THE BULL SPERMATOZOA VIDEO IMAGE CYTOTOXICITY TEST

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INTRODUCTION

The cytotoxicity test has been found to be the most sensitive of the biocompatibility tests. It is widespread for evaluating a large range of devices and materials. To perform a test, permanent mammalian cell lines are commonly used. Studies have shown that bull spermatozoa video image cytotoxicity test has performance characteristics which meet the requirements of Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity, 2001 and ISO 10993-5 Third edition 2009-06-01Biological evaluation of medical devices — Part 5:Tests for in vitro cytotoxicity. The bull spermatozoa video image cytotoxicity test yields results similar to the results obtained by means of cytotoxicity tests using permanent mammalian cell lines. Wherein the experiment is 3 hours, no laborious cell maintenance process (periodical thawing, subculturing and freezing), no labling procedure, no requirements for sterility of cell suspension and extracts, simple preparation of cell suspension and quality check of assay.

METHOD

Free from mycoplasma frozen bull spermatozoa are received from artificial insemination centers. For cytotoxicity test use batches of bull spermatozoa checked and meet sensitivity acceptance criteria: IC50 for sodium lauryl sulfate is within the interval 0,070 mg/ml to 0,116 mg/ml. One dose is needed for quality check of the whole batch. Less than 5% of the tested batches are rejected.

The bull spermatozoa video image cytotoxicity test work flow is presented in Table 1. To prepare cell suspension it is necessary to thaw one dose of frozen bull spermatozoa in glucose-citrate medium. Thawing process takes less than 10 minutes. The isotonicity of the test samples is adjusted by adding dry glucose and sodium citrate. Test and blank replicate samples are filled in disposable transparent glass flat capillaries of 200 micron channel height and of 25 mcl volume which are applied as chambers (see Figure 1). Capillaries are placed in a carriage which is installed in the Cytotoxicity analyzer (see Figure 2).

Bull spermatozoa suspension viability is evaluated by measuring suspension motility with real-time computer microscope video image analysis. Dynamic method of measurment permits to find the cytotoxic effect at early stages. Data automaticaly captured throughout the entire time course of an experiment (see Figure 3) are used for cytotoxic effect evaluation. The cytotoxic effect (toxicity index) is equal to the ratio of the weighted average time of spermatozoa suspension motility in the test sample to that in the blank sample.

RESULTS

11 recommended reference chemicals were tested to verify the adequacy of the bull spermatozoa video image cytotoxicity test. Figure 4 shows the Registry of Cytotoxicity (RC) prediction regression (black bold line) ± log 5 interval (black thin lines) and the 11 reference chemicals (blue triangles). The new IC50 /LD50 points obtained with the bull spermatozoa video image cytotoxicity test are shown (red squares) with the new linear regression line determined from these data (gray dashed line). The regression line obtained with the bull spermatozoa video image cytotoxicity test parallels the RC regression and is within the ±log 5 interval. Regression coefficients of the experimentally obtained new regression do not differ significantly from the literature-based RC regression equation:

RC regression: log (LD50) = 0.435 x log (IC50) + 0.625

New test regression: log (LD50) = 0.517 x log (IC50) + 0.259 [R2 = 0.9001]

The determination coefficient (R2) of the new regression is rather high. Data are comparable with the data for NHK NRU and 3T3 NRU tests.

To establish interlaboratory reproducibility IC50 for sodium lauryl sulfate (SLS) was measured in two laboratories for two different cell batches. Five independent repeat tests were conducted per laboratory. Interlaboratory reproducibility determined according to ISO 5725 is 0.035mg/ml. Mean IC50 for SLS is 0.078mg/ml. That is somewhat better than the results for 3T3 NRU and NHK NRU tests.

CONCLUSIONS

Results obtained indicate that the bull spermatozoa video image cytotoxicity test and permanent mammalian cell lines cytotoxicity tests have close metrological performances. In actual use the bull spermatozoa video image cytotoxicity test is much faster, cheaper and less labor-consuming than cytotoxicity tests using permanent mammalian cell lines. The effectiveness of the test is confirmed by the experience of its use in more than 160 laboratories within a few years.

Table 1. Bull spermatozoa video image cytotoxicity test work flow

Time, h:min	Procedure
00:00	Thaw one dose of bull spermatozoa suspension in glucose-citrate medium, 40 °C ↓
00:10	Prepare test-tubes containing 0,4 ml either of test sample or blank (glucose-citrate medium), 37 °C Per test-tube, add 0,1 ml of bull spermatozoa suspension, 37 °C Place samples into capillaries, 37 °C ↓
00:20	Measure bull spermatozoa suspension motility in 9,5 min interval, 37 °C ↓
02:50	Evaluate toxicity index, and variation coefficients

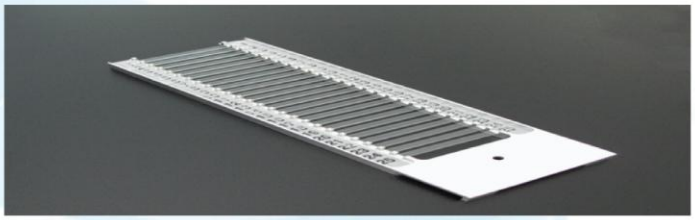

Fig. 1: Carriage with glass capillaries

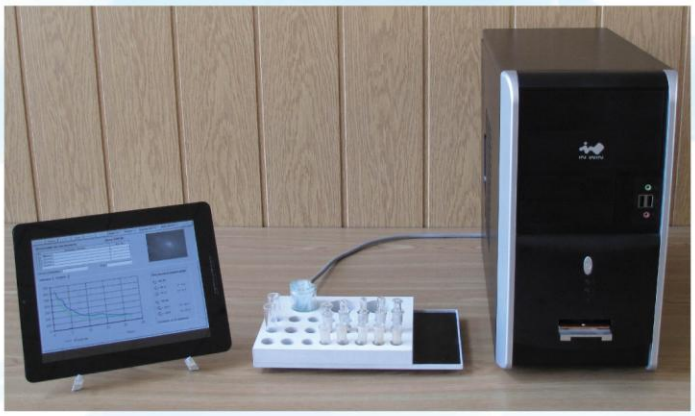

Fig. 2: Cytotoxicity analyzer

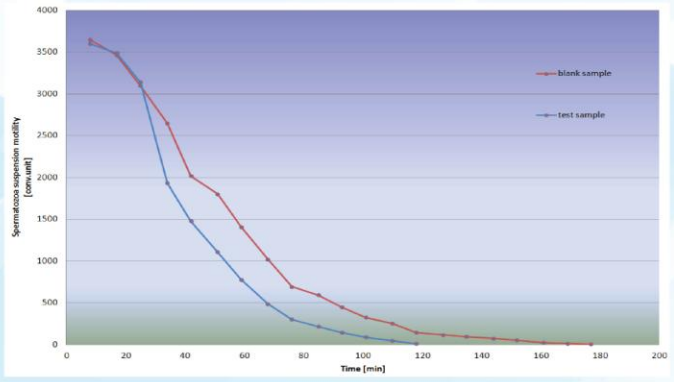

Fig. 3: Dynamics of bull spermatozoa suspension motility in the test and blank samples

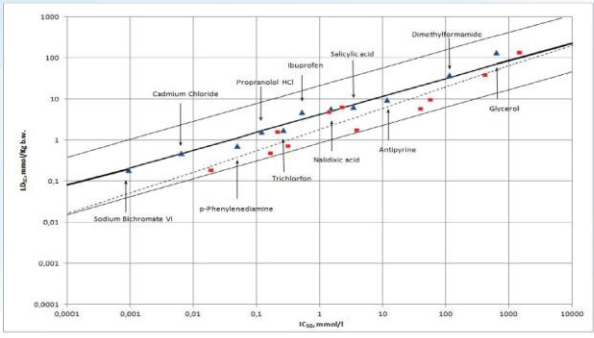

Fig. 4: The regression line obtained with the bull spermatozoa video image cytotoxicity test and the RC regression line