



TÜV Product Service GmbH
Masurenweg 1-3

30163 Hannover

**Cytotoxicity Test to DIN EN ISO 10993-5
SOP 09-001**

16.09.2002

Test Protocol

Sample number: SN 2785
Delivery date: 06.09.2002
Product: Handle 44, 2002-007, material PVC 50 Shore A
Customer: TÜV Product Service GmbH
Manufacturer: Firma B.Hermans, FIN-68601, Jakobstad-Pietarsaarie
Test method: Cytotoxicity of eluates according to the DIN EN ISO 10993-5, 1999-11
Biological assessment of medicine products
Part 5: Examination of Cytotoxicity
SOP 09-001
Test time period: 09.09. – 11.09.2002
Test conditions: Examining climate: 22°C / 25% rel. humidity
Incubation: 24 hours
The samples were checked in the delivery state.

Description of the method

- Extraction conditions:** 5 cm² material (approx. 1g) into 4 ml MEM + 1 % serum +1 % antibiotic solution at 37°C for 24 h = **extraction medium**
- cell culture** FI-cells are derived from the human amnion. 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 culture tubes.
The MEM culture medium containing 1 % antibiotic solution (endconcentration: 100 IE Penicilline G and 100 µg Streptomycin sulfate/ml) and 8 % calf serum supplemented with 70 % Lactalbumin hydrolysate.
The pH was adjusted to 7.2 with 1 M NaOH.
- Exposition** 1.5 ml of medium per tube was changed carefully after 72 h of culture. The MEM medium contains 1% antibiotics and 1% calf serum (control) which is also used as extraction medium (see above).
Following an incubation for 24 h was carried out.
- Take off / count the cells** The medium was decanted, the confluent cell culture was trypsinised with 0,3 ml enzyme solution (0,05% 1:250 trypsin and 0.02 % EDTA in PBS without Ca²⁺ and Mg²⁺). After 10 min incubation at 37°C the cells were resuspended in 1 ml MEM containing 1% calf serum and examined directly in an cell counter.
- Evaluation** The cell counts of 19 parallel tests were determined and used for statistical evaluation.

Results

Figure 1: box plot of the cells/ml

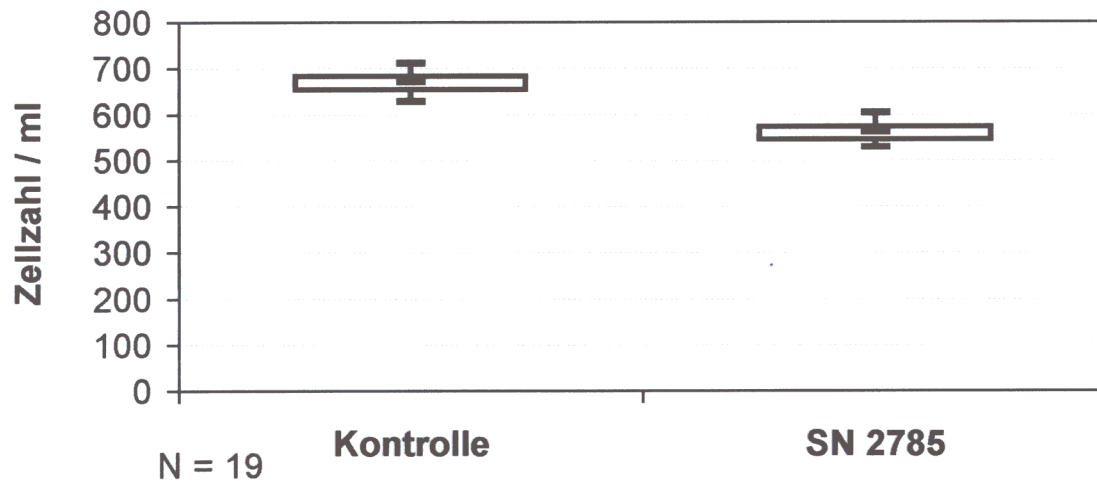


Table 1: Descriptive statistics (cells/ml)

	N	Mean	cell count (%)	minimum	maximum	Std. Deviation	p*
control	19	670	100,00	629	711	23,56	-
SN 2785	19	561	83,74	528	603	22,50	0,000

*U test (Man Whitney) vs. Control

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Handle 44, 2002-007, material PVC 50 Shore A

Judgement

After testing the Cytotoxicity of the handle 44, 2002-007, material PVC 50 Shore A according to the DIN EN ISO 10993-5, 1999-11-- our test report of 16.09.2002 (SN 2785) -- we give the following statement:

From the tested material only minimal cytotoxic compounds were extracted at 37°C. The extract of the test material reduced the cell growth to 83,74% of control. This is statistically significant. (Fig. 1 and Tab. 1).

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



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