Test Report

Sponsor	EnviteC-Wismar GmbH, Mr. Michael Schiffner, Alter Holzhafen 18, 23966 Wismar
Date of order	11-07-08/11-08-02
Test	Cytotoxicity, L 929-Proliferation EN ISO 10993-5, -12, LM P 4-06, LM SOP 4-06-01
Test material	T-adapter 22 mm/15 mm [Specified by the sponsor. Storage light-protected at 20-25 °C.]
Arrival of material	11-07-11
Study director	Dipl.-Ing. (FH) Tanja Halter
Beginning of study	11-07-21
End of study	11-08-16
Quality statement	This test was conducted according to Directive 93/42/EEC, 90/385/EEC, EN ISO/IEC 17025 (ZLG-P-870.96.08 accredited), and Good Laboratory Practices (GLP).
Data storage	All raw data of this study and a copy of this report in the archives of the supplier, samples of the test material by the sponsor.
Note	This report shall not be reproduced except in full without the written approval of Medical Device Services. The test results shown in this report relate only to the items tested.

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ZLG-P-870.96.08

Summary

The results indicate that the T-adapter 22 mm/15 mm does not release substances in cytotoxic concentrations during a permanent 24 h contact of 4.5 cm² surface area to 1 ml physiological fluid (1.5 % v/v dimethylsulfoxide in complete cell culture medium).

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Test method

In order to remove superficial dirtying, the test material was cleaned with a common used detergent solution and tap water and placed for 10 min into 70 % v/v isopropanol. The isopropanol was allowed to evaporate for 15 min. Afterwards, the test material was rinsed with distilled water and complete cell culture medium (DMEM-FBS). Then, it was extracted light-protected with 1.5 % v/v dimethylsulfoxide (DMSO) in DMEM-FBS for 24 ± 2 h at 37 ± 1 °C. A surface area to volume ratio of 4.5 cm²/ml extraction medium was used (corresponding to a final ratio of 3 cm²/ml in the cell culture assay). 1.5 % v/v DMSO in DMEM-FBS without test material was incubated 24 ± 2 h at 37 ± 1 °C as reagent control. DMSO (6.0 % v/v) was used as positive control (PK-DMSO). As further positive control polyvinyl chloride (PK-PVC) was extracted light-protected with DMEM-FBS 24 ± 2 h at 37 ± 1 °C. As negative control polyethylene was extracted light-protected with DMEM-FBS 24 ± 2 h at 37 ± 1 °C.

Test extract and reagent control were diluted in five steps with DMEM-FBS (dilution ratio 2:3). 100 µl of the dilution steps of the test extract and the reagent control as well as 100 µl of the positive control (PK-DMSO), respectively, were added in triplicates to wells of a 96-well tissue plate. The extracts of positive (PK-PVC) and negative control were tested in triplicates analogous the test extract. Then, 50 µl of a freshly prepared cell suspension (7.0×10^4 - 7.5×10^4 cells/ml) were seeded in all wells with the exception of wells used for background determination. The final concentrations of the test extract in the cell cultures were 66.7, 44.5, 29.6, 19.8, 13.2, and 8.8 % v/v. The tissue plate was incubated for 72 ± 6 h in humidified air (5 % CO_2/95 % air) at 37 ± 1 °C. Afterwards, the protein content of each well was determined colorimetrically at the wave length of 560 nm (BCA protein assay method).

As test organisms L 929 cells (DSM ACC 2, mouse connective tissue fibroblasts, clone of strain L) were used. The culture medium (Dulbecco's modified Eagle medium, DMEM) was supplemented with 10 % v/v fetal bovine serum (FBS), 100 U/ml penicillin (P), and 100 µg/ml streptomycin (S). DMSO (REF 1.02950) was purchased from Merck, Darmstadt, DMEM (REF FG 1445), FBS (REF S 0615), and P/S (REF A 2213) from Biochrom, Berlin, and BCA protein quantitation kit (REF UP40840A) from Interchim, France. The PVC press plate (RAU-PVC 7500) was purchased from Raumedic, Münchberg. The PE bag (bürkle SteriBag, REF 339 28 47) was purchased from Fisher Scientific Deutschland, Schwerte.

Calculation

The average absorption at 560 nm (A_{560}) with standard deviation was determined for each set of three wells. Per cent inhibition of proliferation was calculated as

follows:

$$\% \text{ inhibition of proliferation} = 100 - 100 \times \frac{(A_{560} \text{ sample}) - (A_{560} \text{ background})}{(A_{560} \text{ reagent control}) - (A_{560} \text{ background})}$$

The protein content is an indirect measure of cell proliferation respective to the inhibition of proliferation induced by toxic substances. An inhibition of proliferation of more than 30 % compared to untreated cultures (reagent control) is considered as a clear cytotoxic effect.

Result

Proliferation of L 929 cell cultures was not affected in presence of the test extract compared to untreated reagent control cultures (table). The results of the reagent control and the experimental controls confirm the sensitivity and accuracy of the test system.

In the present investigation the test material was extracted for 24 h at a physiological temperature of 37 °C. An organic solvent (dimethylsulfoxide) was added to complete cell culture medium and used as extraction medium in order to intensify the migration of toxic leachables. L 929 cell cultures were incubated for 72 h in presence of constant concentrations of the extract to ensure high sensitive detection of toxic effects on cellular level.

The results indicate that the T-adapter 22 mm/15 mm does not release substances in cytotoxic concentrations during a permanent 24 h contact of 4.5 cm^2 surface area to 1 ml physiological fluid (1.5 % v/v dimethylsulfoxide in complete cell culture medium).

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References

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Gesetz zum Schutz vor gefährlichen Stoffen (Chemikaliengesetz): 2002, Anhang 1, Grundsätze der guten Laborpraxis (GLP).

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DIN EN ISO 10993-5: 2009-10, Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity.

DIN EN ISO 10993-12: 2009-08, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials.

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Table

Cytotoxicity, L 929-Proliferation

Test material T-adapter 22 mm/15 mm

Extraction 4.5 cm²/ml 1.5 % v/v DMSO in DMEM-FBS, 24 ± 2 h, 37 ± 1 °C

		Relative protein content [$A_{560\ nm}$]						Proliferation inhibition [%]
		1	2	3	x	±	s	
Background		0.307	0.231	0.278	0.272	±	0.038	
Positive control		0.479	0.362	0.377	0.406	±	0.064	82
Reagent control	66.7 %	0.785	0.736	0.725	0.749	±	0.032	0
	44.5 %	0.833	0.823	0.797	0.818	±	0.019	0
	29.6 %	0.887	0.875	0.862	0.875	±	0.013	0
	19.8 %	1.044	0.917	0.908	0.956	±	0.076	0
	13.2 %	1.023	0.961	0.952	0.979	±	0.039	0
	8.8 %	1.111	0.986	1.004	1.034	±	0.068	0
Test extract	66.7 %	0.732	0.763	0.734	0.743	±	0.017	1
	44.5 %	0.840	0.836	0.797	0.824	±	0.024	0
	29.6 %	0.945	0.869	0.852	0.889	±	0.050	0
	19.8 %	0.958	0.922	0.927	0.936	±	0.020	3
	13.2 %	1.042	0.975	0.960	0.992	±	0.044	0
	8.8 %	1.075	1.000	1.003	1.026	±	0.042	1

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L 929 cell cultures were incubated for 72 ± 6 h in presence of the test extract (final concentrations in % v/v are shown). DMSO (final concentration 4 % v/v) was used as positive control (PK-DMSO). After incubation per cent inhibition of proliferation was calculated on the measured protein content values.