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HYGCEN GERMANY®

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Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten www.zlg.de ZLG-AP-314.10.23

Pro Seating BV
Rooseindsestraat 19

5705 BP Helmond

Cytotoxicity Test to EN ISO 10993-5:2009
SOP 09-001

2017-03-17

TESTREPORT

Identification of the test laboratory:	SN 23033
Delivery date:	2017-03-09
Product:	Pro Skin®
Customer:	Pro Seating BV
Test method:	Cytotoxicity of eluates according to the EN ISO 10993-5:2009 Biological evaluation of medical devices Part 5: tests for cytotoxicity: in vitro SOP 09-001
Test time period:	2017-03-14 until 2017-03-17
Test conditions:	Examining climate: 23,4 °C / 30 % rel. humidity Incubation: 24 hours The samples were checked in the delivery state.

Description of the method

Extraction conditions: 2,1 cm^2 material into 10,5 ml MEM + 9 % serum +1 % antibiotic solution at 37°C for 24 h = **extraction medium**

Cell culture

Fl-cells are derived from the human amnion. The stock cultures were carried out into 250 ml culture flasks (Sarstedt). The cells were trypsinised all 4 days. Only cells up to 100 passages were used.
Trypsinised cells were seeded in tissue culture plates.
The culture medium consists of MEM (Minimum Essential Medium) supplemented with 9 % calf serum, 1 % antibiotic solution (Penicilline G, Streptomycin sulfate, Neomycin) and L-glutamine.

Exposition

After 24hours of cultivation the cells were available as monolayer. A medium change with extraction medium was accomplished. Therefore the culture medium was decanted and the extraction medium carefully pipetted into the wells (0.1 ml per well).
An incubation for 24h is following.

Measuring principle

Vital cells incorporate the dye neutral red. Destroyed cells cannot incorporate the dye and remain unstained. The intensity of colour of the elution solution can be measured with a photometer.

Measurement

At the end of the incubation time the microtiterplate will be washed with PBS (Phosphate Buffered Saline). Culture medium containing the dye neutral red (50µg/ml) was given to the cells. After an incubation time of 3 hours the microtiterplate was washed again to remove the spare dye. With a special elution solution (1% acetic acid in 50% ethyle alcohol) the dye was solved out of the cells. After 1 hour of elution the photometric measurement was conducted.

Controls

As a negative control culture medium without a test solution was established.
To verify the sensitivity of the test system a positive control (1.5mg/ml Sodiumdodecylsulfate) in culture medium was exposed in the cell culture system.

Evaluation

The optical density of 12 parallel tests was determined and used for statistical evaluation.
A cell vitality of <70% relative to the negative control is considered to be significantly cytotoxic result.

Results

Figure 1: Box plot of the cellvitality

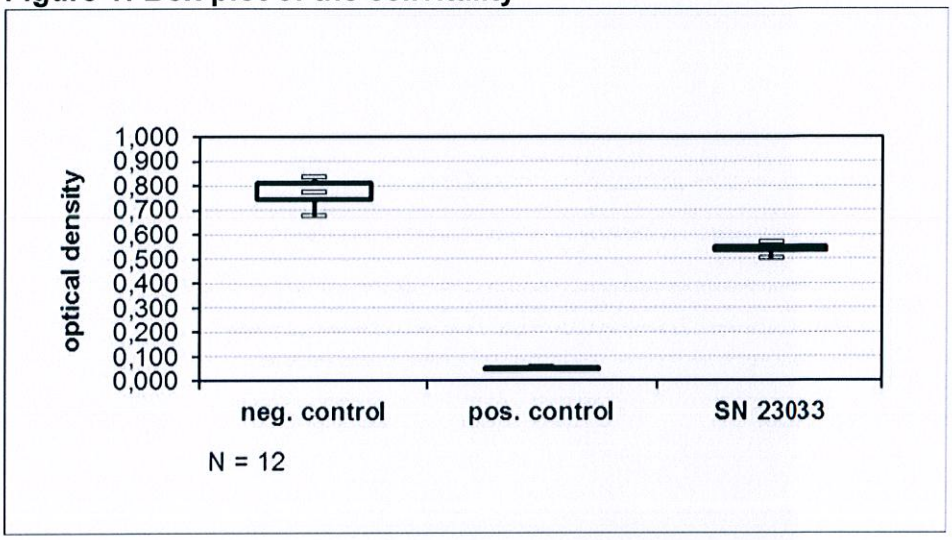

Table 1: Descriptive statistics (cellvitality)

	N	Mean	Cell vitality (%)	Minimum	Maximum	Std. Deviation	p*
Negative control	9	0,771	100,00	0,675	0,834	0,052	-
Positive control	9	0,053	6,83	0,049	0,058	0,003	-
SN 23033	11	0,540	70,00	0,500	0,566	0,021	0,8695

*U test (Man Whitney) vs. Control

Archiving: The raw data with respect to this test and a copy of the report will be stored in the archive of HygCen.

Information: The test results exclusively refer to the samples described above. Account of extracts of this test report is only possible by written approval from HygCen.

Prof. Dr. med. H.-P. Werner
Head of Scientific-Technical Affairs

Dipl. Umweltwiss. J. Köhnlein
Division Manager

Annex of testreport SN 23033 of 2017-03-17

Figure 2: Pro Skin®

Prof. Dr. med. H.-P. Werner
Facharzt für Hygiene

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Pro Skin®

Judgement

After testing the cytotoxicity of the Pro Skin® according to the EN ISO 10993-5:2009 -- testreport SN 23033 of 2017-03-17 I give the following statement:

An evaluation of the scope of biological testing was carried out as per EN ISO 10993-1:2009.

The intended use of the product, declared by the producer, involves contact with intact skin for a period of less than 24 hours. To evaluate the biocompatibility of the product, a cytotoxicity test as per EN ISO 10993-5:2009 was therefore considered sufficient.

Any knowledge to be gained from further biocompatibility testing with this product would not justify the unnecessarily high level of harm to experimental animals involved. As per EN ISO 10993-1:2009, chapter 4.6 and chapter 6.2.1 8, such tests will therefore not be performed in these cases.

The type and scope of the tests performed complies with the specifications as per EN ISO 10993-1:2009.

From the tested material only minimal cytotoxic compounds were extracted at 37°C. The extract of the test material reduced the cell growth to 70,00% of control. This is statistically not significant (testreport SN 23033, Fig. 1 and Tab. 1).

Using the test material as mentioned before described by the manufacturer no cytotoxic effects should be expected.

Prof. Dr. med. H.-P. Werner