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Comparative Performance Analysis of CHO and HEK Cells Using Vi-CELL BLU Analyzer and Roche Cedex® HiRes Analyzer

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Introduction

In this application note, the Vi-CELL BLU cell counter and the Roche Cedex® HiRes analyzer were tested for their counting performance with CHO and HEK cells across a wide measurement range. Both devices show excellent counting performance and – with some adjustments to the standard cell type setting – comparable results were obtained. The Vi-CELL BLU cell viability analyzer did show some advantages in terms of throughput, range, measurement time and sample volume. Also, the Vi-CELL BLU cell counter allowed for changing the amount of mixing and aspiration cycles, which can improve the performance when working with clumpy cell lines.

Methods

Prior to measurements with cell suspensions, calibration standards were used to confirm both devices were set up properly and performing within specification. The standards listed in Table 1 were tested in quadruplicates, according to the manufacturer's instructions.

Device	Control standard	Lot	Expected value
Vi-CELL BLU cell viability analyzer	0.5 M concentration	148	0.498 *10^6/mL ± 10%
	10 M concentration	168	9.9 *10^6/mL ± 10%
	50% viability	162	48.7% ± 10%
Roche Cedex® HiRes analyzer	Control Beads 5*10^5	-	0.5*10^6/mL ± 10%
	Control Beads 1*10^6	-	1*10^6/mL ± 10%
	Control Beads 5*10^6	-	5*10^6/mL ± 10%
	Viability Beads 60%	-	60% ± 10%

Table 1. Control standards used on both cell counting devices.

Afterwards, dilution series with FreeStyle CHO-S (Thermo Fisher, Catalog Number R800-07) and FreeStyle 293-F (Thermo Fisher, Catalog Number R790-07) cells were made and measured on both devices to test for accuracy and linearity across a wide concentration range. Both cell lines were cultivated for four days at 37°C, 250 rpm at 5% CO_2 in FreeStyle CHO or 293 Expression medium, respectively. Then, the cells were harvested, and the cell density was determined before pelleting the cells at 180 rcf for 5 minutes. The supernatant was removed, and the cells were resuspended in fresh medium and the following dilution steps **(Table 2)** were made. The Roche Cedex® HiRes analyzer´s maximum concentration (10*10^6 cells/mL) is depicted as the 100% dilution, to reflect this is the upper range where accurate and comparable results are to be expected.

Dilution Factor [%]	Target TCD [*10^6 cells/mL]	CHO-S	HEK 293-F
150	15		x
100	10	x	x
50	5	x	x
25	2.5	x	
20	2		x
10	1	x	
5	0.5		x
2.5	0.25	x	

Table 2. Dilution series with CHO-S and HEK 293-F cell line. Dilution steps marked with an x were prepared for the respective cell line.

All cell suspensions were measured in quadruplicates on the Vi-CELL BLU device using the 96 well sample plate, and – due to capacity limitations – in triplicates on the Roche Cedex® HiRes analyzer. To avoid effects from sedimentation and cell growth in the fresh medium, the cell suspensions were loaded onto the Roche Cedex® HiRes analyzer immediately after preparing the dilution series. The samples were organized such that the complete series was measured before proceeding with the (next) replicate measurements such that any potential effects – like sedimentation – induced by longer wait-times on the sample deck were mitigated.

Results

The measurements with the concentration control beads were within the expected value range and showed an excellent linearity. The trendline was forced through (0,0) to simulate a blank measurement.

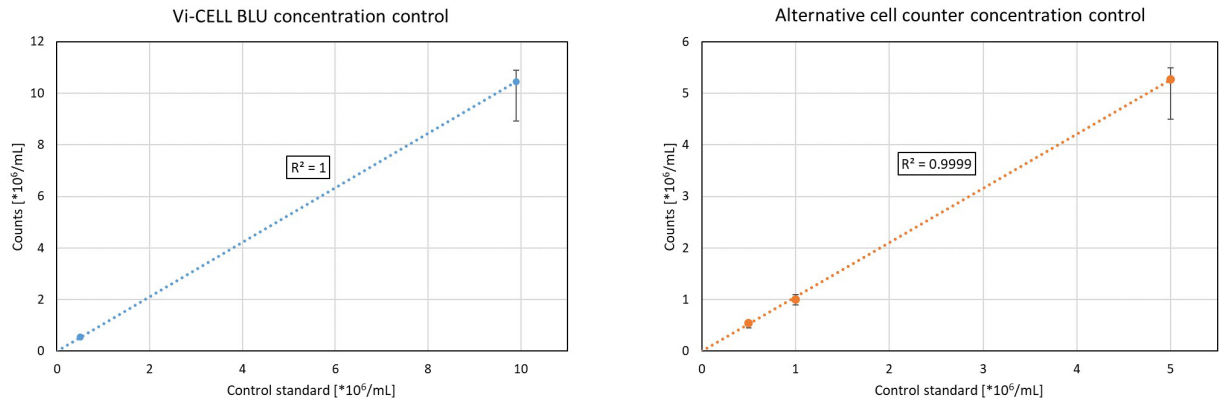

Figure 1. Concentration control results of both cell counting devices. The linear trend lines were forced through (0,0) and the R^2 values are shown in the graphs. The error bars represent the allowed 10% deviation from the expected value.

The viability control standard for the Vi-CELL BLU device resulted in an average measured viability of 46.4% at an expected value of 48.7%, and for the Roche Cedex® HiRes analyzer a 63% viability was measured at an expected 60%; meaning both devices performed within specifications (see table 1 for specifications).

Now, the CHO dilution series were measured on both devices. On the Vi-CELL BLU cell viability analyzer, measurements were performed in quadruplicates using Normal Wash (200 µL sample volume). The Vi-CELL BLU analyzer used for these experiments already had a custom-made cell type for measuring CHO cells, and this optimized cell type was used for these measurements **(Figure 2)**.

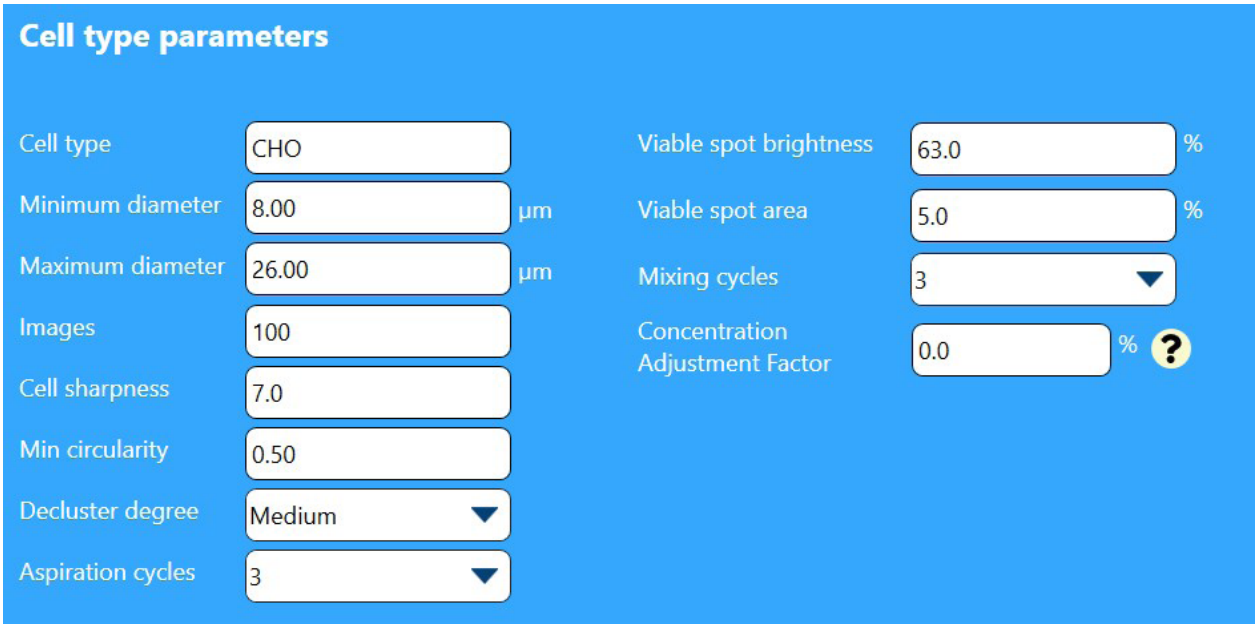

Figure 2. Custom-made CHO cell type parameters on the Vi-CELL BLU cell viability analyzer.

On the Roche Cedex® HiRes analyzer, the samples were run in triplicates using the default settings, with 300 µL of sample volume. The Vi-CELL BLU cell viability analyzer measured 20 samples in approximately 35 minutes, the Roche Cedex® HiRes analyzer needed around 60 minutes for 15 samples. Average total and viable cell densities were calculated per dilution and plotted against the dilution factor to determine the linearity of the devices across their measurement range **(Figure 3)**.

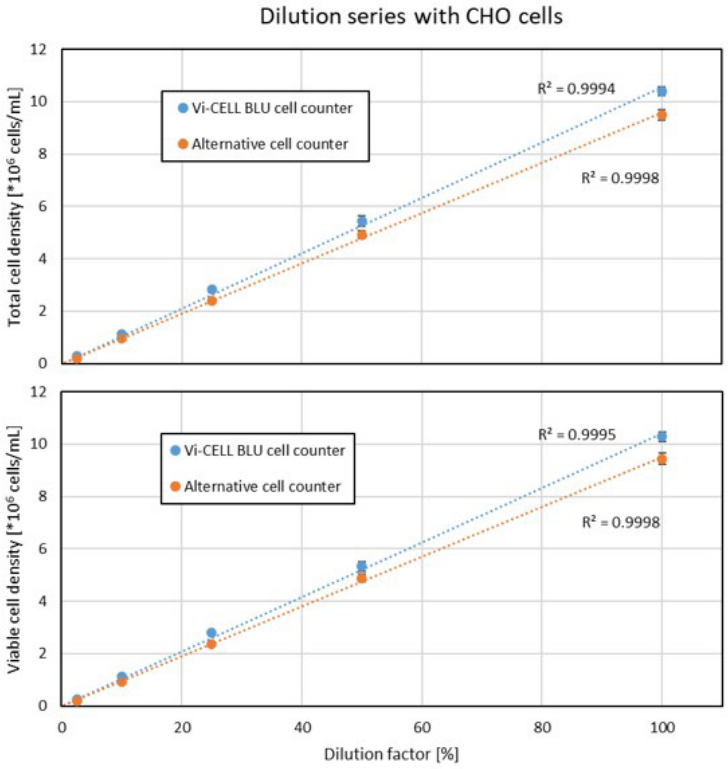

Figure 3. CHO dilution series measured on both cell counting devices. The linear trend lines were forced through (0,0) and the R^2 values are shown in the graphs. The error bars represent the standard deviation calculated from the replicate measurements.

As seen in Figure 3, both devices show excellent linearity across the tested range with R^2 values > 0.999. The Vi-CELL BLU cell viability analyzer reported slightly higher cell densities for all dilution steps, but some discrepancy is to be expected since the default cell type settings on the Roche Cedex® HiRes analyzer were not optimized.

Afterwards the HEK dilution series was measured. As HEK suspension cell lines are known to be prone to clumping, the Vi-CELL BLU device's default *Mammalian* cell type was edited: additional aspiration and mixing cycles were included **(Figure 4)**. The Roche Cedex® HiRes analyzer did not allow for additional aspiration and mixing steps, so the default cell type settings were used.

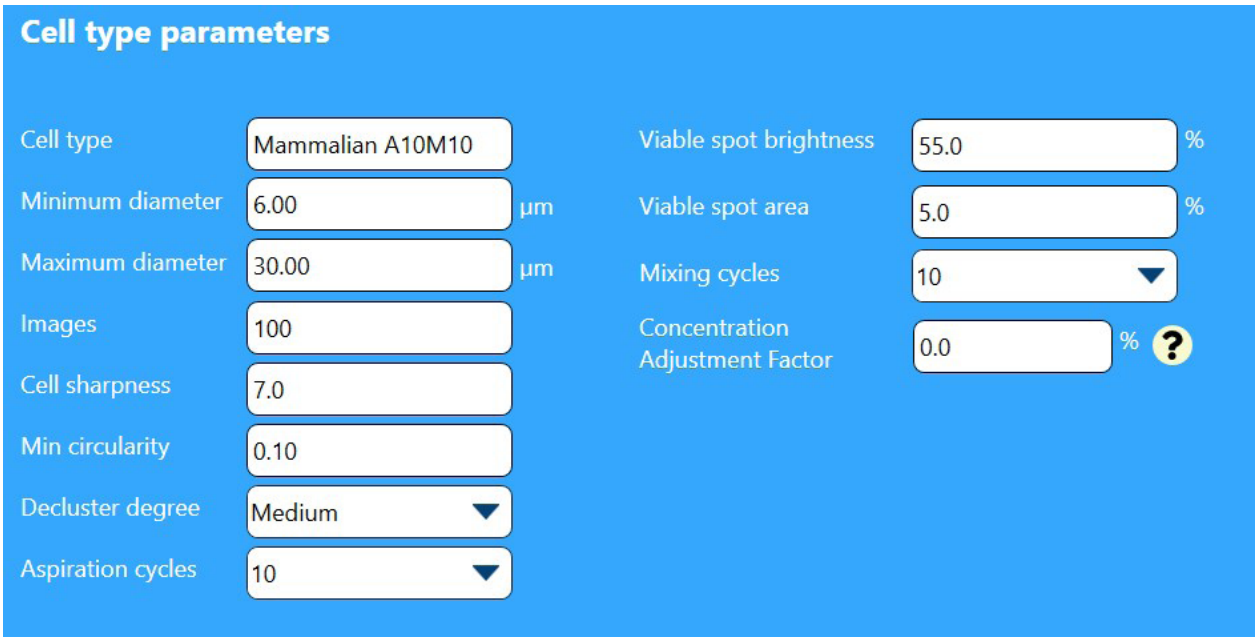

Figure 4. Custom-made Mammalian cell type parameters on the Vi-CELL BLU cell viability analyzer.

The Vi-CELL BLU cell viability analyzer managed to measure 24 samples in just under 55 minutes, using normal wash mode. On the Roche Cedex® HiRes analyzer, a maximum of 20 samples could be loaded at once. Therefore, the dilution series was measured in triplicates instead of quadruplicates and the resulting 18 samples were measured in about 1.5 hours. Again, 200 and 300 µL of cell suspension were used on the Vi-CELL BLU cell counter and the Roche Cedex® HiRes analyzer, respectively.

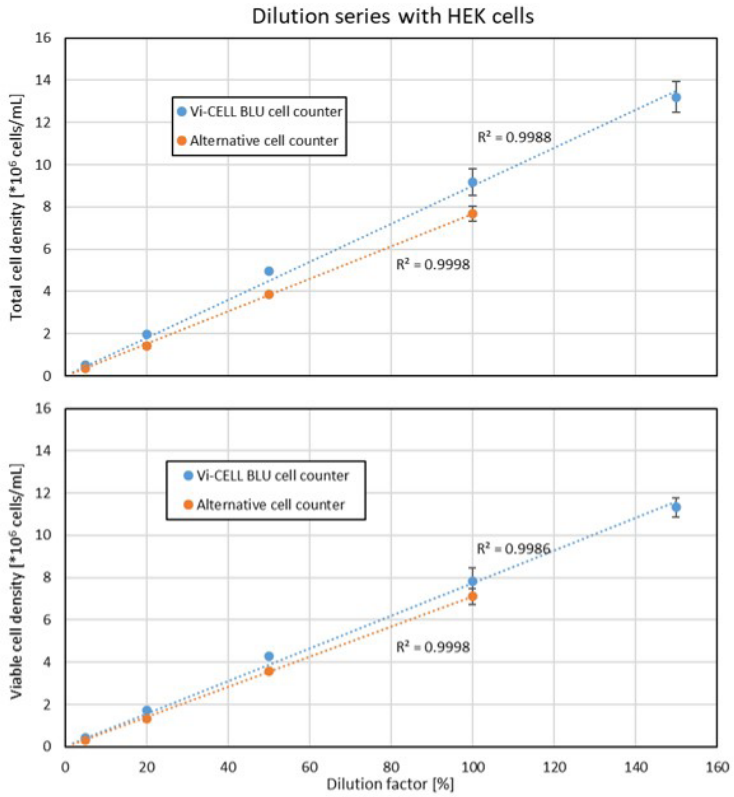

Figure 5. HEK dilution series measured on both cell counting devices. The linear trend lines were forced through (0,0) and the R^2 values are shown in the graphs. The error bars represent the standard deviation calculated from the replicate measurements.

The average TCD values in Figure 5 show excellent linearity (R^2 > 0.999) for both devices within their respective measurement ranges. However, the measured total cell density is significantly higher in the Vi-CELL BLU cell viability analyzer. The clumpiness of the HEK cells was confirmed by the Vi CELL BLU device's cluster count values, with e.g., an average of 50.5 clusters for the 50% diluted samples. The Roche Cedex® HiRes analyzer too reported aggregating cells across all dilutions.

Clumpy cells are more difficult to count and cell counting results with clumpy cultures could underestimate the cell concentration (ISO 20391, 1). Therefore, most cell analyzers allow users to define their own (optimized) cell counting parameters. For example, on the Vi-CELL BLU cell viability analyzer the *Decluster degree* influences how cell clusters are counted. In Figure 6, the effect of different *Decluster degree* settings is shown by comparison of an image from one of the 50% diluted samples on the Vi-CELL BLU device. Clearly, the analysis with the *High Decluster degree* results in additional cells being counted.

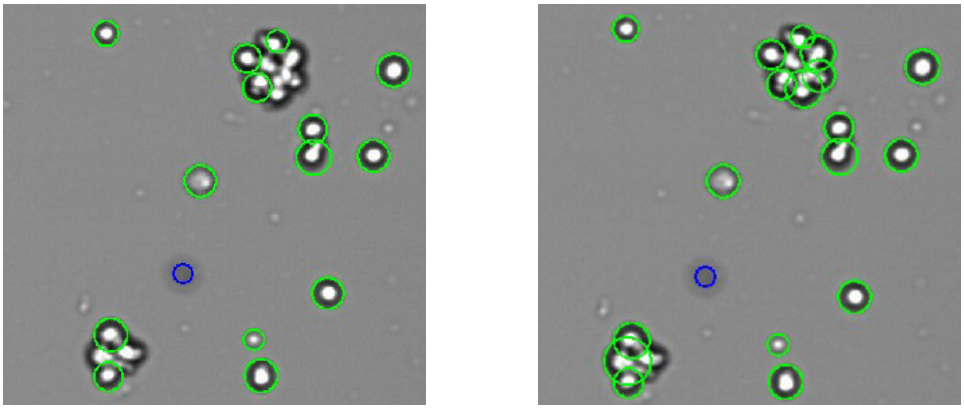

Figure 6. Image analysis on the Vi-CELL BLU with *Decluster degree* set to Normal **(left)** and High **(right)**.

To demonstrate the effect of cell type optimization on both devices, the results from Figure 5 were reanalyzed with altered parameters. The Roche Cedex® HiRes analyzer did not allow for any changes in the mixing and aspiration, but it did allow for increasing the recognition of both living and dead cells and for optimizing the analysis of cell clusters. The corresponding parameters were set to obtain the maximum total and viable cell concentrations. Similarly, the samples on the Vi-CELL BLU cell viability analyzer were reanalyzed with the *Decluster degree* set to *High*. The other parameters on the Vi-CELL BLU cell counter were left unchanged.

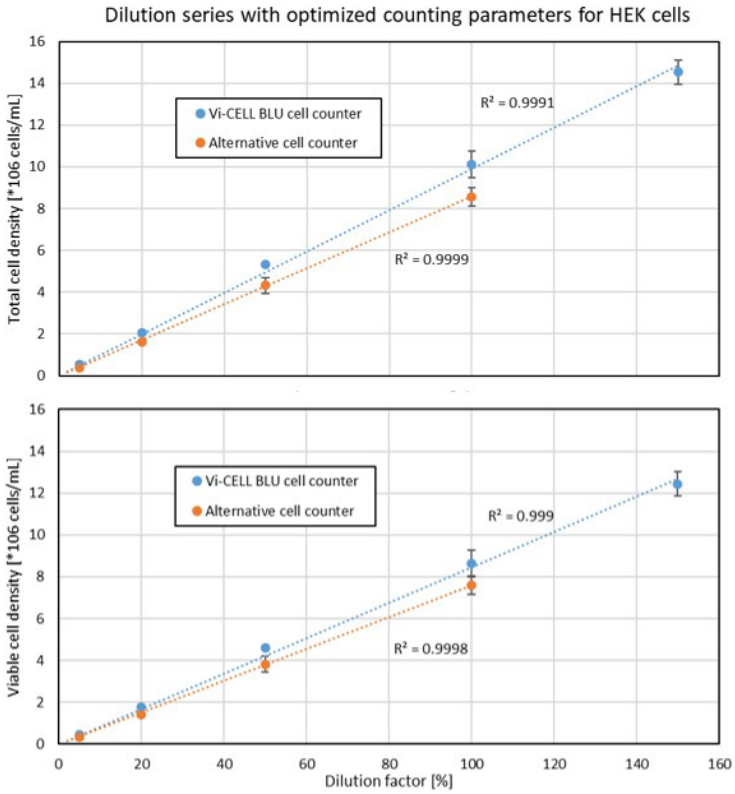

Figure 7. HEK cell dilution series with adjusted counting parameters on both cell counting devices. The linear trend lines were forced through (0,0) and the R^2 values are shown in the graphs. The error bars represent the standard deviation calculated from the replicate measurements.

The reanalyzed results in Figure 7 still show excellent linearity, but with increased cell concentrations for both devices. Further investigation would be necessary to find the optimal settings for counting these HEK cells. However, the Vi-CELL BLU analyzer was able to count more cells compared to the Roche Cedex® HiRes analyzer, even with the relevant counting parameters of the other analyzer set to the maximum. In fact, the initial run with the *Medium Decluster* degree setting resulted in very comparable counts compared to the optimized settings on the other cell counting device. Here, the increased aspiration and mixing cycles on the Vi-CELL BLU device likely helped breaking up some of the clumps present in the sample, leading to increased cell recognition. In addition, for further optimization on the Vi-CELL BLU analyzer some of the other parameters such as the *Cell sharpness, Min circularity* or *Viable spot brightness* could be investigated.

Conclusion

Both devices showed excellent linearity measuring both the CHO and HEK cell lines. The initially used cell counting parameters resulted in higher counts on the Vi-CELL BLU cell viability analyzer. Furthermore, both devices allowed for increasing the measured cell concentrations by altering the cell type parameters used for image analysis. However, the upper limit for the alternative cell counting device was well below the observed counts on the Vi-CELL BLU cell viability analyzer after setting the *Decluster degree* to *High*.

The Vi-CELL BLU cell viability analyzer allows for optimizing the amount of mixing and aspiration cycles, which might help with accurately counting clumpy cell lines. Furthermore, the Vi-CELL BLU device had comparably higher throughput at lower measurement times while using lower sample volumes.

References

1. *ISO 20391-1:2018.* **International Organization for Standardization.** s.l. : ISO/TC 276 Biotechnology, 2018-01.

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