ≥ 3

negative control grade < 3

test item extract grade < 3

**Table 6. Results of cytotoxic potential assessment.**

| Sample | Grade | |
|---|---|---|
| **Blank** | 0 | Valid |
| **Negative control** | 0 | Valid |
| **Positive control** | 4 | Valid |
| **Test item** | 0 | No cytotoxic potential |

## 13. DEVIATIONS

No incident that could have affected the quality of the raw data obtained was observed.

## 14. CONCLUSIONS

On the basis of the results interpreted according to ISO 10993-5:2009(E), the test item "Walletmor Payment Implant with Vivokey biopolymer (REF: N/A, LOT: N/A)" should be considered non-cytotoxic.

## 15. LIST OF RECORDS AND MATERIALS SUBMITTED TO THE ARCHIVE

- Raw data,
- Study report,
- Laboratory journal,
- Data on monitoring the testing environment - temperature and humidity records at the time of testing.

All the above-mentioned records and documents are to be filed to the archives of the research facility for 10 years after issuing the study report. The Test Facility does not store test items in the archive.



## 16. RELATED DOCUMENTS

- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22nd December 1978 and subsequent amendments,
- ISO 10993-5:2009(E), Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity,
- ISO 10993-12:2021(E), Biological evaluation of medical devices – Part 12: Sample preparation and reference materials.

| Study Director | Quality Assurance |
|---|---|
| Gołaszewska Kamila<br>2021-07-02 11:48:10 | Konrad Jankowski<br>2021-07-02 10:47:15 |