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Life Sciences

Evaluation of Instrument to Instrument Performance of the Vi–CELL BLU Cell Viability Analyzer

Beckman Coulter Life Sciences is proud to introduce our new Vi-CELL BLU Cell Viability Analyzer. The Vi-CELL BLU leverages the key performance features of the Vi-CELL XR but incorporates many design improvements that our customers have requested over the years.

While a seemingly straightforward application, automated cell counting performance can be influenced by a variety of conditions and variables arising from both the sample and instrument. It is therefore important to ensure that the instrument is performing within specifications so that any instrument variability can be eliminated from the sample measurements. However, this becomes more complicated when multiple instruments may be in operation within a department as instrument to instrument variability is possible.

One of the key strengths of the Vi–CELL BLU is the ability to fine tune the performance to minimize variability between different instruments. This is particularly valuable in facilities that utilize multiple instruments but can also be important when instruments are occasionally shared between departments.

Due to the primary use of the Vi–CELL instruments being within regulated and GMP manufacturing environments, it is critical that the new Vi–CELL BLU provide acceptable instrument to instrument performance. The following information and data serves to illustrate that the Vi–CELL BLU has improved instrument to instrument variability by comparing several instruments using a series of standard test samples.

Figure 1. New Vi–CELL BLU Cell Viability Analyzer

Bead and Cell Counting Data Comparisons

Sample materials utilized

6602796 (lot 9747455F) Coulter CC L10 Standard, nominal 10 µm, Latex Particle (NIST Traceable), 1 x 15 mL

Cell Type Profile: BCI L10 Beads

Instrument Settings for Bead Analysis

Cell Type Profile	BCI L10 Beads
Minimum Diameter (µm)	5
Maximum Diameter (µm)	15
Images	100
Cell sharpness	22
Minimum circularity	0.5
Decluster degree	Medium
Aspiration cycles	3
Viable spot brightness (%)	50
Viable spot area (%)	1
Mixing cycles	3

Data were recorded as averages of 24 samples for each dilution on a 96 well plate.

Data were recorded as an average of 20 runs per sample and reported as the average ± standard deviation of the results. Instrument settings are given below.

Control Bead Results

Sample Type: L10 Size Beads Control (3 instruments with replicate plates)

Instrument	Dilutions	Average Bead count	% CV of Bead count	Average Concentration (x10^6) beads/mL	% CV of Total (x10^6) beads/mL	Average Diameter (µm)	% CV of Diameter	# Samples
A	100%	5501	3.45%	2.08	3.03%	10.40	0.16%	24
	50%	2881	3.32%	2.17	3.31%	10.41	0.18%	24
	25%	1468	2.83%	2.21	2.85%	10.42	0.24%	24
	5%	303	4.54%	2.28	4.59%	10.43	0.55%	24
B	100%	5666	4.18%	2.12	4.18%	10.31	0.13%	24
	50%	3011	1.83%	2.25	1.88%	10.32	0.16%	24
	25%	1501	2.70%	2.25	2.64%	10.33	0.18%	24
	5%	316	6.35%	2.37	6.32%	10.34	0.50%	24
C	100%	5906	2.97%	2.13	2.98%	10.37	0.14%	24
	50%	3054	3.08%	2.21	2.76%	10.39	0.12%	24
	25%	1515	2.33%	2.19	2.27%	10.38	0.26%	24
	5%	314	6.60%	2.27	6.52%	10.40	0.52%	24

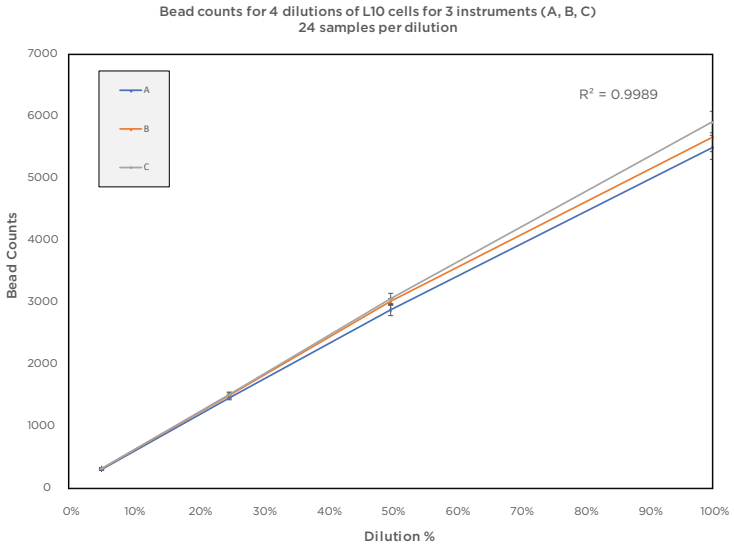

Figure 2

Cell Counting Analysis

In addition to bead standards the instruments were compared using a variety of standard cultured cells. The cells were prepared following dilution guidelines outlined by NIST (Evaluating the quality of a cell counting measurement process via a dilution series experimental design. Sarkar, Sumona et al. (2017) Cytotherapy , Volume 19, Issue 12, 1509 – 1521). Some cell were run without dilution data analyzed using standard protocols for the cell type. Cell type parameters for the protocols are given below.

Instrument Settings for Cell Culture Analysis

Cell type	Mammalian
Minimum Diameter (µm)	6
Maximum Diameter (µm)	30
Images	100
Cell sharpness	7
Minimum circularity	0.1
Decluster degree	Medium
Aspiration cycles	3
Viable spot brightness (%)	55
Viable spot area (%)	5
Mixing cycles	3

Cell Count Results

A dilution protocol of 8 serial dilutions of CHO cells was established to test cell counting performance over different concentration ranges. The lower range concentrations were 3 replicate plates were run on 3 Vi-CELL BLU instruments. Higher concentration ranges were run as 3 sets of replicate samples of 10 tubes per dilution using the carousel due to limited sample availability.

Dilution	Nominal Concentration (x10^6) cells/mL
100%	5.50
80%	4.40
60%	3.30
50%	2.75
40%	2.20
30%	1.65
20%	1.10
10%	0.55

Instrument	Dilutions (n=12)	Average of Cell count	%CV of Cell count	Average of Concentration (x10^6 cells/mL	%CV of Total (x10^6) cells/mL	Average of Viability (%)	%CV of Viability (%)	# Samples
B01	100%	14524	2.51%	5.47	2.50%	63.97	1.69%	12
	80%	11099	2.79%	4.18	2.78%	64.77	0.70%	12
	60%	8528	4.15%	3.21	4.10%	64.39	1.28%	12
	50%	6848	2.81%	2.58	2.72%	64.33	1.20%	12
	40%	5795	9.84%	2.18	9.83%	61.93	2.22%	12
	30%	3960	2.93%	1.49	2.82%	62.07	1.33%	12
	20%	2617	4.71%	0.99	4.63%	60.88	2.10%	12
	10%	1185	4.25%	0.45	4.14%	58.41	3.49%	12
B02	100%	14736	3.44%	5.52	3.43%	65.81	1.17%	12
	80%	11535	3.62%	4.32	3.60%	64.58	0.79%	12
	60%	8479	2.27%	3.17	2.27%	65.79	1.15%	12
	50%	7087	3.94%	2.65	3.92%	64.50	1.36%	12
	40%	5547	3.31%	2.08	3.32%	62.36	1.22%	12
	30%	4234	7.24%	1.59	7.19%	64.33	1.68%	12
	20%	2820	6.27%	1.06	6.33%	61.88	1.87%	12
	10%	1250	6.55%	0.47	6.42%	58.28	3.70%	12
B03	100%	14999	2.24%	5.42	2.34%	65.50	1.16%	12
	80%	11834	2.39%	4.27	2.40%	64.80	0.94%	12
	60%	8776	1.91%	3.17	1.88%	65.51	0.91%	12
	50%	7281	2.78%	2.63	2.79%	64.40	1.48%	12
	40%	5658	3.12%	2.04	3.11%	61.48	1.13%	12
	30%	4138	4.71%	1.49	4.74%	62.58	2.08%	12
	20%	2644	4.10%	0.96	3.93%	60.79	1.91%	12
	10%	1237	4.25%	0.45	4.14%	58.36	3.81%	12

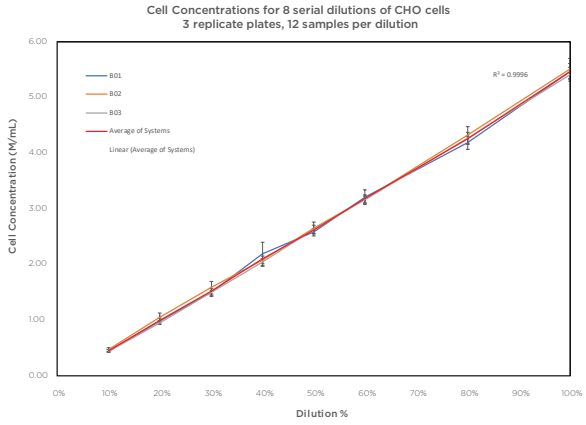

Figure 3

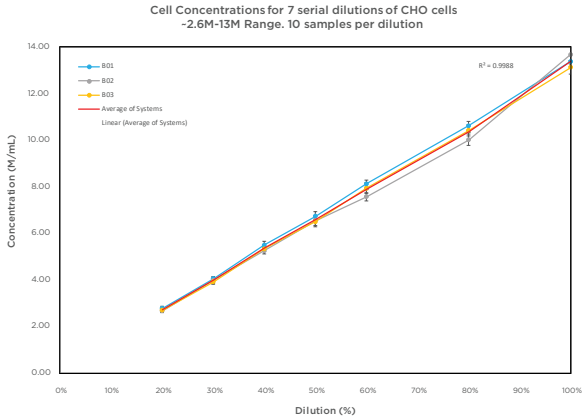

Figure 4

Additional sample plates were run using a smaller range of dilutions to confirm the performance of the instruments for both plate and carousel.

Plate runs were repeated 3 times within a 16 hr period using the same stock supply of cells run on the same instrument. During this period cell population increase is considered minimal and the source material for analysis effectively the same.

Three plates were run in triplicate on 3 different Vi-CELL BLU instruments (9 plates total, n = 864 samples). The data below shows the 3 runs from one instrument.

The data collected from these runs was subjected to an ANOVA analysis to determine the degree of variability between the runs and within each run across sample replicates. The results show no statistical significance (p value >90) between all runs, across all instruments. The data is presented as the average cell concentrations and viability for all concentrations.

Dilution	Nominal Concentration (x10^6) cells/mL
100%	2
50%	1
25%	0.5
10%	0.2

Instrument	Plate	Average of Concentration (x10^6) cells/mL	%CV of Total (x10^6) cells/mL	Average of Viability (%)	%CV of Viability (%)
A	1	1.86	1.52%	92.40	0.004%
	2	1.71	1.11%	88.70	0.012%
	3	1.81	1.41%	85.57	0.038%
B	1	1.85	0.78%	92.54	0.008%
	2	1.72	0.86%	89.39	0.007%
	3	1.82	1.06%	85.64	0.014%
C	1	1.87	1.87%	92.40	0.010%
	2	1.67	1.98%	88.39	0.011%
	3	1.69	2.04%	85.53	0.019%

The charts below shows the averages from each instrument for each dilution across all 3 replicates.

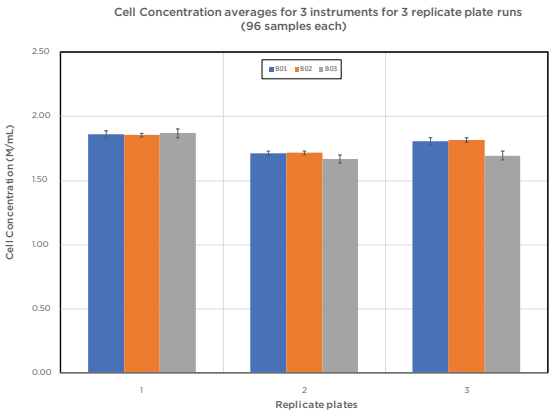

Figure 5

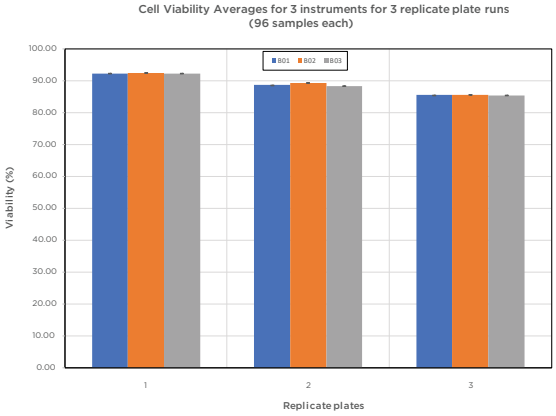

Figure 6

Results

The counting performance of the Vi-CELL BLU shows excellent linearity over several dilutions. As expected cell counts below 0.5M cells per mL do show a higher variability due to low overall numbers of cells per image frame. Even so the variability remains within allowable limits (10%) for instrument performance. When using standard L10 size beads the variability in counts is significantly lower due to the more uniform nature of the sample material.

Replicate samples across 3 instruments consistently show no statistical difference between the replicates of equivalent samples indicating the instruments are performing equivalently with the samples provided.

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