Quick Cellular Toxicity Testing

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INTRODUTION

Cellular toxicity testing is required for many practical and research toxicological applications. Usually, mammalian cells are seeded into well plates and maintained in culture for 24 h to form a semi-confluent monolayer. They are then exposed to the test compound. After 24 h exposure, cells are labelled and using different techniques the inhibition of growth percentage is calculated. Such single end point assays have some limitations. They are long-continued, labor intensive, involve labelling step. To overcome these disadvantages we have developed test method based on the influence of the test compound extract on bull spermatozoa suspension motility. Test method is performed on Cytotoxicity Analaser AT-05.

METHODS AND MATERIALS

It takes 10 minutes to defrost granule of the bull's frozen sperm in glucose-citrate media, prepare control and test samples, place them in disposable glass flat capillaries of 25 mcl (Fig. 1) and start motility measurement. Cell concentration is about $8 \cdot 10^5$ ml^{-1}. Temperature $(37 \pm 1)^{o}C$ is maintained. Motility of spermatozoa suspension is proportional to the moving spermatozoa concentration c_m and average modulus of the cell velocity v, $m = c_m v$. It is measured straight in suspension by means of real-time microscopic videoimage analysis. Five replicates of five test samples and control can be evaluated simultaniously. Such technique is harmless to the cells. The operator can control the process visually on monitor (Fig. 2). The values of bull spermatozoa suspension motility for reference and test samples are measured in equal intervals and accumulated (Fig. 3). In 3 h motility becomes close to zero and measurments are terminated. Capturing data throughout the entire time course of an experiment we exceed the limits of endpoint analysis and obtain more physiologically relevant data. Scoring for cytotoxicity is the value of the toxicity index which is equal to the ratio of the weighted average time of spermatozoa suspension motility in the test sample to that of spermatozoa suspension motility in the control sample. Inter-experiment variation is (10-15) %.

Table 1. Inhibiting concentrations IC_{50}

№	Compound	IC_{50} mmol/1	
		Bull spermatozoa	Literature data*
1	Methanol	790	930
2	Ethanol	560	379
3	Cyclohexanon	12.3	26.3
4	Phenol	9.55	3.01
5	Formaldehyde	0.04	0.12
6	Acetone	510	444

* The Registry of Cytotoxicity: Testing in Cell Cultures To Predict Apute Toxicity (LD50) and Reduce Testing in Animals ALTA 31, 89-198, 2003

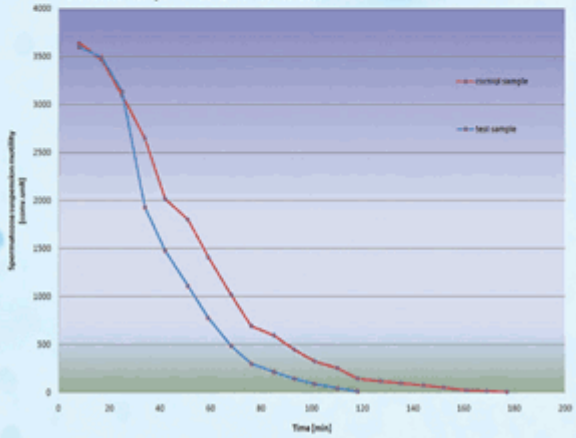

Fig.3 Cytotoxic effect of test compound extracton spermatozoa suspension motility

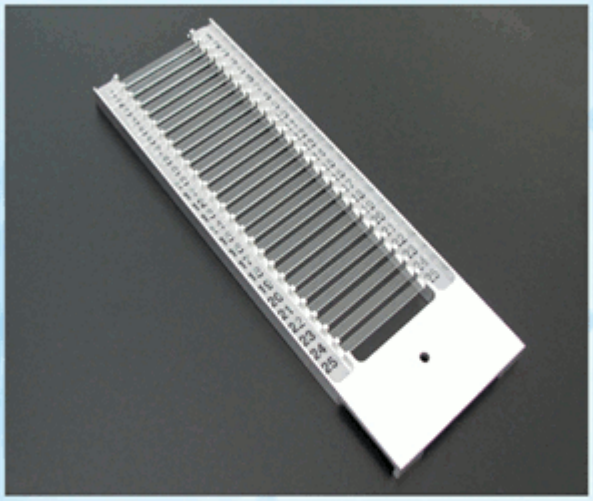

Fig. 1 Carriage with glass capillaries

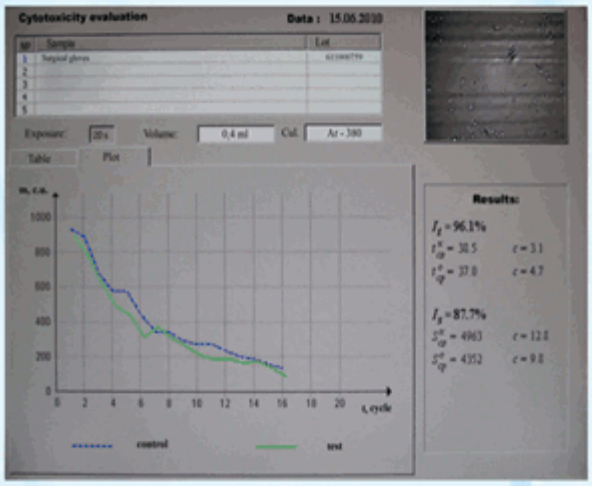

Fig.2 Measurement of spermatozoa suspension motility

RESULTS

Half inhibitory concentrations IC_{50} for some compounds were evaluated (Table 1). Results are very close to results obtained by the end point technology with different cell cultures. Correlation coefficient is about 0.75.

CONCLUSION AND DISCUSSION

The test method appeared to be simple, quick, dynamic, real-time, label free cytotoxicity assay. Usage of defrosted cells and structure peculiarity of bull spermatozoa (all of its mitochondria become aligned in a helix around the first part of the tail) permit to evaluate cytotoxicity in 3 h. The cytotoxic effect is found at early stage. Short duration of expirement permits to test not steril extracts. There are no requirements for sterilyty of materials, instruments and equipment used. Frozen cells in liquid nitrogen are stored unlimitedly and can be used at any moment. Pretesting time is reduced up to a few minutes. The possibility to get results in 3 h is comfortable for ready production testing. Quick cytotoxicity evaluation is especially necessary in emergency situations.