Effects of Microplates on Cancer Assay Performance

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Abstract

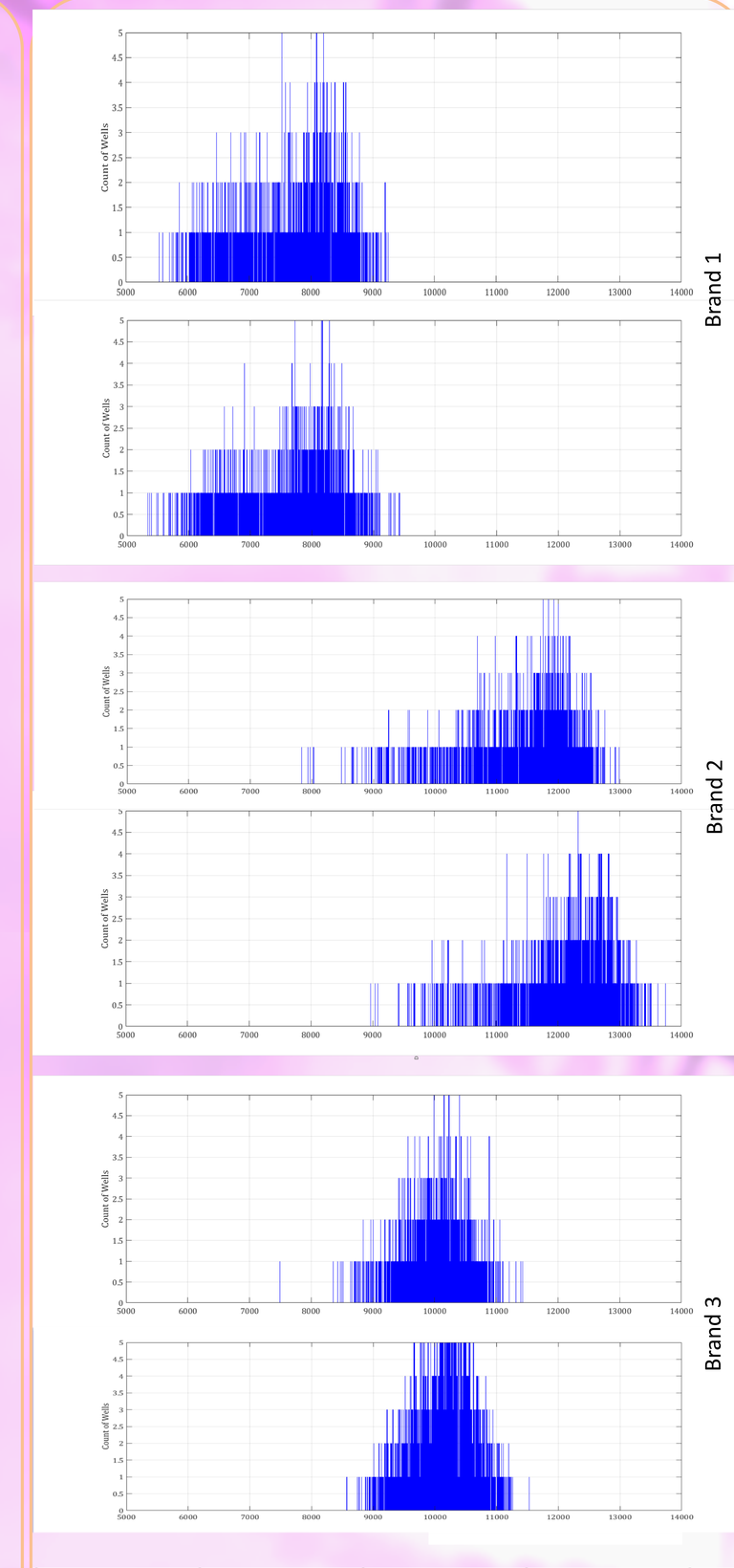
This poster presents results of a cancer cell assay with an experimental design that isolated three brands of microplate as the sole variable. The results showed substantial differences between the brands for the rate of cell growth and uniformity of cell growth. ToF-SIMS analysis of the chemicals on the surface of each microplate is a possible explanation for the differences.

In conjunction with NIH/NCATS, IonField Systems used a PlasmaKnife System to rinse and plasma process the three brands, two polystyrene one CPC, as part of an experiment of repeat testing to measure carry using a Be2C cell assay. The test detected no carryover but did measure differences between the three brands of microplates. The first and second assays were each run as single batches with uniform conditions for all microplates. No compound in the second assay so effectively the second assay results represent the assay's Z' max signal. The lowest wells in each plate from the first run were compared post plasma treatment and were found be within +/- 0.5 SD of Z' max signal mean of the plate.

Subsequent analysis of each microplate's results by frequency distribution, showed that each brand's microplates had a unique curve shape, two brands having unique skew values and curve shapes and one brand with a normal Gaussian distribution curve and no skew. The distribution curve shapes are repeatable and unique enough to allow identification of the manufacturer.

Experimental Method

- The assay was uniformly plated with 500 cells of Be2C cells in six microplates.
- No cytotoxic compounds were added to any well in the data reported here.
- All plates were handled as a group with no differences at any step in the protocol.
- After cells were added, the microplates were incubated at 37 degrees for 72 hours.
- CellTiter-Glo® assay reagents were used for the assay post incubation following manufacturer's (ProMega) instructions.
- Assay plates was read on PE ViewLux® instrument.
- CVs for the two higher cell growth microplates were under 6% (one PS and one CPC) and the lower growth microplate were 10% (one PS).



Background

The assay described was the second use for each of the microplates shown. A similar assay was run several weeks prior with a more standard protocol having a significant number of wells having cytotoxic compounds plated in them. While no carryover was found in any plate, the experiment has shown a number of effects on the growth rate of a cancer cell line that is commonly reported in the literature. Nothing in the literature reports the Be3C as being particularly sensitive to the brand of microplate used.

Correlation with ToF-SIMS

IonField and NIH/NCATS had previously run ToF-SIMS (Time of Flight, Secondary Ion Mass Spectrometry) on all three brands of microplates. Data from those runs is shown in the bottom section of this poster. The authors have not conclusively shown a linkage to specific chemicals for the growth rate variation of 35% from high to low or the unique frequency distribution curve shapes and skews.

Conclusions

The authors consider the data, without further testing, as not supporting any firm conclusions as to why the cell growth rate between brands and well to well uniformity of growth vary.
The observation that each brand of microplate appears to provide a unique environment for cell growth is worth noting. Changing brands or lots within brand can have very significant alteration on a cell assay's metrics and may preclude comparisons between what otherwise appear to be identical assays.

						amino acid		
ammonium						Pro	TMAH	
18	23	27	28	39	40	70	74	
NH4	Na	Al	Si	K	Ca	C4H8N	C4H12N	
0.18	29.07	17.35	2.15	0.03	3.57	1.99	0.15	
0.26	4.26	13.10	23.04	0.32	0.98	2.92	0.33	Brand 1
0.35	3.17	25.04	8.65	0.17	1.23	2.19	0.68	
8.42	1.34	0.26	0.61	0.09	0.01	2.55	7.49	
1.83	1.09	2.00	1.25	0.47	13.34	1.75	1.68	Brand 2
1.45	0.53	2.38	4.02	0.13	4.01	1.72	1.39	
4.81	99.50	11.80	7.41	0.86	0.06	2.10	2.18	
3.78	61.85	50.34	14.16	1.82	5.95	2.06	4.50	Brand 3
2.36	30.77	27.72	20.50	1.43	8.36	2.06	2.60	

Phe	amine	PDMS	phthalate	amine		alkyl amide	EBS	Irgafos	
120	130	147	149	168	208	228	310	648	
C8H10N	C8H20N	Si2OC5H15	C8H5O3	C11H22N	Pb	C14H30NO	C20H40NO	C42H64PO3	
0.15	0.07	0.29	0.19	0.26	0.02	0.10	1.96	0.02	
0.34	0.06	2.83	0.82	0.18	0.22	0.19	0.94	0.43	Brand 1
0.44	0.14	0.92	0.30	0.40	0.14	0.08	1.74	0.00	
1.07	4.18	2.47	2.43	0.67	0.06	0.05	0.01	0.00	
0.96	1.46	2.65	1.74	0.54	0.97	0.05	0.01	0.00	Brand 2
1.04	1.64	2.79	2.22	0.15	1.16	0.05	0.01	0.00	
0.59	2.22	1.65	1.21	0.22	0.14	0.07	0.00	0.00	
0.47	2.54	1.73	0.96	1.67	0.93	0.06	0.02	0.01	Brand 3
0.51	2.40	3.68	3.47	0.72	0.97	0.06	0.01	0.00	