Determination of the cytotoxicity of potential medical substances by using three colorimetric assays for mammalian cells

"Bestimmung den Zytotoxizität auf die möglich Arzneimittel Substanzen mit drei farblich Messwert Technik imMammalia Zellen"

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INTRODUCTION

During the last decades many substances which have been obtained from animals or combinations of already known agents or chemically synthesized structures such as oligo - peptides, are part of the basic research projects as potential medical agents. All of them need to be extensively screened in order to discover their effect against cancer. The present study presents three different colorimetric methods (MTT (methyl – tetrazolium) assay, SRB (Sulforhodamide B) assay and CVE (crystal violet)) which are being used in order to measure the number of living cells at the end of the incubation period with the potential drug candidate. Furthermore, it has been shown the repeatability as well as the linearity of the results that are exported.

MATERIALS AND METHODS

In order the assays to be performed, three commercial cancer cell lines (obtained from breast (T47D), colon (HCT15) and lung (COLO699N) carcinoma) were used, plated and treated with a known anti-cancer substance for 0, 24 and 48h treatment, via its optical density at 570nm by using the appropriate equipment (μQuant™ Biomolecular Spectrophotometer and Gen5™ Microplate Data Collection & Analysis software, BioTek®). In order to subtract the noise and deviations a second wavelength of absorbance was studied. In MTT assay, the absorbance value at A570nm was corrected by a second measurement at 630nm. The same method was used for SRB and CV assay with an additional measurement at 690nm.

RESULTS

It has been shown that the results in both MTT and SRB as well as in CVE were repeatable and the correlation between absorbance and incubation time was linear (y=ax+b) with $R^2 \approx 0.85 - 0.99$. The three figures represent the results of the three assays concerning the effect of a chemo-substance in a 24h incubation period of time (1mg/ml).

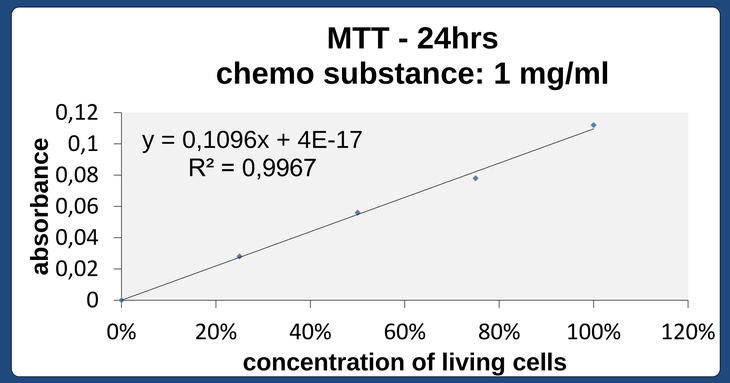

Figure 1. MTT assay. The graph represents the correlation between absorbance – concentration of living cells in COLO699N lung cancer cell line. The function that describes this correlation is y=ax+b with $R^2 \approx 0,9967$

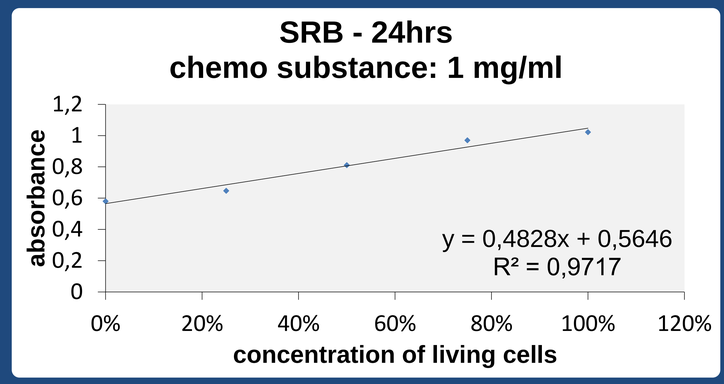

Figure 2. SRB assay. The graph represents the correlation between absorbance – concentration of living cells in COLO699N lung cancer cell line. The function that describes this correlation is y=ax+b with $R^2 \approx 0,9717$

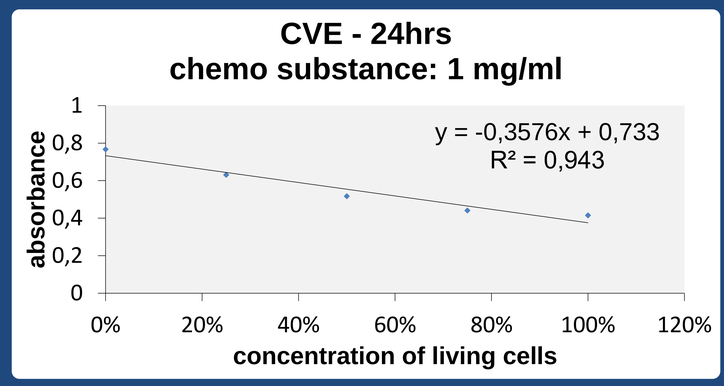

Figure 3. CVE assay. The graph represents the correlation between absorbance – concentration of living cells in COLO699N lung cancer cell line. The function that describes this correlation is y=ax+b with $R^2 \approx 0,9717$

CONCLUSION

It has been well –pointed out that the three chemosensitivity - cytotoxicity assays (MTT, SRB and CVE) are easy to be performed, sensitive, quick and the results are repeatable between them. The mathematical function that describes the exported results is y=ax+b.

Disclosure of Potential Conflicts of Interest

None of the authors of the above study has declared any conflict of interest

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