BECKMAN COULTER
Life Sciences

Cell Counting Performance of Vi-CELL BLU Cell Viability Analyzer

The Vi-CELL BLU analyzer leverages the key performance features of the Vi–CELL XR analyzer but incorporates many design improvements that our customers have requested over the years.

While a seemingly straightforward application, automated cell counting 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.

To determine the cell counting performance of the Vi-CELL BLU analyzer we adapted the protocol outline 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).

By utilizing a dilution series one can assess instrument performance by determining the proportional cell count across the dilutions and replicate samples.

In addition to using cells we also evaluated the system using standardized beads that can be used for Concentration, Viability and Size calibration and as cell-independent samples for assessing instrument performance.

Figure 1. Vi–CELL BLU Cell Viability Analyzer

Bead and Cell Counting Data Comparisons

Sample materials used

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 72 samples for each dilution from 3 replicate 96-well plates.

Control Bead Results

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

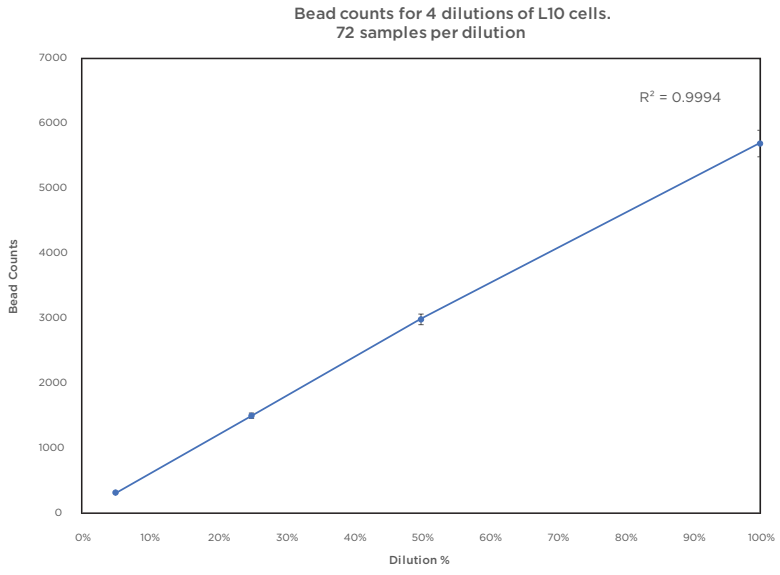

Figure 2. L10 Bead Counting Dilution Series Results

Cell Counting Analysis

Instrument performance for Cell Counting was determined using standard cultured CHO cells. The cells were prepared following dilution guidelines outlined by NIST illustrated below, and data analyzed using standard Mammalian 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 9 replicate plates run on 3 Vi-CELL BLU instruments (3 plates per instrument). 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	Nominal Concentration (x10^6) cells/mL
	Low-Mid Range	Mid-High Range
100%	5.50	13
80%	4.40	10M
60%	3.30	8M
50%	2.75	6.5M
40%	2.20	5.2M
30%	1.65	3.9M
20%	1.10	2.6M
10%	0.55	-

Results

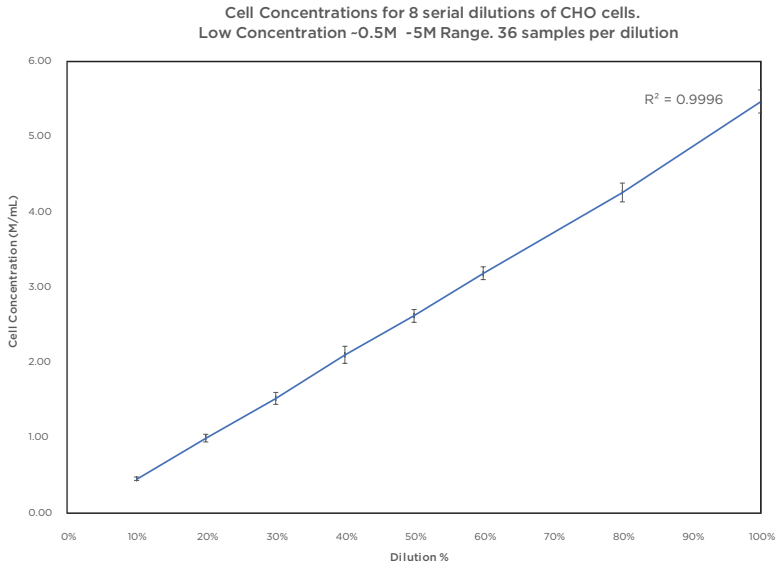

Figure 3. CHO Cells Low Concentration Range Dilution Series Data

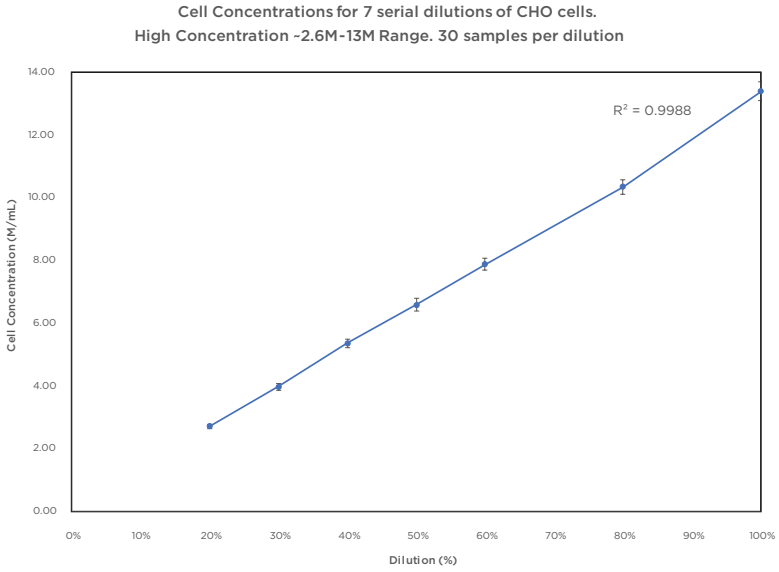

Figure 4. CHO Cells High Concentration Range Data

Additional sample plates were run using a smaller range of dilutions to confirm the performance of the instruments. These 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.

3 plates were run in triplicate on 3 different Vi-CELL BLU instruments (9 plates total, n=864 samples). The data below shows the average of 216 samples for each dilution.

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.

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

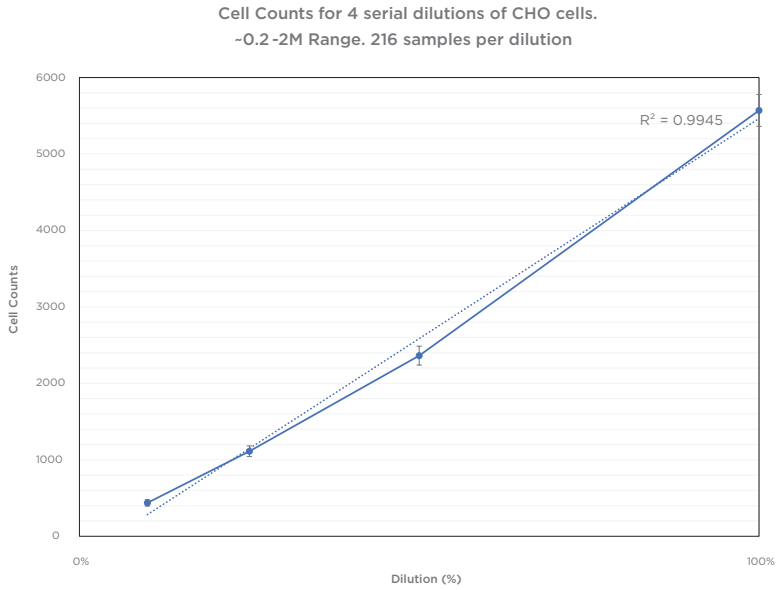

Figure 5. Dilution Series of CHO Cells, Multiple Replicates

Conclusions

The counting performance of the Vi-CELL BLU analyzer shows excellent linearity over several dilutions covering a range of 0.5M–15 Million Cells per mL. 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.

Utilizing the recommended NIST protocol, the proportional dilution acts as an internal control for cell count, ensuring that instrument is counting accurately across different cell concentration ranges. Correlation of the counts and concentration across dilutions has an R^2 value of >0.99 showing a consistent counting performance across dilutions. It should be noted that the large number of samples that can be run on the Vi-CELL BLU analyzer also allows for increased confidence in the cell counts and for easier identification of anomalous results.

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