| | |
|---|---|
| FİRMA ADI :<br>Company Name | **FELİKS PLASTİK LAMİNASYON VE AMBALAJ MALZEMELERİ SANAYİ VE TİCARET ANONİM ŞİRKETİ** |
| ADRESİ :<br>Adress | **75. YIL OSB MH. 26. CD. NO:9<br>ODUNPAZARI - ESKİŞEHİR / TÜRKİYE** |
| TESTİN ADI :<br>Test Name | **In vitro Cytotoxicity Test** |
| TEST STANDARDI :<br>Test Standart | **ISO 10993-5** |
| LOT NUMARASI :<br>Lot Number: | **20200907** |
| TİCARİ MARKA (VARSA) :<br>Commerical Brand (If You Have) | **BODYGARD** |
| ÜRÜN ADI:<br>Name of the Product | **Bodygard Surgical Gown Level 3 - KNIT CUFF** |
| RAPOR NUMARASI:<br>Report Number | **2021-04/BIYO/1368FBG-002** |

** Felix Plastic companies tests were made in accordance with GLP (Good Laboratory Practice) standards.

BIOCOMPATIBILITY TEST RESPONSIBLE

Vet. Hekim Simge GARLI

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# CYTOTOXICITY TEST ANALYSIS REPORT

**TEST NAME**

**In Vitro Cytotoxicity Test Performed According to TS EN ISO 10993-5 Standard**

**TEST REQUESTED INSTITUTION AND SAMPLE NAME**

Felix Plastik - **Bodygard Surgical Gown**
**Level 3 -Knit Cuff Sample**

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## SUMMARY:

Biocompatibility testing according to TS EN ISO 10993-5 Biological evulation of medical devices - Part 5 : Extracorporeal cytotoxicity tests was performed on Bodygard Surgical Gown Level 3 Knit Cuff sample numbered 20200907. Samples are prepared in accordance with TS EN ISO 10993-12 Biological evaluation of medical devices - Part 12: Sample preparation and extraction according to the reference materials standard. L929 cells were seeded in 96-well plates and kept in culture for 24 hours to farm a semi-confluent monolayer. After 24 hours of exposure, the formation of the forest was determined for each treatment concentration and compared with the results determined in the control cultures. Growth inhibition percentage was calculated for each treatment. **There was no evidence of cytotoxic effect** on the cells of the sample extract under these working conditions.

## 1. INTRODUCTION

**Purpose:** This test was performed to evaluate the viability of the cells by mitochondrial dehydrogenesis in the case of indirect contact of the sample described below with mammalian cells.

**Test Guide:** This study was conducted according to the requirements of the International Organization for Standardization. 10993: Biological Assessment of Medical Devices, Part 5: Extracorporeal cytotoxicity assays.

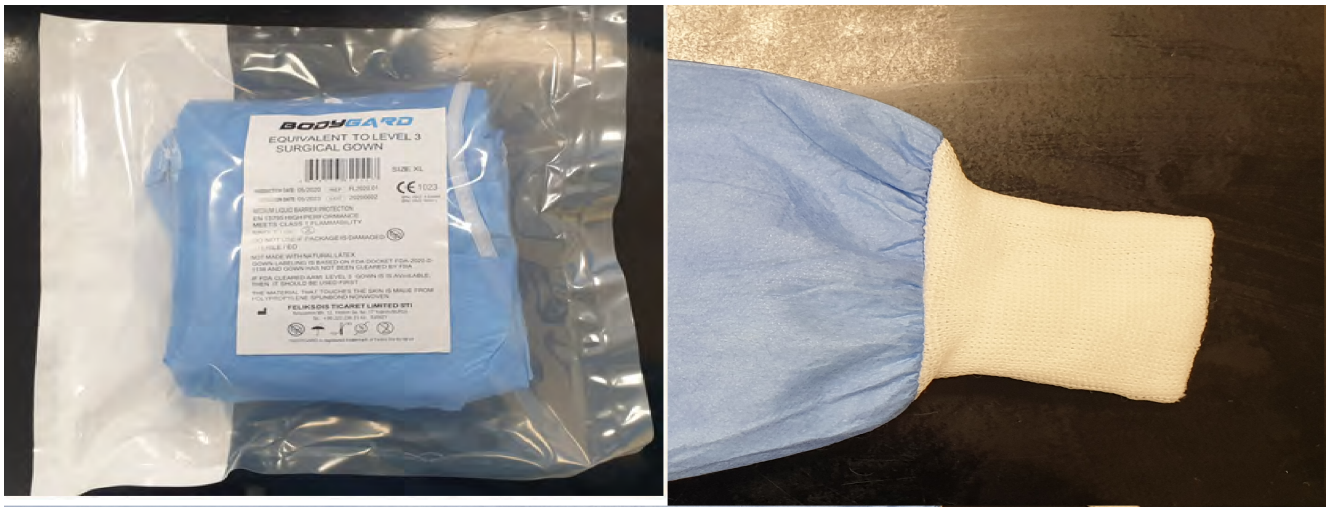
**Dates**

**Sample Acceptance Date:** **29.03.2021**
**Test Date:** **05.04.2021**
**Observation Date:** **05.04.2021 – 09.04.2021**

## 2. SAMPLE INFORMATION

| | |
|---|---|
| **Company Name:** | Felix Plastik |
| **Date of the Sample Acceptance:** | 29.03.2021 10:00 |
| **Sample Record Number:** | FPBG0004/2021 |
| **Sample Lot Number:** | 20200907 |
| **Number of Sample:** | 6 Pocket |
| **Packaging Information:** | CLOSED PACKED |
| **Delivery Method of the Sample:** | CARGO |
| **Expiration Date of the Sample:** | 09/2023 |
| **Production Date of the Sample:** | 09/2020 |
| **Description of the Sample:** | Surgical Gown Knit Cuff |
| **Characteristics of the Sample Use/Application:** | Surgical Gown level 3 - Knit Cuff sample |

**Numune Görseli:**



## 3. TEST SYSTEM

| | | |
|---|---|---|
| **Cell Line Used in the Test Line** | : | L2929 (Mice Fibroblast Cells)<br>DMEM+L-Glutamin, Fetal Bovine Serum,<br>Penisilin-Streptomycine |
| **Culture Medium** | : | Streptomycine |
| **Blank** | : | Sterile cell culture medium |
| **Negative Control** | : | Poliethylene Cryo Tube + Cells |
| **Positive Control** | : | Lanolin + Cells |

## 4. EXTRACTION METHOD

**Extraction Standard:** Extraction of the sample is carried out as specified in TS EN ISO 10993-12 standard.

**Extraction Procedure:** Samples taken from the sample are kept in a water bath that oscillates at a speed of 50 rpm at 37°C for 24 hours in 10% serum cell medium at the dimensions specified in the standard, extraction is terminated and used within 24 hours.

## 5. MATERIALS

| | | |
|---|---|---|
| **Devices and Raw Materials** | : | -20°C deep-freezer, -80°C deep-freezer, Laminaar Hood, Centrifuge, Micropipet, Invert Microscope, Liquid nitrogen, Carbondioxide incubator, Vortex |
| **Chemicals, Standard Materials** | : | MEM, DMEM<br>TOX2 in vitro toxicology assay kit,<br>XTT based mL-glutamin,<br>Penicillin-streptomycin Trypsin/EDTA,<br>DMSO, FBS, Phospate Buffer Saline Hemositometre lame,<br>Sodium hydrogen bicarbonate |

## 6. METHOD

### 6.1 Qualitative Evaluation

- Cells were seeded in 96-well plates and allowed to become confluent.
- In the next stage; Cells were exposed to – control + control and sample extracts in a 37°C 5% CO2 incubator for 24 hours.
- After incubation, microscopic examination was performed and evaluated according to TS EN ISO 10993-5 / XTT Cytotoxicity Test Standard.

### 6.2 Quantitative Evaluation

- According to TS EN ISO 10993-5 / XTT Cytotoxicity Test Standard, 96/ well plates were counted as 100 / well and incubated for 24 hours to ensure 80% confluency.
- In the next step, the sample was exposed to 1/1 - dilutions of the extract for 4 hours.
- At the end of the process, 1 mg / mL XTT was added to the wells and plates were incubated for 3 hours in a 37°C 5% CO2 incubator.
- The experiment was terminated by adding isopropylalcohol to the wells.
- Color change in plates was measured by spectrogotometer (570-650 nm) and % viability values were calculated.

## 7. EVALUATION

### 7.1 Qualitative Evaluation

Qualitative evaluation was made by taking into consideration Table 1 which was prepared considering TS EN ISO 10993-5 / XTT Cytotoxicity Test Standard.

**Table 1.** Qualitative morphological grading of cytotoxicity of extracts

| DEGREE | REACTION | CULTURE STATE |
|---|---|---|
| 0 | - | • Separate intracellular granules<br>• No cell destruction<br>• No reduction in cell proliferation |
| 1 | Verry Little | • more than 20% of the cells are non-round, poorly adherent, rarely contain cells that do not contain intracellular granules or show changes in morphology<br>• only mild growth inhibition is observed |
| 2 | Mild | • the number of round cells is less than 50%<br>• no intracellular granules<br>• no excessive cell destruction<br>• observable cell inhibition not more than 50% |
| 3 | Moderate | • the number of rounded and destroyed cells is not more than 70% of the cell layer<br>• Cell layers are not completely fragmented<br>• observable cell inhibition more than 50% |
| 4 | Severe | • all or almost all cell layers are destroyed |

**Table 2.** - control, + control and sample reaction and qualitative evaluation

| TEST MATERIAL | REACTION SCORE | QUALITATIVE CULTURES EVALUATION |
|---|---|---|
| NEGATIVE CONTROL | 0 | • Separate intracellular granules<br>• No cell destruction<br>• No reduction in cell proliferation |
| POSITIVE CONTROL | 5 | • all or almost all cell layers are destroyed |
| SAMPLE | 0 | • Separate intracellular granules<br>• No cell destruction<br>• No reduction in cell proliferation |

## 7.2 Quantitative Assessment

**Table 3.** XTT Test Results

| DILUTION RATE | | %100 | %75 | %50 | %25 |
|---|---|---|---|---|---|
| TEST SAMPLE | 1st TRIAL | 1,108 | 1,002 | 1,003 | 1,330 |
| | 2nd TRIAL | 1,023 | 1,071 | 1,171 | 1,298 |
| | 3rd TRIAL | 1,100 | 1,074 | 1,017 | 1,258 |
| | MEAN | 1.077 | 1.049 | 1.063 | 1.295 |
| POSITIVE CONTROL | 1st TRIAL | 0,146 | 0,176 | 0,222 | 0,280 |
| | 2nd TRIAL | 0,128 | 0,134 | 0,244 | 0,295 |
| | 3rd TRIAL | 0,136 | 0,112 | 0,265 | 0,213 |
| | MEAN | 0.136 | 0.140 | 0.243 | 0.262 |
| NEGATIVE CONTROL (%100 EKSTRACT) | 1st TRIAL | 1,138 | - | - | - |
| | 2nd TRIAL | 1,155 | - | - | - |
| | 3rd TRIAL | 1,156 | - | - | - |
| | MEAN | 1.149 | | | |

**Viab.%** = 100x OD450e/OD450b
**OD450e:** % 100 optical density average value of the sample extract
**OD450b:** Optical density average value of blanks
**Test Sample Viab.% = %93**
**Positive Control Viab.% = %11**
**Negative Control Viab.% = %108**

## 8. RESULT

Qualitative evaluation result; Based on the protocol and evaluation criteria specified in the standard TS EN ISO 10993-5 biological evaluation of medical devices, part 5, extracorporeal cytotoxicity assays; As indicated in Table 2, the negative control had no toxic effect on cells (0), the positive control had a high toxic effect (4), and the sample extract had no toxic effect. When the scoring indicated in Table 1 prepared in accordance with the criteria specified in the standard is taken into consideration, it has been determined that the test specimen "Bodygard Surgical Gown Level 3 - Knit Cuff sample" **does not have cytotoxic effect**.

Quantitative evaluation results; Based on the protocol and evaluation criteria specified in the standard TS EN ISO 10993-5 biological evaluation of medical devices, part 5, extracorporeal cytotoxicity assays; As stated in Table 3, when the negative control and positive control results are taken into consideration, it is seen that test validity criteria are met. In the experiment, the effects of 1/1 dilutions of the sample extract on the cells were examined and the viability was determined as 96% by the full dilution (1/1) of the sample extract. In line with the criteria specified in the standard; viability ratio was determined to be over 70% of the value obtained from the blind, so the test specimen "Bodygard Surgical Gown Level 3-Knit Cuff Sample" **was found to have no cytotoxic effect.**

## 9. RECORD

All raw data and a copy of the final report are stored in the Medicert archive files.

## 10. REFERENCES

- Guide For The Care And Use Of Laboratory Animals Eighth Edition National Research Council of The National Academies
- TS EN ISO 10993-1 Biological evaluation of Medical Devices - Chapter 1: Evaluation and experiment in a risk management process
- TS EN ISO 10993-5 Biological evaluation of Medical Devices – Chapter 5: Extra-body cytotoxicity assays
- TS EN ISO 10993-12 Biological evaluation of Medical Devices – Chapter 12: Sample preparation and reference materials