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DAkkS Deutsche Akkreditierungsstelle D-PL-18818-02-01 D-PL-18818-02-02

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

BEO Berlin
Helmholtzstraße 2

D-10587 Berlin

2019-08-23

TESTREPORT

Identification of the test laboratory:	SN 27939b
Test ID No.:	2019-1772
Date of order:	2019-07-10
Delivery date:	2019-07-19
Product:	Levabo SP – 004, PE laminated nonwoven, (90 gsm White Laminate Fabric)
Customer:	BEO Berlin
Test method:	Biological evaluation of medical devices Cytotoxicity of eluates according to the EN ISO 10993-5:2009 Part 5: tests for cytotoxicity: in vitro Tests for irritation and sensitization according EN ISO 10993-10:2013 Part 10: Tests for membrane integrity SOP 09-001
Test time period:	2019-07-16 to 2019-07-18
Test conditions:	Examining climate: 21.3°C / 53% rel. humidity Incubation: 24 hours The samples were checked in the delivery state.

Description of the method

Extraction conditions: 60cm² material into 10ml MEM + 9% serum +1% antibiotic solution at 37°C for 24h = extraction medium

Cell culture L929-cells (ATCC CCL1) are derived from murine tissue. The stock cultures were carried out into 250 ml culture flasks (Greiner GmbH). 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 Lactatdehydrogenase (LDH), a stable cytoplasmatic enzyme, exists in all cells and will be released into the culture medium, if the cell membrane is damaged or in case of cell lysis. LDH reduces pyruvate to lactate, by oxidation of NADH to NAD^+. The uptake rate of NADH was determined photometric with a cinetic about 25 min.

Control As negative control culture medium without extraction medium was applied. To prove the maximum LDH-release Triton X was used as a positive control.

Evaluation The LDH-release of 6 parallel tests was determined and used for statistical evaluation. A LDH-release of <30% of the positive control shows no statistical significant damage of the cellmembranes.

Fig. 1: Release of LDH

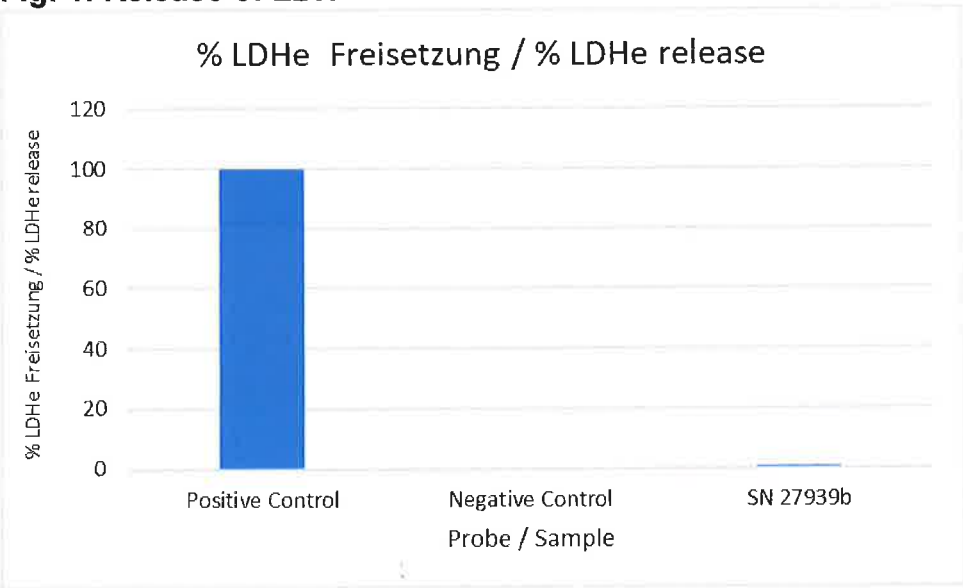

Table 1: Descriptive statistics

	N	LDH-release (%)	Standarddeviation
Triton X	6	100.0	0.13
Negative control	6	0.0	0.00
SN 27939b	6	0.6	0.01

Conclusion: The extract of the material „Levabo SP – 004, PE laminated nonwoven, (90 gsm White Laminate Fabric)" resulted in a LDH release of less than 30% in comparison to the control and is therefore considered to be not irritative.

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
Medical Devices

Dr. Michael Koch
Division Manager Biological Test Methods

Annex of test report SN 27939b of 2019-08-23

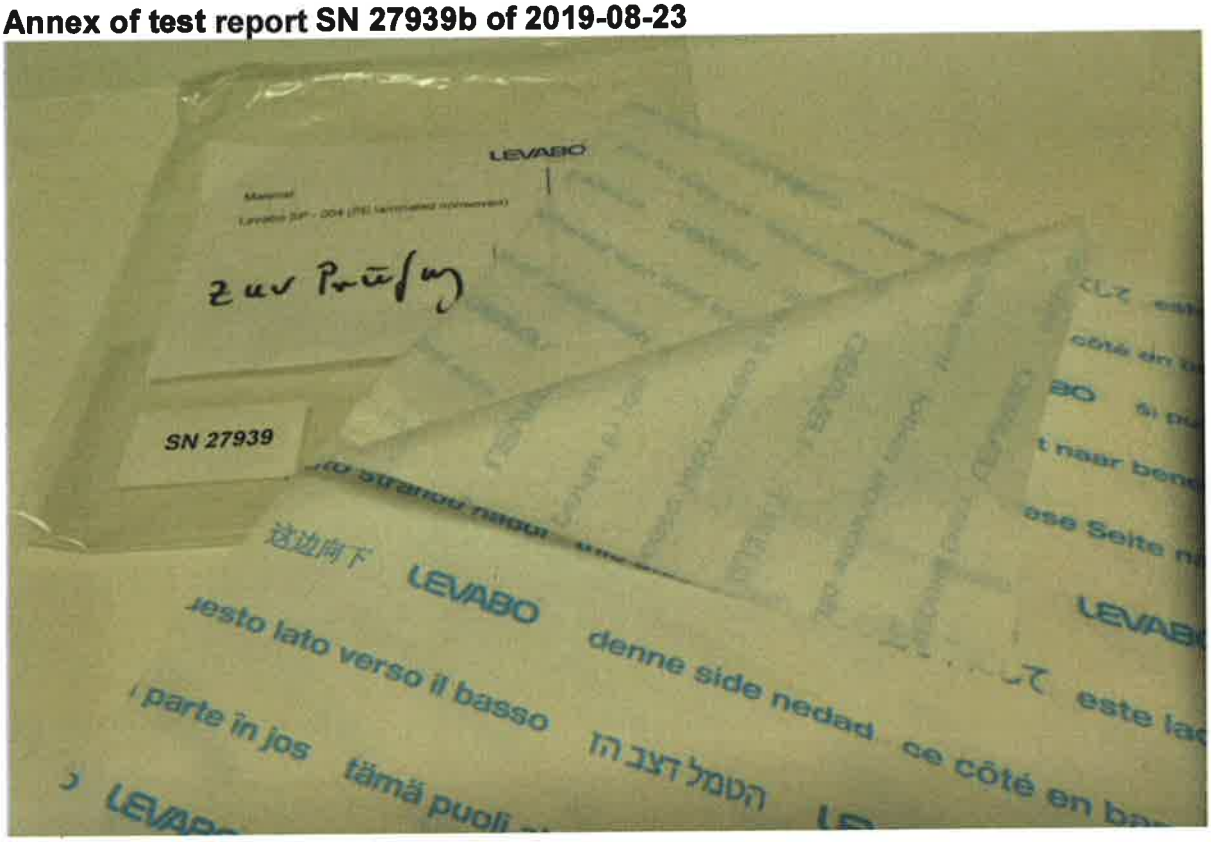

Figure 2: Levabo SP – 004, PE laminated nonwoven,
(90 gsm White Laminate Fabric)

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BEO Berlin
Helmholtzstraße 2

D-10587 Berlin

2019-07-22

TESTREPORT

Identification of the test laboratory:	SN 27939a
Test ID No.:	2019-1769
Date of order:	2019-07-10
Delivery date:	2019-07-12
Product:	Levabo SP – 004, PE laminated nonwoven, (90 gsm White Laminate Fabric)
Customer:	BEO Berlin
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:	2019-07-16 to 2019-07-18
Test conditions:	Examining climate: 21.3°C / 53% rel. humidity Incubation: 24 hours The samples were checked in the delivery state.

Description of the method

Extraction conditions:	60cm² material into 10ml MEM + 9% serum +1% antibiotic solution at 37°C for 24h = **extraction medium**
Cell culture	L929-cells (ATCC CCL1) are derived from murine tissue. The stock cultures were carried out into 250ml culture flasks (Greiner GmbH). 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 (0.1ml 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

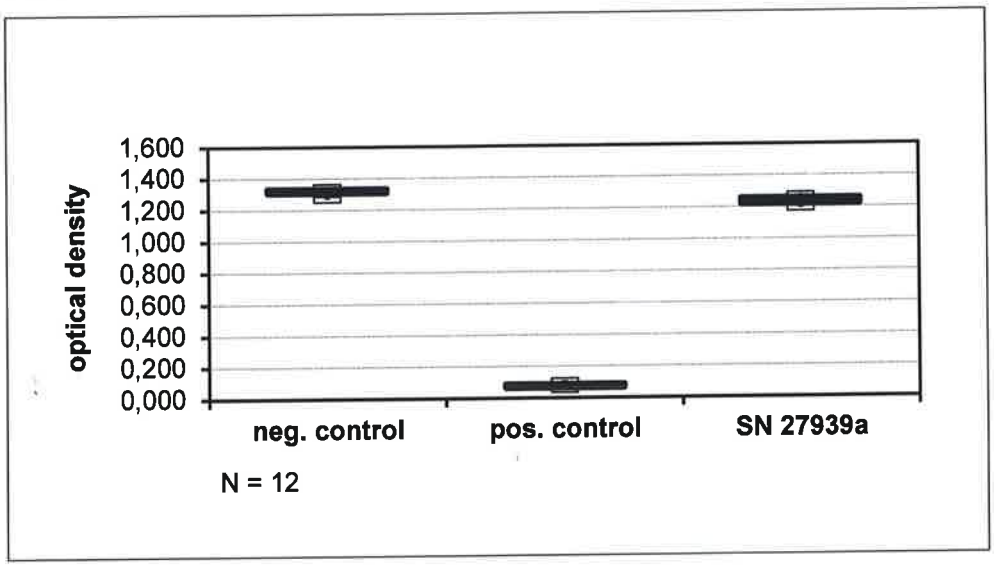

Table 1: Descriptive statistics (cellvitality)

	N	Mean	Cell vitality (%)	Minimum	Maximum	Std. Deviation	p*
Negative control	9	1.319	100.00	1.269	1.349	0.025	-
Positive control	9	0.076	5.75	0.054	0.109	0.017	-
SN 27939a	12	1.236	93.74	1.189	1.276	0.025	0.8286

*U test (Man Whitney) vs. Control

Conclusion: The extract of the material "Levabo SP – 004, PE laminated nonwoven, (90 gsm White Laminate Fabric)" resulted in a cell vitality of more than 70% in comparison to the control and can therefore be considered to be not cytotoxic.

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
Medical Devices

Dr. Michael Koch
Division Manager Biological Test Methods

Annex of test report SN 27939a of 2019-07-22

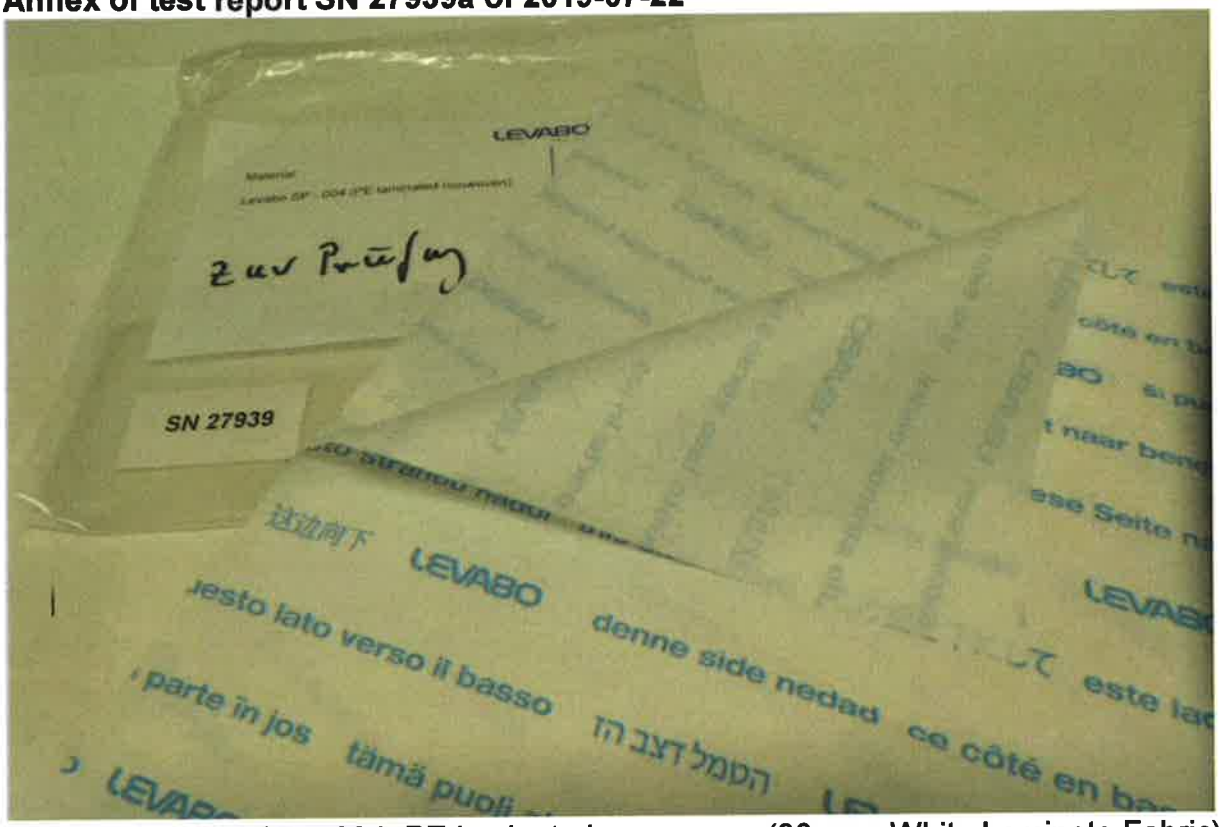

Figure 2: Levabo SP – 004, PE laminated nonwoven, (90 gsm White Laminate Fabric)

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Ihre Zeichen / Ihre Nachricht vom	Rückfragen an	Projekt	Datum
	Ines Dannemann	19-037 Heel Up Fix	05.09.19

Testreport SN 27939a and SN 27939b

Dear Mr Egelund,

Please find the original Testreport SN 27939a and SN 27939b for your documents.

Best regards

i. A. Ines Dannemann