Validation Study of the Vi-CELL™ XR for Automated Cell Counting of Dendritic Cells

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Dr.Iveta Bottova is a Process Development Specialist at SOTIO, a biotechnology company developing a next generation Active Cellular Immunotherapy drug. In this application note, Dr. Bottova shares the Validation Study conducted to change from a manual counting process for dendritic cells to an automated counting process using the Vi-CELL XR.

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Abstract

Dendritic cells (DC) population is a key functional constituent of cell based immunotherapy drugs. The correct cell count and adequate viability of DC are one of the quality control criteria for the final product release. Number of viable DC is historically determined by microscopy using manual counting method – Bürker chamber and trypan blue dye for dead cell exclusion. The manual method can have significant variability between cell counts determined by different people performing the manual counting, which may contribute to an unstable manufacturing process. The manual method is also time consuming for the operator. An automated cell counting process would remove the variability between operators[2] and could free up the operator for other tasks. The Vi-CELL XR (Vi-CELL) is an automated cell counting and viability analyzer that uses the trypan blue dye exclusion method. The Vi-CELL was evaluated as a suitable method for Quality Control of DC count and viability for a dendritic cell-based drug. The test for Vi-CELL counting accuracy was performed on concentration control beads of known concentration three times under the same operating conditions. Diameter and circularity of DC and lymphocyte was determined by NIKON™ Eclipse microscope to set up the correct recognition of DC to exclude lymphocytes for Vi-CELL counting. The size range for DC was established so lymphocytes were excluded and number of total DC, number of viable DC and DC viability were analyzed and compared to Bürker chamber counting. There was no significant difference between DC count obtained by Vi-CELL and by Bürker chamber. Vi-CELL automated cell counter was established as a method which is accurate and suitable for DC counting.

Introduction

SOTIO is a biotechnology company that is developing a next generation Active Cellular Immunotherapy ("ACI"), focusing on the treatment of cancer and autoimmune diseases. The company is in the process of developing new medical therapies using an immunotherapy platform based on activated dendritic cells with the view to significantly improving these treatments and making them available to patients. SOTIO is currently focused on developing a new medicinal product, named DCVAC/PCa, for prostate cancer patients. The Phase I and Phase II clinical trials, already involving several hundred patients with prostate cancer at various stages, indicate promising preliminary results.

In this application note we present a validation study where the Vi-CELL XR was evaluated as a method for quality control of DC count and viability for the DCVAC/PCa final product.

Results

Evaluation of Vi-CELL measurement accuracy

The accuracy of the automated counting method – Vi-CELL was determined by calculating inter and intra operator CV% (Vi-CELL vs. Bürker chamber). Vi-CELL accuracy was tested using concentration control beads of known concentration ($1x10^6$/ml). Bead concentration was determined by two operators, three times under the same operating conditions. To evaluate the counting accuracy, the results obtained with Vi-CELL were compared to those

obtained using Bürker chamber. Coefficient of variation between each counting and also between operators did not exceed 5% for both counting methods. The final concentration of control beads determined by manual and automated counting method was comparable and in the correct range of $0.9x10^6$count/ml – $1.1x10^6$count/ml ($1x10^6$count/ml ± 10%) as required in the concentration control manual (Figure 1).

Vi-CELL	Number of beads [$x10^6$]	CV % intra
Op1 average	1.03	1.68
Op2 average	1.02	4.10
Op1-2 average	1.02	2.89
SD Op1-2	0.005	
CV % inter Op1-2	0.46	

BURKER	Number of beads [$x10^6$]	CV % intra
Op1 average	1.03	1.12
Op2 average	0.99	6.44
Op1-2 average	1.01	3.78
SD Op1-2	0.028	
CV % inter Op1-2	2.81	

Figure 1: Accuracy test. The test was performed on concentration control beads (1x10e6count/ml), by Operator 1 and 2, three times under the same operating conditions on both counting devices. The average of three counts and their SD was calculated for each operator for both counting devices to obtain CV% intra value. The average number of beads counted by two operators and its SD (Op1-Op2) was calculated to obtain CV% inter value between two operators. Vi-CELL count was comparable to Bürker chamber counting. CV is defined as the ratio of the standard deviation to the average. Op = Operator, CV = coefficient of variation, SD = standard deviation.

Assessment of precise cell diameter and circularity for optimal setting of Vi-CELL for DCVAC analysis

DCVAC/PCa contains several cell populations but mainly DC and lymphocytes. Since Vi-CELL imaging system analyzes different cell types based on their size and circularity, diameter and circularity of DC and lymphocyte were determined to set up a correct recognition of DC to exclude lymphocytes for Vi-CELL counting. NIKON™ Eclipse microscope at 20x magnification (same as Vi-CELL microscope) and NIS Elements BR imaging software were used to determine both parameters.

Cell circularity was analyzed on five DCVAC/PCa lots (two control and three clinical study subjects) measuring twenty DC and ten lymphocytes for each lot and the average circularity was calculated for each lot (Figure 2A). The circularity for each cell type was compared and no significant difference was found between DC and lymphocytes (P=0.6560; paired t-test) and therefore making it impossible to differentiate those cell types based on their circularity.

Cell diameter was analyzed on nineteen DCVAC/PCa lots (six control batches and thirteen clinical study subjects) measuring twenty DC and ten lymphocytes for each lot (Figure 2B). Diameters of both cell populations were compared to establish the size range that would distinguish DC from lymphocytes so lymphocytes were excluded during Vi-CELL counting. The characteristic DC size range was determined to be between 11-30 µm and lymphocytes were within the range of 5-12.5µm overlapping partially with lower limit for DC population, however the percentage of overlapping was not high (4%).

A

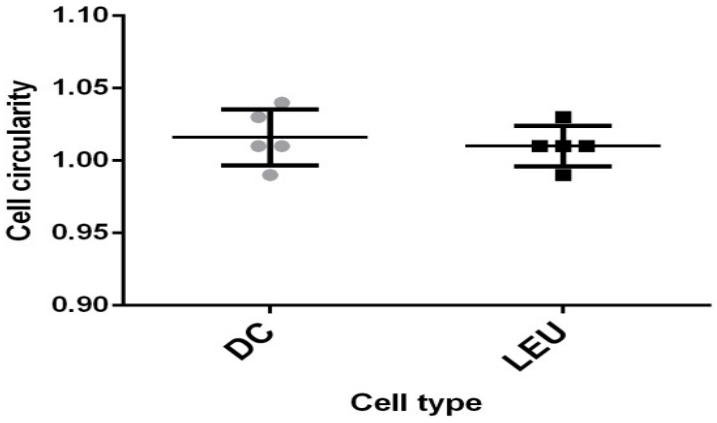
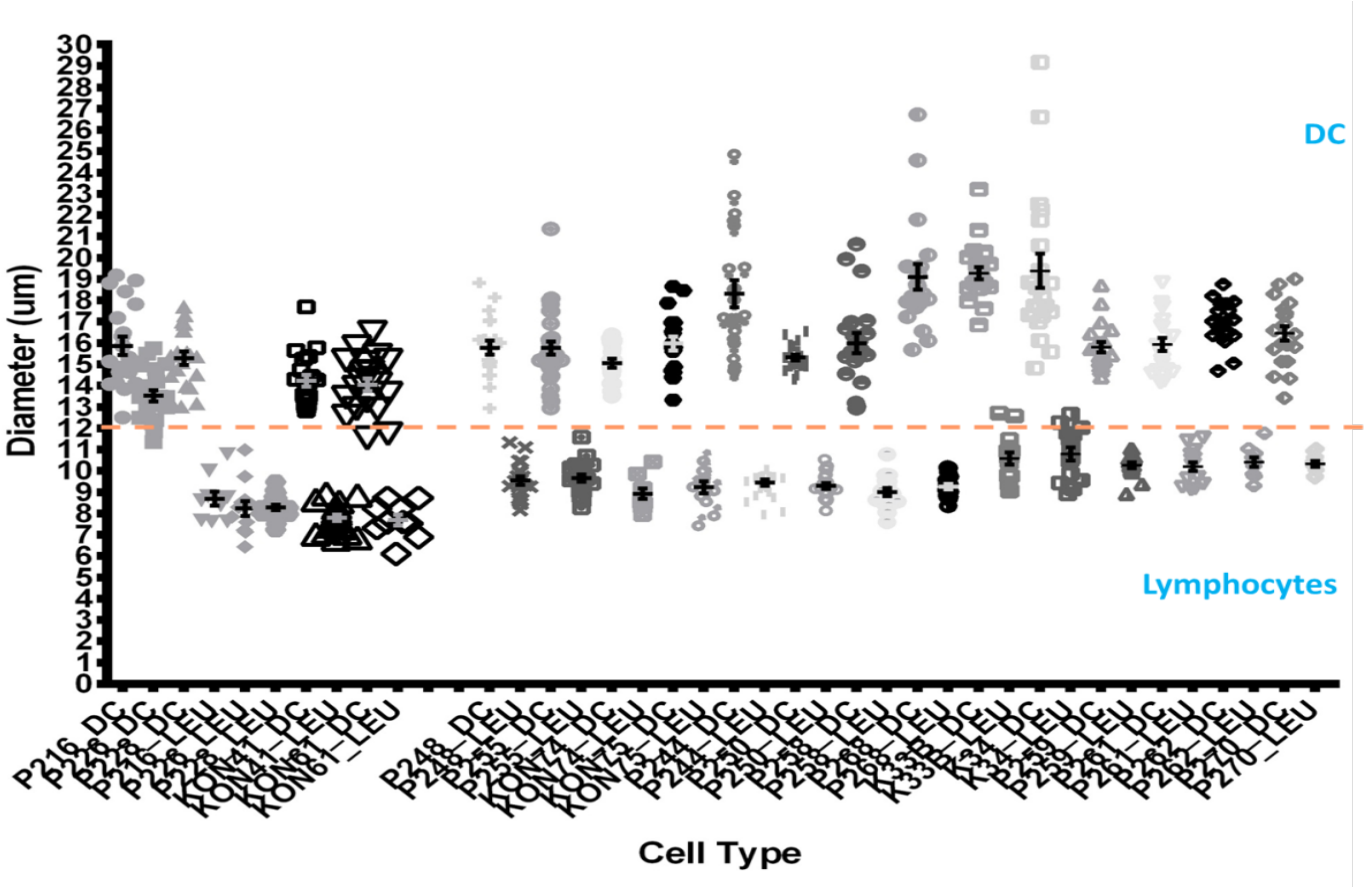


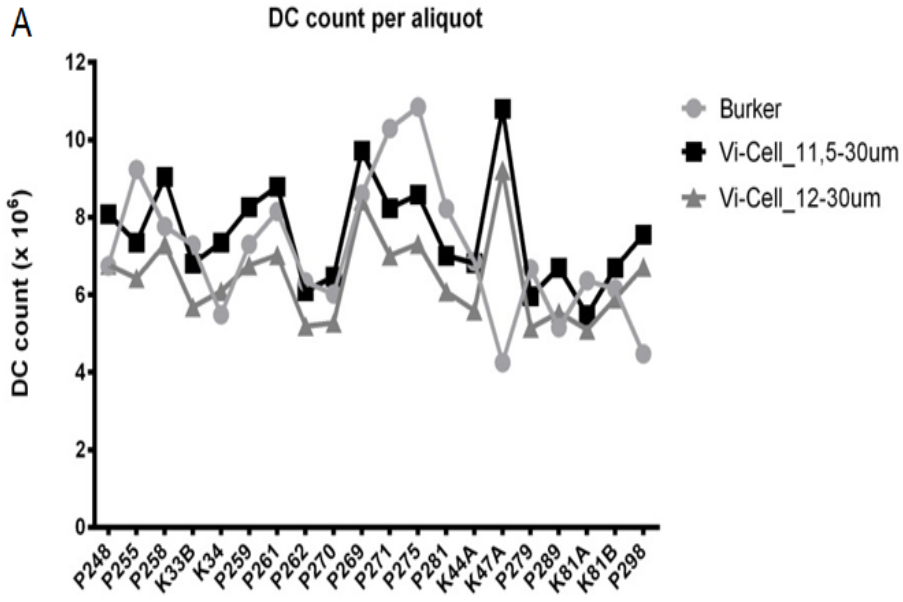
Figure 2: Cell circularity and diameter determination in DCVAC/PCa. Both parameters were determined by NIKON™ Eclipse at 20x magnification and NIS Elements BR imaging software. **A)** Circularity was determined on 5 DCVAC/PCa lots when 20 DC (gray points) and 10 lymphocytes (black squares) where measured and average was calculated for each lot. **B)** Diameter of DC and lymphocytes was determined and based on those data a Vi-Cell criterion for DC size was established: 11.5-30µm and 12-30µm. P = clinical study subject DCVAC/PCa, KON = control DCVAC/PCa, DC = dendritic cells, LEU = lymphocytes.

B



Validation of cell counting using Vi-CELL automated counter

Since the size separation between both populations was not completely conclusive, two size parameters: 11.5-30µm and 12-30µm were tested as possible Vi-CELL setting parameters for DC counting. Number of total DC, number of viable DC and DC viability was determined on twenty DCVAC/PCa lots (five control and fifteen clinical study subjects) for both established size parameters in Vi-CELL in parallel with Bürker chamber counting. Obtained values by the Vi-CELL were compared to Bürker counting values using paired t-test (level of significance α=0.05) to determine which size parameter is more suitable for DC recognition by the Vi-CELL and not significantly different from Bürker counting. It was shown that there is no significant difference between DC counts (total and viable) in Vi-CELL and Bürker chamber (Figure 3). Both proposed size parameters were suitable for Vi-CELL set up for DC recognition, however the range 11.5-30µm was less significantly different than 12-30µm when compared to Bürker chamber counting (Figure 3A, 3B). Moreover, when the difference between a pair of measurements (Vi-CELL value-Bürker value) was plotted against their mean (Vi-CELL value+ Bürker value/2), the differences were smaller and more centered around zero in the case of 11.5-30µm range compared to 12-30µm range thus confirming 11.5-30µm range to be more suitable as a Vi-CELL size parameter for DC counting (Figure 4). DC viability values (%) determined by Vi-CELL were significantly different from those obtained by Bürker chamber counting (Figure 3C). This is most probably related to the fact that a different number of cells were analyzed by each method. Vi-CELL system was counting around 1000 cells per run compared to proximately 100 cells counted in Bürker chamber suggesting the automated method to be more precise in percentage determination.



ViCELL_11,5um vs. Burker
Paired t test
P value = 0,2953
Significantly different? (P < 0.05) = **No**
Number of pairs = 20

ViCELL_12um vs. Burker
Paired t test
P value = 0,1255
Significantly different? (P < 0.05) = **No**
Number of pairs = 20

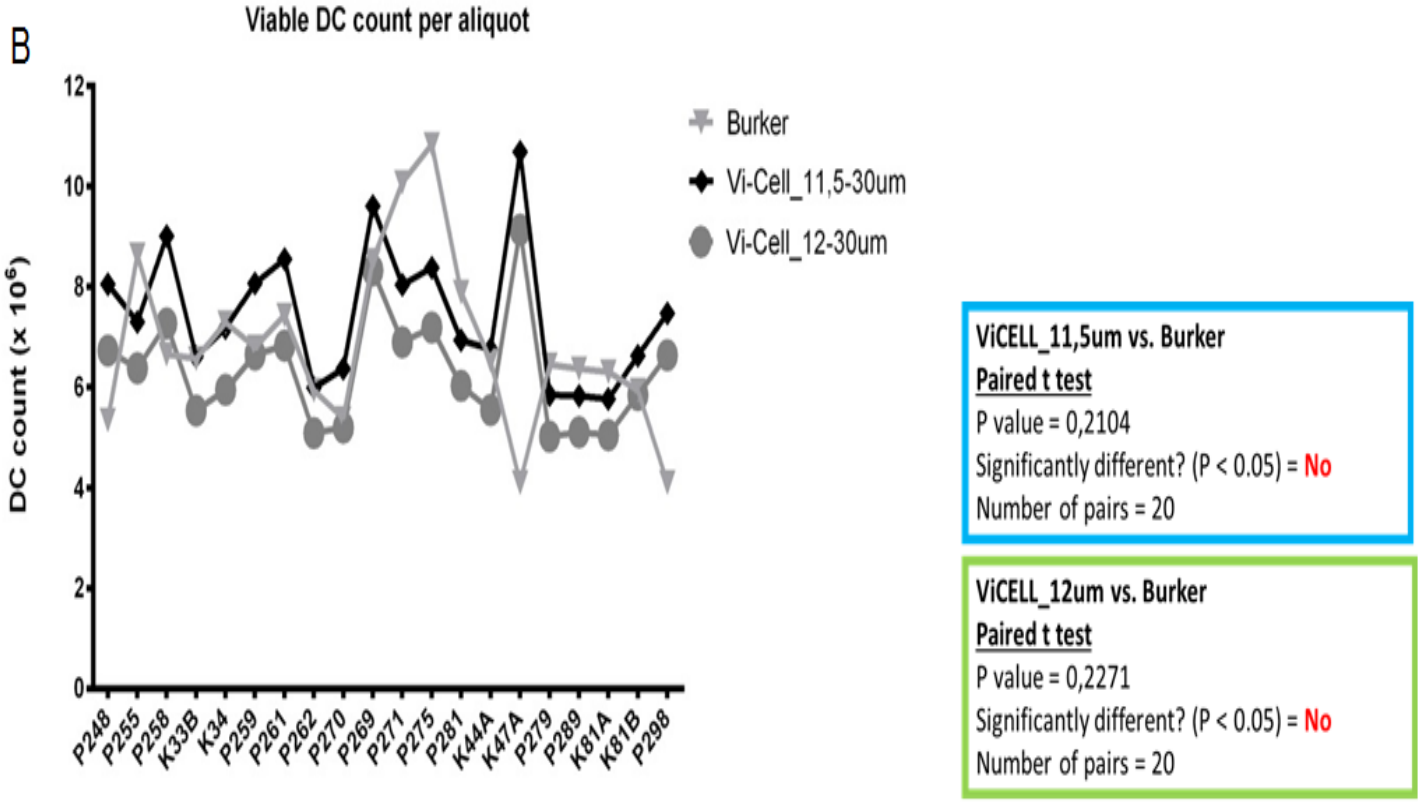


Figure 3: DC count and viability. DCVAC/PCa aliquots of 20 batches were thawed according to SOP 11-KON and cell count and viability were measured using Burker chamber (SOP 16-KON) and using Vi-CELL counter in parallel. Two size ranges for DC recognition: 11.5-30µm and 12-30µm were tested as a possible Vi-CELL setting parameters. Number of total DC per aliquot (A), number of viable DC per aliquot (B) and DC viability (C) in DCVAC/PCa were counted by both methods Vi-CELL and Bürker chamber. Obtained values were compared in paired t-test with the level of significance α=0,05. P = clinical study subject DCVAC/PCa, K = control DCVAC/PCa, DC = dendritic cells.

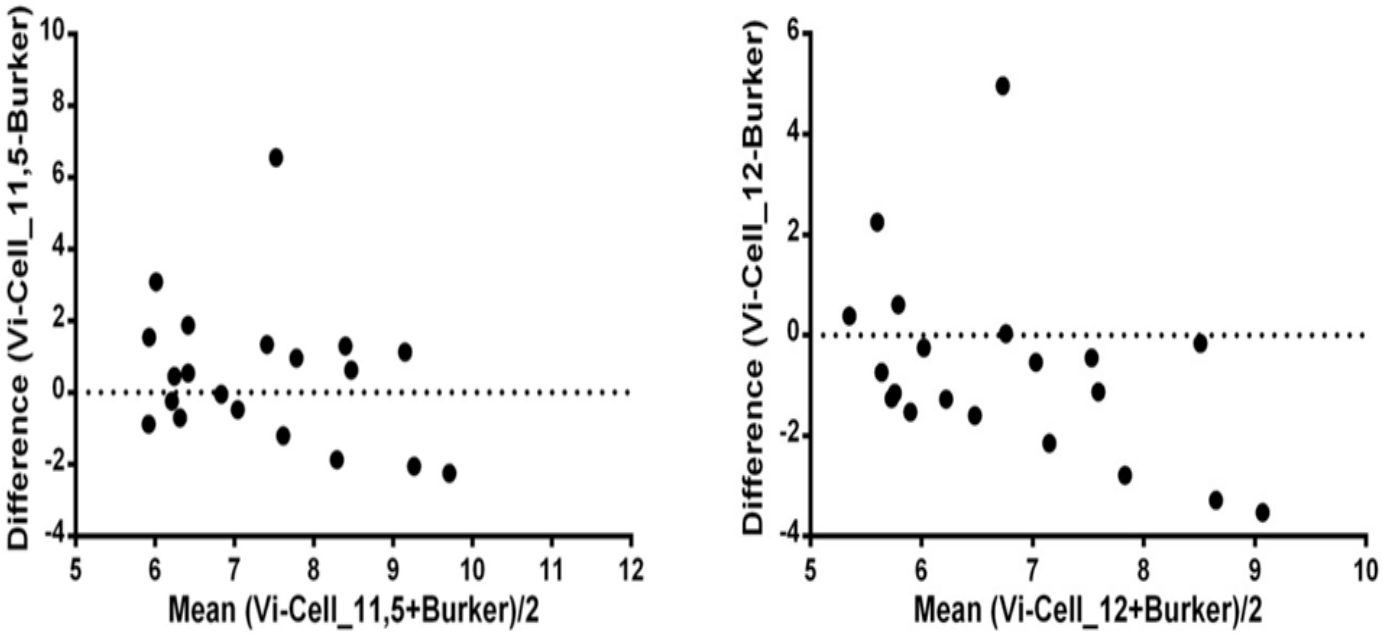


Figure 4: XY plot. The difference between a pair of measurements (Vi-CELL value-Bürker value) was plotted against their mean (Vi-CELL value+Bürker value/2) for both of the size range parameters 11.5-30µm and 12-30µm. The level of differences and its position towards the zero was analyzed. The differences were smaller and more centered around zero in case of 11.5-30µm.

Conclusion

The Vi-CELL automated cell count and viability analyzer was established as a method which is accurate and suitable for DC counting and comparable to the currently used quality control method Bürker chamber. There was no significant difference between DC counts values obtained by Vi-CELL and by Bürker chamber, moreover the size range 11.5-30µm is important for DC recognition in the Vi-CELL and shown to be suitable as DC size set up parameter.

References

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2. Comparison of Manual versus Automated Trypan Blue Dye Exclusion Method for Cell Counting. Kristine S. Louis, Andre C. Siegel, Gary A. Levy. Application Note – Industrial BioDevelopment Laboratory (www.ibdl.ca)

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