A new method to improve microplate selection criteria for cell assays

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Abstract

The selection of the microplate brand and type to be used with a cell assay poses many challenges. It is common when running the same assay in multiple brands to find differences in readout in a Z' run as well as differences in SD values, when adjusted for assay mean (many labs use CV = SD/Mean).
A selection can come down to which plate provides the best signal to noise ration which to many labs indicates maximum cell growth. We are going to suggest a method to augment current approaches that precisely measures consistency of plate noise and read out.

Introduction

In research IonField has conducted to better understand how microplates interact with assays, it was found that with today's high resolution assay technologies, minor inconsistencies of microplates add to the noise in cell assays. It has long been known that pharmaceutical packaging, where the contents have long exposure time to polymers, chemicals on and near the surface leech into the liquid and are implicated as the cause of cellular toxicity, carcinogenicity and decreased stability of enzymes and other proteins. As microplate densities has increased, the wall surface to well volumes have increased, increasing chemical concentrations in reactions. Considering that assays continue to have increasing sensitivities, it is only a matter of time before there is a convergence where interference is a routine problem.

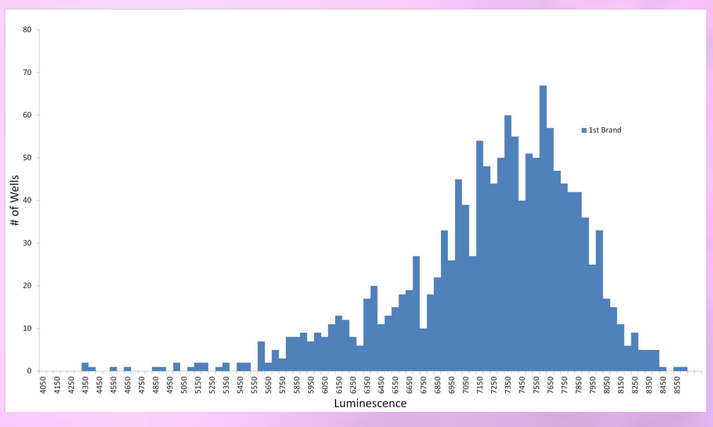


Figure 1. Graph of 1536 results from a uniform Z' assay. All wells should report the same result.

ToF-SIMS Analysis

Time of Flight Secondary Ion Mass Spectrometry measured the surface chemicals on various brands of microplates. The data showed considerable differences between brands. Those differences resulted in large differences in the level of assay precision, measured by increased CVs.
One brand had elevated levels of positive ions: C3H5, Al, Ca, Zn, EBS (C20H44NO), C21H44NO, IRGAFOS (C42H46PO3) and reported CVs roughly 2X the other brands in the testing. EBS is a mix of animal and vegetable fats. IRGAFOS is a secondary antioxidant and analogue of a pesticide.

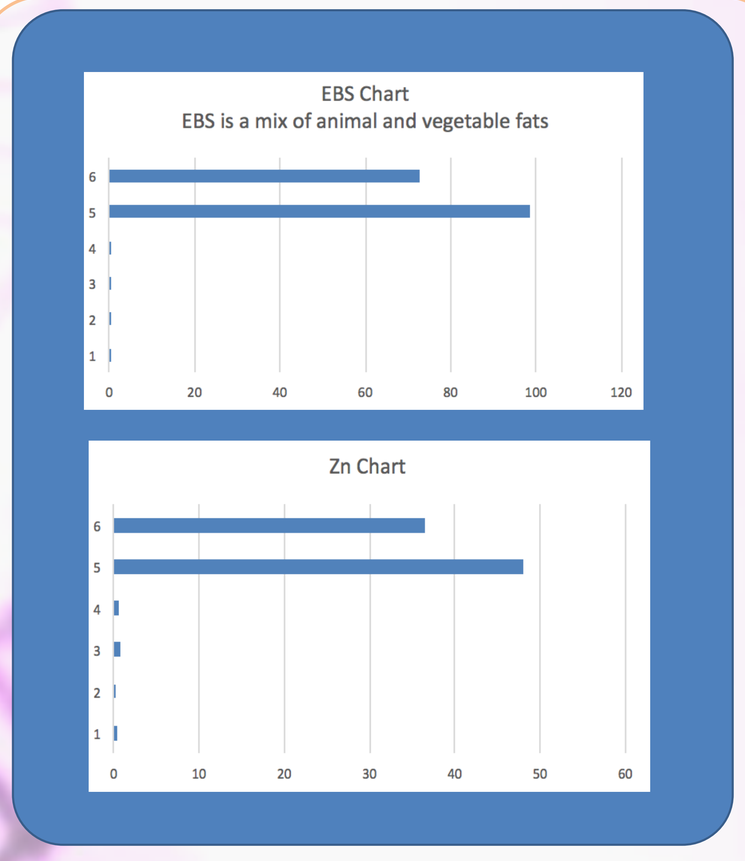
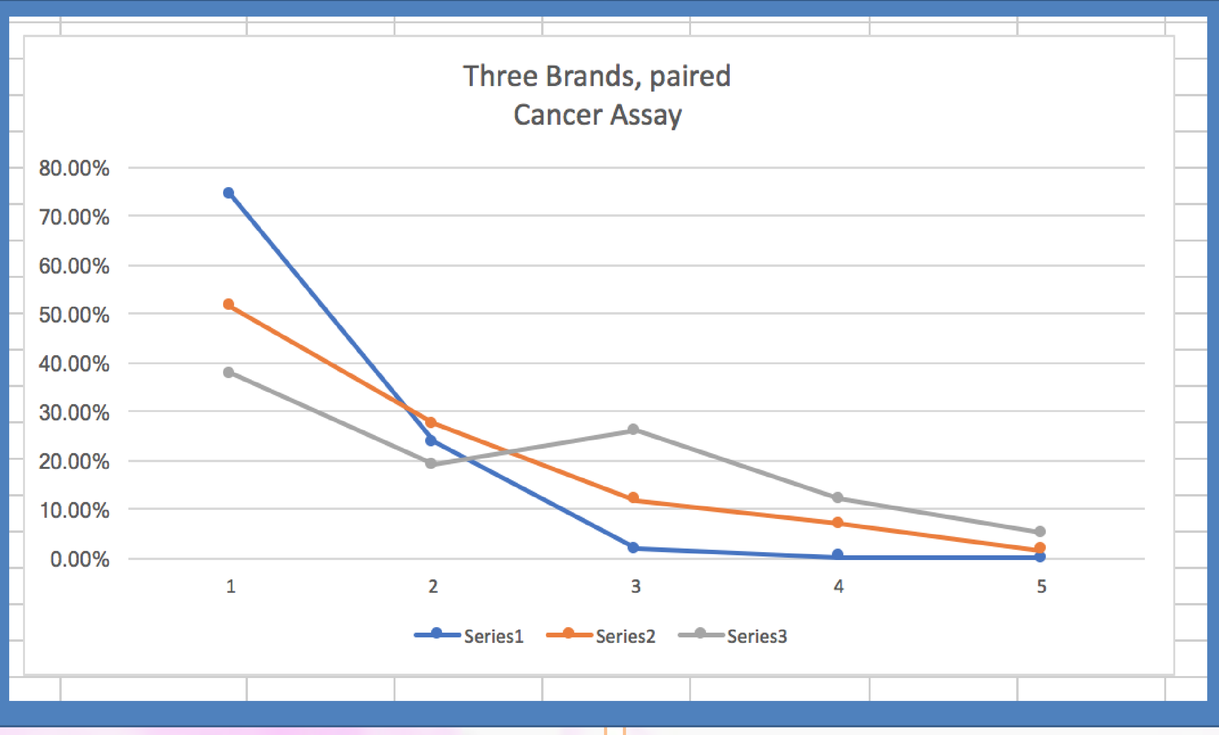


Figure 2. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Vivamus



Testing of Wells by Position

An analysis of the reproducibility of assay results in pair plates by brand was conducted with results in Figure 3. A uniform Z' assay was run in pairs of three brands of microplates. Plates were normalized by mean and then results of well positions were subtracted individually then divided by the SD. The results were grouped into ½ SD sets and graphed. The results show by brand the consistency of assay readout by well position. Brands that have more wells with lower scores have fewer differences between the microplates.

Figure 3. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Vivamus justo leo, tristique vitae lorem sed

Conclusions

- Cell Assay SDs are related to surface chemicals.
- All microplates have surface chemicals so matching cell lines is an important step in assay optimization.
- Intra and inter plate can be measured and minimized easily with this new technique.
- Labs should reanalyze when changing microplate lots.