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LETTER TO THE EDITOR

MTT assays can underestimate cell numbers

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Sir,

The use of the yellow tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) as an indicator of cell proliferation and viability in in vitro studies have increased. The simplicity of the methodology and the rapidity at which results are attained, make the MTT assay a useful operator-independent method of studying cell number [1]. The principle premise of the assay is the intrinsic ability of mitochondrial dehydrogenases to reduce MTT to a purple-coloured formazan, which is detected colorimetrically. This renders the assay a choice for many groups studying drug activity in high-throughput screening studies. Therefore, we have read with interest the report by Simms and Plattner [2] who describe interference of the MTT assay when using imatinib. Specifically, the effects of imatinib were studied in vitro using tritiated thymidine and by the MTT assay. Most worryingly, their data reported that although imatinib reduces cell numbers and increases apoptosis in a dose-dependent manner, the MTT assay showed a dose-dependent increase in cell number. The authors concluded that the MTT assay was a inappropriate method for testing the activity of the drug in solid tumours. Indeed, this is our experience, and the reason why we did not employ the assay in our studies with imatinib [3].

Another consideration that was not mentioned in the report was that the reduction of MTT may not be entirely dependent upon cell number, but also upon the mitochondrial function and proliferative state. To highlight this possible limitation, non-adherent cell lines CEM, HL60 and

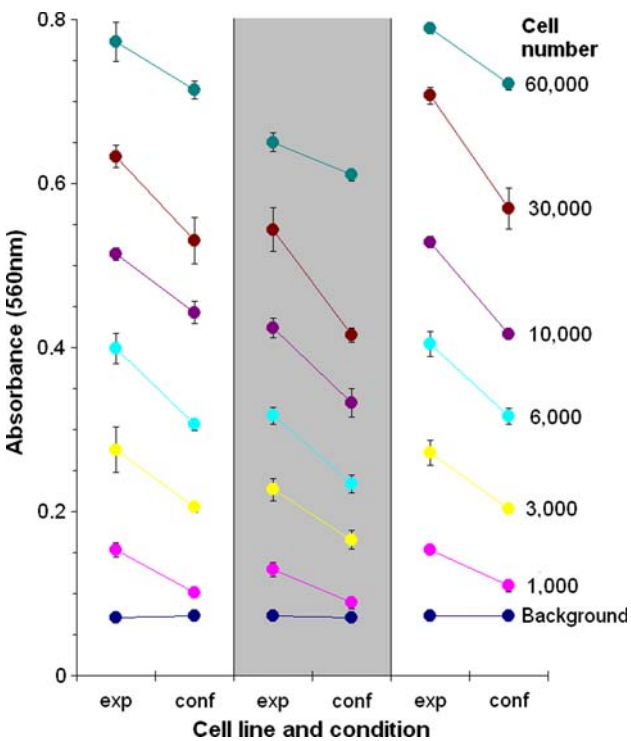

Fig. 1 The effect of cell quality on the MTT assay. CEM (*left*), HL60 (*middle*) and MOLT4 (*right*) cells were reset in 96-well plates at the densities indicated. The growth states of the cells were either exponential (*exp*) or confluent (*conf*). Cells were then subject to the MTT assay, and absorbance at 560 nm assessed. Each data point represents the mean and SD of at least three separate experiments

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MOLT4 were reset in 96-well plates at cell concentrations of between 0.1 and 6×10^4 cells/well. Two distinct conditions of the cells were used—cells that were in exponential growth and those at confluence (cells growing in basal media for 5 days, with a concentration of $>1 \times 10^6$ cells/mL). Cells were allowed to equilibrate to 37°C in an incubator with a humidified atmosphere for 30 min, prior to addition of 0.4 mG/mL MTT and a further incubation for 90 min. Cells were pelleted and washed in ice-cold PBS before dissolving formazan crystals in di-methyl sulphoxide and reading absorbance at 560 nm.

Results suggested that the MTT assay may underestimate cell numbers if the cells studied are positioned towards the upper end of the growth curve—where high cell densities and sub-optimal growth conditions would hinder endogenous enzyme function. Figure 1 shows the average optical densities (ODs) of the wells containing the cells at 560 nm. Although there were equal amounts of cells plated in each well, there was a significantly higher ODs in the cohorts of cells growing at exponential phase ($p < 0.001$—paired t tests). Furthermore, as MTT-values attained from cells treated with drugs are usually compared with the results from untreated cells, it is likely that drug action may be over-estimated.

Taken together, in studies that involve the enumeration of cell number by the MTT assay, appropriate controls should be performed to ensure correlation with other methodologies.

Conflict of interest statement The authors have no financial disclosures/conflict of interest.

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