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Set-up an alamarblue based viability assay as an alternative method to routine MTT tests

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Background and Aims: Alamar-Blue is a water-soluble dye used for quantifying in vitro viability of various cells. Being extremely stable and more importantly non-toxic to the cells, continuous monitoring of cultures over days is possible by this method making it superior to tetrazolium (MTT) tests. The aim of our study was to set-up optimal conditions in running a viability test using AlamarBlue for MDA-MB-231 breast cancer cells.
Methods: Due to a difference in metabolic capacity of cells in reducing AB dye, optimum length of incubation and number of cells must be determined before performing viability/cell proliferation assays. To do so, MDA-MB-231 breast cancer cells were maintained in complete medium. A suspension of 1×106 cell/ml and further dilutions were seeded into 96- well plates (200 µl for each well). Medium without cell was used as negative control. 20 µl AlamarBlue was added to each well after 4 h and absorbance was directly measured immediately and every 2 h at 570 and 600 nm for the first 6 h as well as further 24 h of incubation using plate reader.
Results: AlamarBlue posed no toxic effects on cells as shown by cell morphology. Percentage reduction of the dye was calculated using the online colorimetric calculator provided by manufacturing company (www.abdserotec.com). The standard curve of %AlamarBlue reduction plotted against the logarithm of cell concentrations and time points was linear at 1×105 cell/ml and 4 h of incubation.
Conclusions: AlamarBlue dye was successfully used to measure viability of MDA-MB-231 cells over time when proper cell concentration and incubation time were determined. Not being toxic, viability of cells could be monitored over 24 h making kinetic viability assays possible. Moreover, solubility of the dye which obviates further crystal solubilization with DMSO increased convenience and sensitivity of the test.

Keywords: Alamar-Blue; Standardization; Viability; Sensitivity