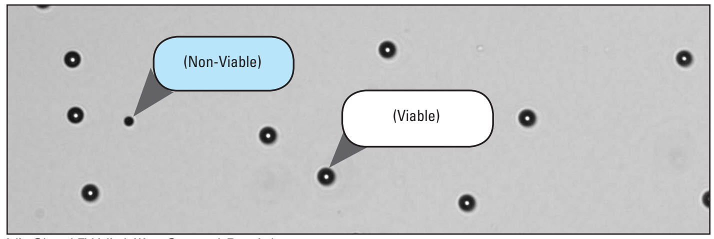
Optimization of Vi-CELL® XR Settings for Calibration Checks Using ViaCheck™ Controls

Kathryn Kilbride, Robert Anglea, Anna Bavender, Bangs Laboratories, Inc.

The Beckman Coulter Vi-CELL® XR is an imaging cell viability analyzer based on trypan blue dye exclusion. Cell samples are aspirated from a cuvette, and automatically stained, washed and analyzed; data regarding cell concentration, %viability, etc. are then returned. Such analyzers serve an important function in biomaufacturing as they support vital decisions (cell line selection, etc.) and process management factors for vaccine development, biopharmaceuticals, and regenerative medicine.



ViaCheck™ Viability Control Particles

Like all analytical instruments, cell viability analyzers require initial qualification and routine QC to demonstrate both that they are suited to their intended use, and demonstrate ongoing capability. ViaCheck™ Concentration and Viability Controls are microsphere-based calibrators that support quality programs for imaging-based cell viability analyzers across a range of cell (bead) concentrations and %viabilities. Though ViaCheck have long been used to demonstrate agreement between the Vi-CELL XR and "gold standard" Coulter Counter, recent exponential growth in biomanufacturing has placed an even greater emphasis on the controls utilized in the validation and QC of cell viability analyzers.

The current effort was undertaken as a fresh examination of instrument settings using latest instrumentation / software to ensure highest agreement between concentration results from the Coulter Z2 and Vi-CELL XR. (Beckman Coulter)

Method and Results

From prior studies *(unpublished data)*, it was known that both user-configured and locked instrument settings impact results returned by the Vi-CELL, most notably for concentration. The present study sought to examine concentration results generated using ViaCheck Concentration Controls under different Concentration Factor (CF) and user-defined settings, and level of agreement with the primary Coulter Z2 method.

Concentration Determinations: User-Defined Settings

User-defined setting sets were selected through historical data and discussions with Beckman Coulter. These are provided in Table 1, below.

Table 1 : User-Defined Settings

USER-DEFINED SETTINGS	CONCENTRATION CONTROL SETTINGS SET 1	CONCENTRATION CONTROL SETTINGS SET 2
Minimum cell diameter (µm)	2	5
Maximum cell diameter (µm)	50	50
Minimum circularity	0.9	0
Dilution factor	1	1
Cell brightness (%)	70	85
Cell sharpness	75	100
Viable cell spot brightness (%)	55	75
Viable cell spot area (%)	1	5
Decluster degree	Low	Low
Aspirate cycles	2	1
Trypan blue mixing cycles	3	3

Concentration determinations: Concentration Factor

As an instrument's CF is known to have an impact on concentration results, we examined the effects of modified CF values under the two sets of settings provided in Table 1. Specifically, ViaCheck Concentration Controls of 1e+6, 4e+6 and 8e+6 were run on the Vi-CELL XR (software version 2.04) using both setting Sets 1 and 2, and several CF values in the range of 0.97 – 1.13. Results were compared to formal Z2 concentration values ("assay values") for each ViaCheck Lot. Results from user-defined settings Set 2 (which was found to yield the best agreement with Z2 concentration) and different CF values are provided below. Multiple Lots were run; results from a single Lot for each ViaCheck Concentration are shown:

Table 2: Effect of changed CF on ViaCheck Concentration

Vi-CELL	ViaCheck Concentration Controls					
	VC60N 1M		**VC70N 4M**		**VC80N 8M**	
	Beads/mL	%Diff Z2	Beads/mL	%Diff Z2	Beads/mL	%Diff Z2
1.13	1.08e+6	11.34%	4.61e+6	14.68%	9.11e+06	10.96%
1.10	1.02e+6	5.15%	4.46e+6	10.95%	9.14e+06	11.33%
1.08	1.03e+6	6.19%	4.23e+6	5.22%	8.84e+06	7.67%
1.05	1.01e+6	4.12%	4.07e+6	1.24%	8.66e+06	5.48%
1.03	1.00e+6	3.09%	4.06e+6	1.00%	8.40e+6	2.31%
1.00	1.01e+6	4.12%	4.06e+6	1.00%	8.29e+6	0.97%
0.97	1.01e+6	4.12%	3.93e+6	-2.24%	8.06e+6	-1.83%
Z2	0.97e+6	---	4.02e+6	---	8.21e+6	---

For this instrument and application, the best agreement with formal ViaCheck Concentration Control results (Lot-specific assay values) was found with a CF of 1.00 and user defined settings Set 2. A total of eighty four samples were run (42 on the Vi-CELL XR and 42 on the Z2) for each of the products (1e+6, 4e+6 and 8e+6):

Table 3: Results with adjusted CF Settings Set 2

PRODUCT	VC60N 1e+6		VC70N 4e+6		VC80N 8e+6	
Mfg Conc Specs	0.9e+6 – 1.1e+6		3.6e+6 – 4.4e+6		7.2e+6 – 8.8e+6	
RESULTS	Vi-CELL	Z2 [final]	Vi-CELL	Z2 [final]	Vi-CELL	Z2 [final]
Average Conc (n= 42)	9.54e+5	9.69e+5	3.81e+6	3.92e+6	7.70e+6	7.88e+6
%CV	3.73%	1.07%	1.73%	1.11%	1.85%	1.05%

***Note:* CF settings are instrument (flow cell) specific and must be determined empirically.** The CF value from this study is specific to the featured Vi-CELL XR, and should not be applied in a general fashion to other instruments.* CF adjustments constitute an instrument re-calibration, which should be performed by a service technician or after a thorough investigation of different settings across concentrations and sample types. Additionally, the user-defined settings featured in this study are specific for ViaCheck microsphere calibrators, and such settings should be determined empirically for each cell line.

*The CF setting must also be confirmed or recalibrated with instrument repair, e.g. replacement of flow cell, pump, etc.

Figure 1: Concentration Factor

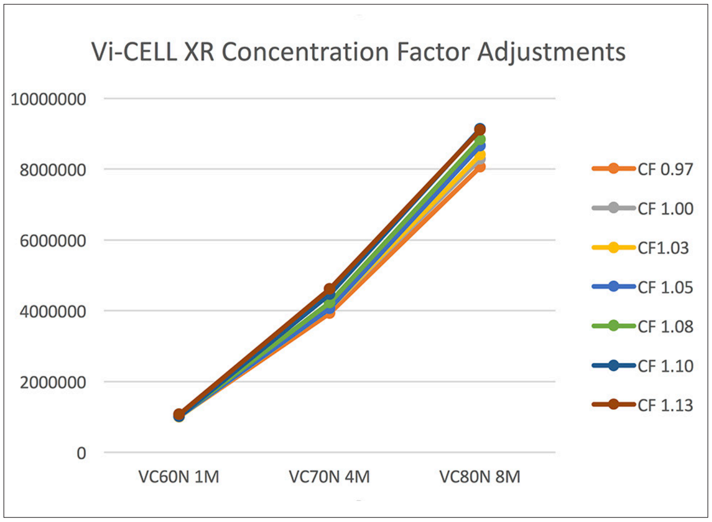
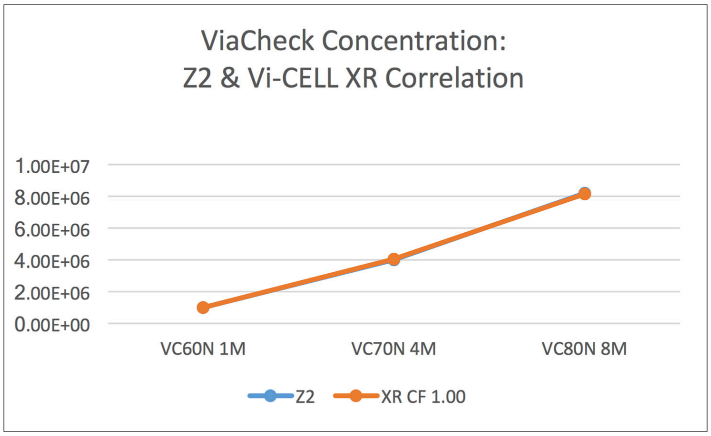


Figure 2: Agreement of XR & Z2 with optimized settings.



Viability Determinations: Concentration Factor

In the case of image-based cell viability analyzers, concentration is key. This is the primary parameter used by algorithms for the calculation of sample concentration and %viability. The Vi-CELL determines %Viability based on the ratio of "live" and "dead" particles counted. The calculations are thus highly robust. To ensure that a changed CF did not have a negative impact on the accuracy of %Viability determination, ViaCheck Viability Controls of 0%, 25%, 50%, 75%, 90% and 100% were tested using the re-defined CF of 1.00 and established user-defined viability settings of:

Table 4: User defined settings

User-Defined Settings	Viability Control Settings
Minimum cell diameter (µm)	5
Maximum cell diameter (µm)	50
Minimum circularity	0.9
Dilution factor	1.0
Cell brightness (%)	85
Cell sharpness	100
Viable cell spot brightness (%)	60
Viable cell spot area (%)	3.0
Decluster degree	Low
Aspirate cycles	2
Trypan blue mixing cycles	3

Table 5

PRODUCT	VC10B 0%	VC25B 25%	VC20B 50%	VC30B 75%	VC40B 90%	VC50B 100%
Mfg Spec	0 - 5%	20 - 30%	45 - 55%	70 - 80%	85 - 95%	95 -100%
RESULTS						
Avg %Via (N=42)	0.7%	24.4%	50.3%	76.7%	91.9%	99.4%
%CV	53.44%	1.87%	3.03%	1.75%	0.91%	0.26%

%Viability Results

It was found that implementation of a new CF (1.00) did not have a significant effect on %viability determinations, and all results remained within specification and expected variation between runs [Avg(%Via) and %CV, Table 5].

Viability Controls: Concentration

The Vi-CELL XR automatically provides the sample concentration for each run. We found that mixed-population (25%:75% live:dead – 10%:90% live:dead) ViaCheck concentrations were underreported by ~10% (i.e. by ~8-12%, unpublished data) when run on the Vi-CELL XR with a CF of 1.00 and the noted "Viability Control Settings," i.e. compared to formal Z2 counts for the same products. This observation underscores the use of ViaCheck™ Concentration Controls and associated "Concentration Control Settings" in conjuction with Viability Controls for calibration checks (see "*Understanding ViaCheck™ Assignments" in ViaCheck™ for Cell Viability Analyzers: Best Practices"*.

Conclusions

Image-based cell viability analyzers like the Vi-CELL are configurable, and results may vary under different settings. In our work, we found concentration to be particularly sensitive to adjustments. The current study demonstrated that excellent agreement can be achieved between the Vi-CELL XR and Coulter Z2 when determining particle concentration. The study also highlighted the importance of understanding both 'locked' and user-defined instrument settings, and the impact on results.

ViaCheck microsphere standards may be utilized to understand the effects of changed instrument settings, and to determine the optimal configuration for achieving highest agreement with orthogonal methods, such as the Coulter Counter (Coulter Z2). ViaCheck Concentration and Viability Controls additionally provide a means to conduct routine QC to ensure that instruments are performing as expected.

References and Further Reading

1. *ViaCheck for Cell Viability Analyzers: Best Practices.* (TSD 0711). Fishers, IN. Bangs Laboratories, Inc.
2. Janakiraman V, Forrest WF, Seshagiri S. (2006) *Estimation of baculovirus titer based on viable cell size.* Nature Prot; 1(5):2271-2276.
3. Kaminski RW, Wu M, Turbyfill KR, Clarkson K, Tai B, Bourgeois AL, Van De Verg LL, Walker RI, Oaks EV. (2014) *Development and preclinical evaluation of a trivalent, formalin-inactivated Shigella whole-cell vaccine.* Clin Vaccine Immunol; 21(3):366-382.
4. Kilbride K, Meza M, Anglea R. (2014) *Comparison study of the Vi-CELL® XR cell viability analyzer and Z2™ Coulter counter.* Poster presented at the Twenty Fourth Congress of the International Society for Advancement of Cytometry.
5. Lew C, Gomez JA, Rhyner MN. *Instrument-to-instrument variability in the Vi-CELL automated viability analyzer.* (Publication No. IB-17279A). Brea, CA. Beckman Coulter, Inc.
6. Li F, Vijayasankaran N, Shen A(Y), Kiss R, Amanullah A. (2010) *Cell culture process for monoclonal antibody production.* mAbs; 2(5):466-477.
7. Prathalingam N, Ferguson L, Young L, Lietz G, Oldershaw R, Healy L, *et al.* (2012) *Production and validation of a good manufacturing practice grade human fibroblast line for supporting human embryonic stem cell derivation and culture.* Stem Cell Res Ther; 3(12):12.
8. Szabo SE, Monroe SL, Fiorino S, Bitzan J, Loper K. (2004) *Evaluation of an automated instrument for viability and concentration measurements of cryopreserved hematopoietic cells.* Lab Hematol; 10:109-111.