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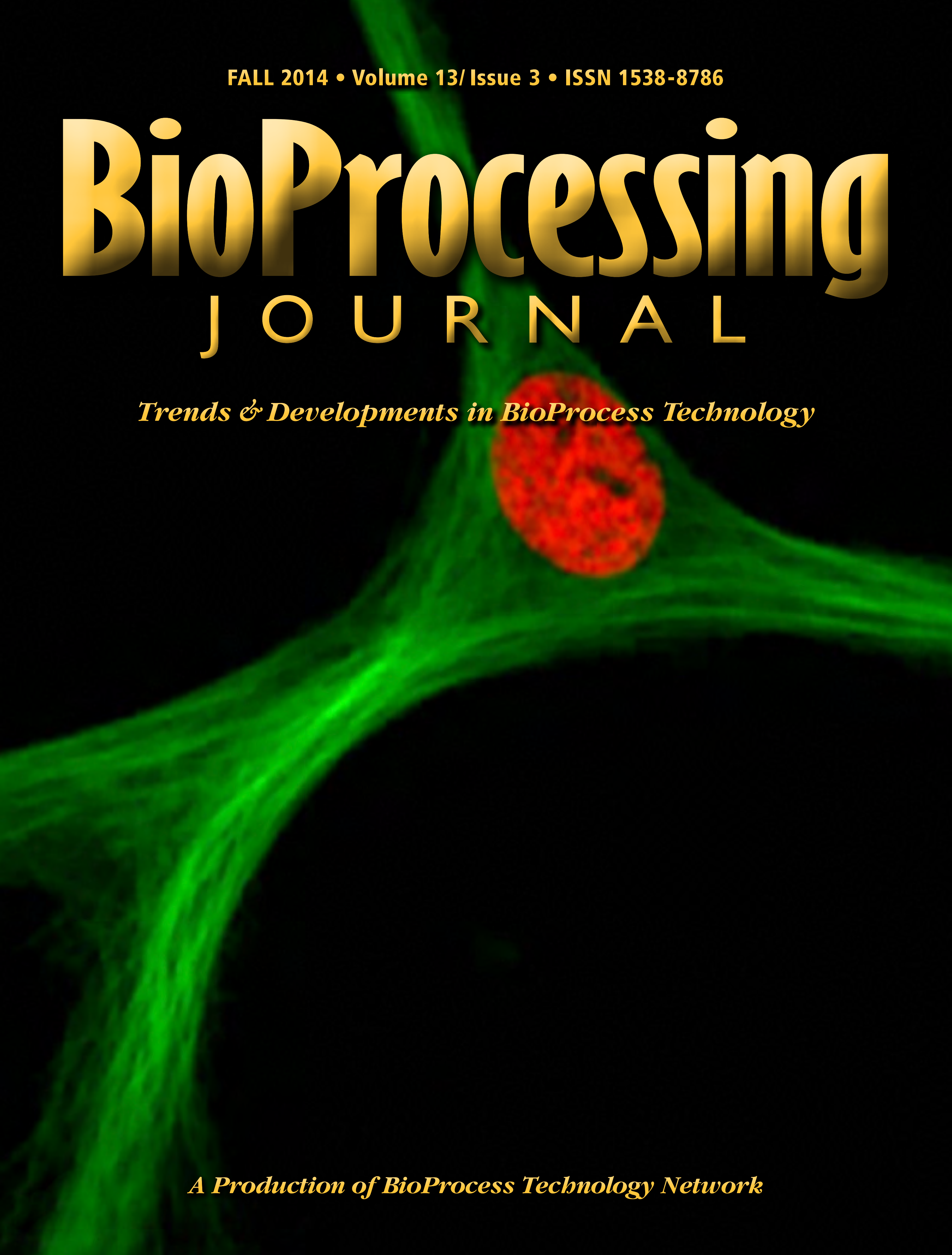
BioProcessing JOURNAL

Trends & Developments in BioProcess Technology



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BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

APPLICATION NOTE

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

By IVETA BOTTOVÁ and LENA LEE

Abstract

Dendritic cell (DC) population is a key functional constituent of cell-based immunotherapy drugs. The correct cell count and adequate viability of DCs are one of the quality control criteria for the final product release. The number of viable DCs is historically determined by microscopy using a 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 procedure, which may contribute to an unstable manufacturing process. The manual method is also time-consuming for the operator. An automated cell counting process helps remove the variability between operators[1] and can free up the operator for other tasks.

The Vi-CELL® XR 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 counts and viability for a dendritic cell-based biologic drug. The test for Vi-CELL counting accuracy was performed three times each on known concentration control beads, under the same operating conditions. The diameter and circularity of dendritic and lymphocyte cells was determined by a NIKON™ Eclipse microscope to set up the "correct recognition of DC. The size range for DC was established so that lymphocytes could be excluded. The number of total DC, viable and also non-viable, were analyzed and compared to Bürker chamber counting. There was no significant difference between the DC count obtained by Vi-CELL and by Bürker chamber. Vi-CELL automated cell counting was established as a method which is accurate and suitable for use with dendritic cells.

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 using an immunotherapy platform based on activated dendritic cells. SOTIO is currently focused on developing a new medicinal product, 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.[2]

Results

Evaluation of Vi-CELL Measurement Accuracy

The accuracy of the automated counting method (Vi-CELL) was determined by calculating inter and intra operator coefficient of variation (CV) % (Vi-CELL vs. Bürker chamber). Vi-CELL accuracy was tested using known concentration control beads (1×10^6/mL). Bead concentration was determined by two operators, performing measurement processes three times each, under identical operating conditions. To evaluate their counting accuracy, the results obtained with Vi-CELL were compared to those obtained using the Bürker chamber. The CV 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 methods was comparable, and in the correct range of 0.9×10^6 count/mL to 1.1×10^6 count/mL (1×10^6 count/mL ± 10%), as required in the concentration control assay sheet (Table 1).

Assessment of Precise Cell Diameter and Circularity for Optimal Setting of Vi-CELL for DCVAC/PCa analysis

DCVAC/PCa contains several cell populations, but mainly DC and lymphocytes. Since the Vi-CELL imaging system analyzes different cell types based on their size and circularity, the diameter and circularity of DCs and lymphocytes were determined to set up a correct recognition of DCs to exclude lymphocytes for Vi-CELL counting. NIKON™ Eclipse microscope at 20× magnification (the same as Vi-CELL's 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 20 DC and ten lymphocytes

TABLE 1. Cell counting accuracy test.

Operations	# of Beads ($\times 10^6$)	CV % Intra Value
Vi-CELL Automated Method		
Operations	**# of Beads ($\times 10^6$)**	**CV % Intra Value**
Average of 3 (operator 1)	1.03	1.68
Average of 3 (operator 2)	1.02	4.10
Average (operators 1 and 2)	1.02	2.89
Standard deviation (SD) % (operators 1 and 2): 0.005		
SD % inter (both operators): 0.46		
Bürker Manual Method		
Operations	**# of Beads ($\times 10^6$)**	**CV % Intra Value**
Average of 3 (operator 1)	1.03	1.12
Average of 3 (operator 2)	0.99	6.44
Average (operators 1 and 2)	1.01	3.78
SD % (operators 1 and 2): 0.028		
SD % inter (both operators): 2.81		

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Vi-CELL XR CELL VIABILITY ANALYZER

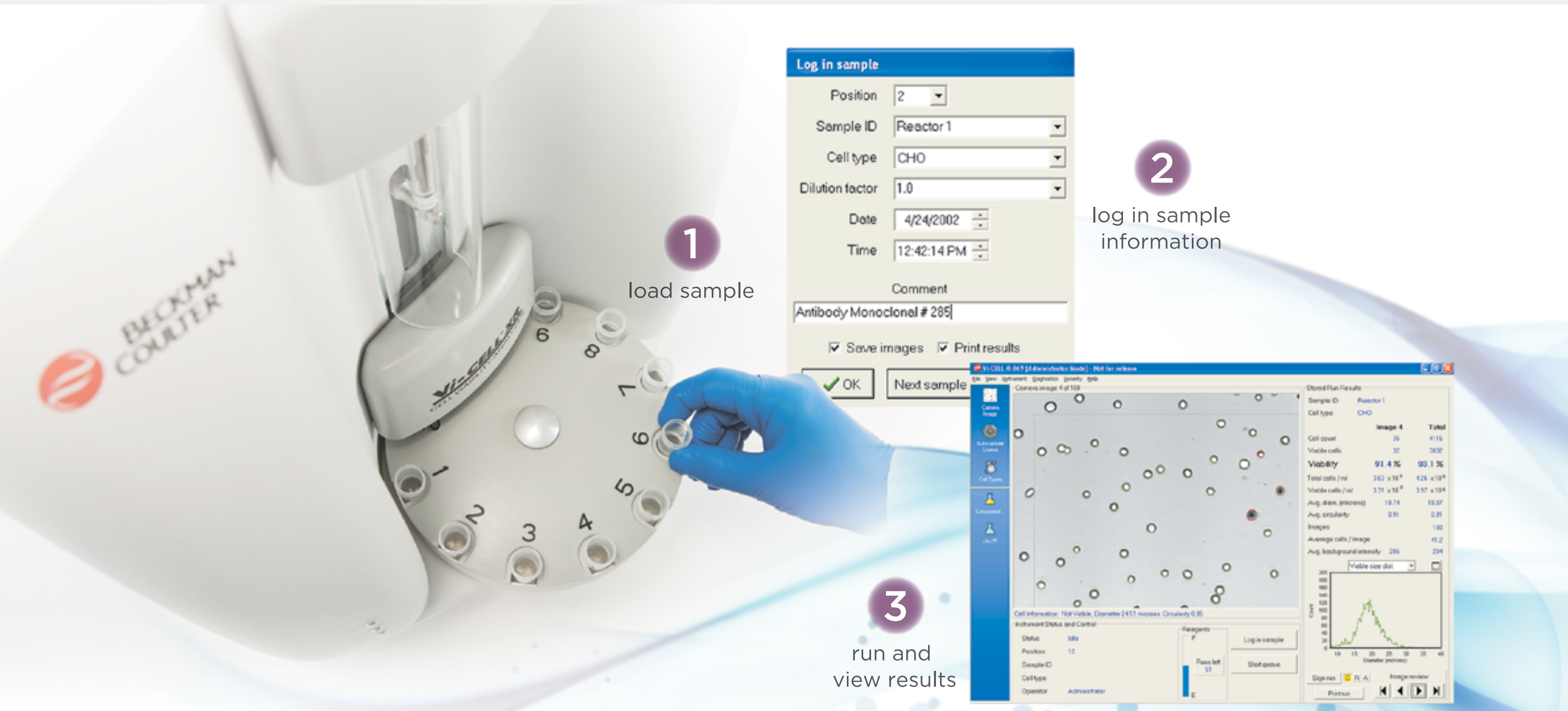

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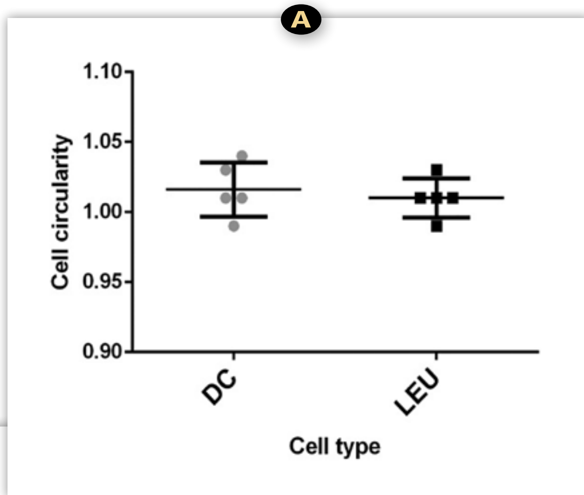
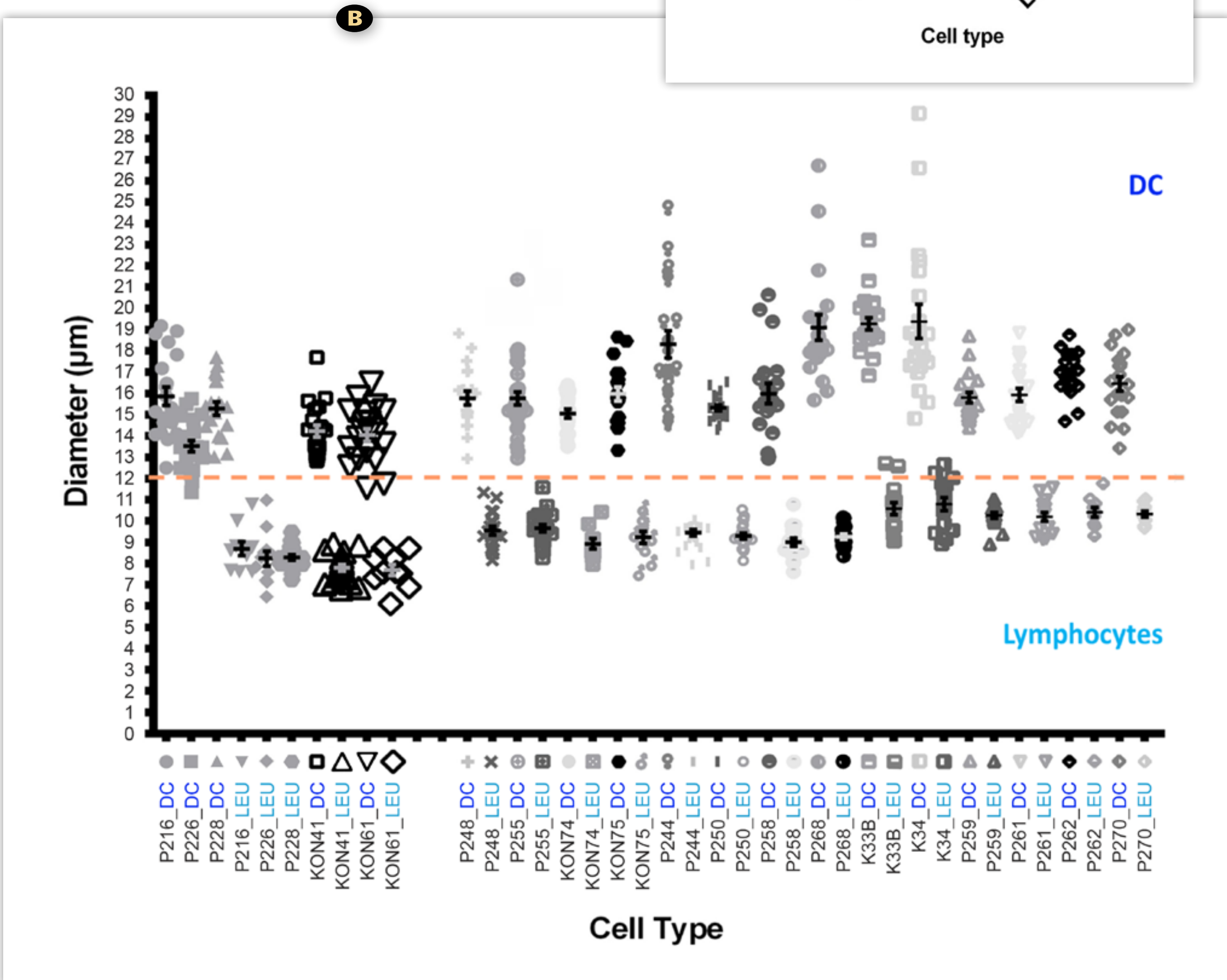
FIGURE 1. Cell circularity and diameter determination in DCVAC/PCa. Both parameters were determined by NIKON Eclipse at 20× magnification and NIS Elements BR imaging software. **(A)** Circularity was determined on five DCVAC/PCa lots when 20 DC (gray points) and five lymphocytes (black squares) were measured and the average was calculated for each lot. **(B)** Diameter of DCs and lymphocytes was determined and, based on those data, a Vi-CELL criterion for DC size was established: 11.5–30 µm and 12–30 µm. (P = clinical study subject DCVAC/PCa; KON = control DCVAC/PCa; DC = dendritic cells; LEU = lymphocytes.)





for each lot, and the average circularity was calculated for each lot (Figure 1A). 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, made it impossible to differentiate those cell types based on their circularity.

Cell diameter was analyzed on 19 DCVAC/PCa lots (six control batches and 13 clinical study subjects) measuring 20 DC and ten lymphocytes for each lot (Figure 1B). Diameters of both cell populations were compared to establish the size range that would distinguish DCs from lymphocytes, so that lymphocytes would be 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 5–12.5 µm, overlapping partially with the lower limit for DC population. However, the percentage of overlapping was not high (4%).

Validation of Cell Counting Using Vi-CELL Automated Counter

Since the size separation between both populations was not completely conclusive, two size parameters: 11.5–30 µm and 12–30 µm were tested as possible Vi-CELL setting parameters for DC counting. The number of total DC, viable DC, and DC viability was determined on 20 DCVAC/PCa lots (five control and 15 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 a paired *t*-test (level of significance: α = 0.05) to determine which size parameter was more suitable for DC recognition by the Vi-CELL, and it was 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 2).

Both proposed size parameters were suitable for the Vi-CELL set up for DC recognition. However, the range 11.5–30 µm was less significantly different than 12–30 µm when compared to Bürker chamber counting (Figure 2, A and B). 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 11.5–30 µm range compared to 12–30 µm range.

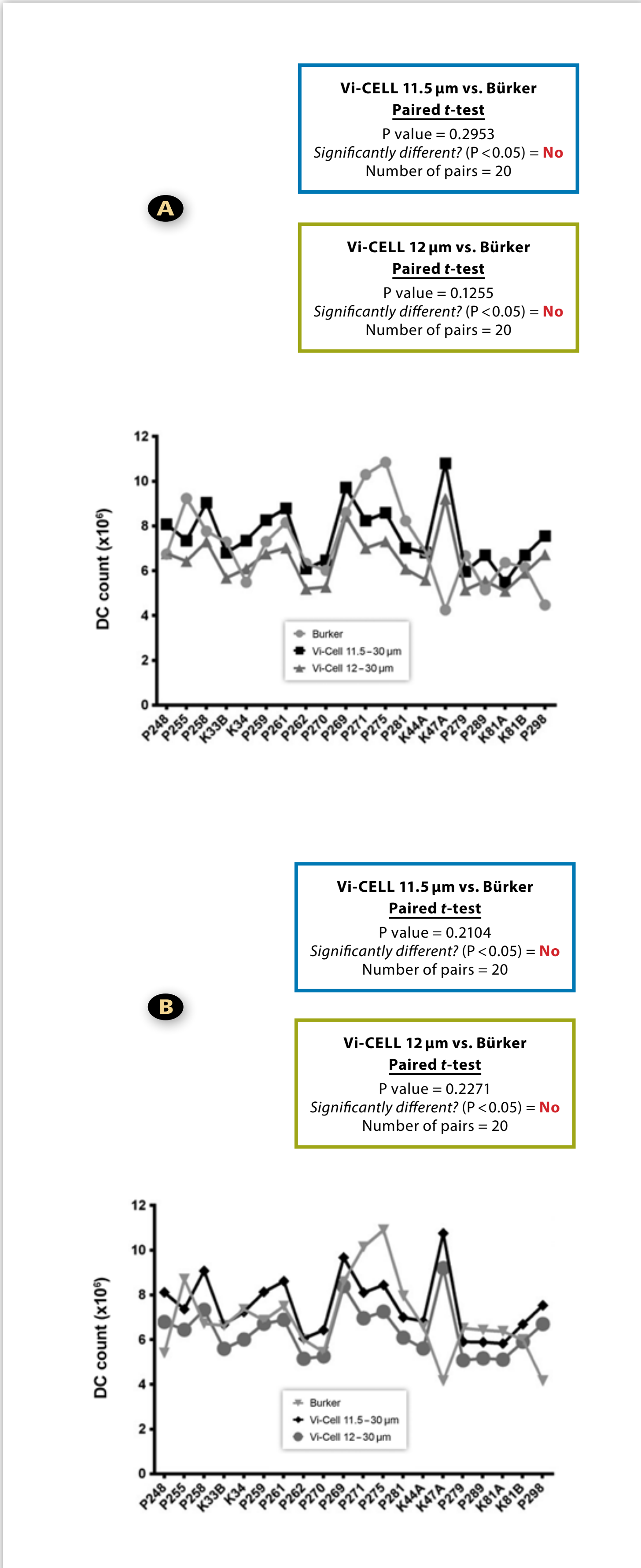


FIGURE 2. DC count and viability. DCVAC/PCa aliquots of 20 batches were thawed according to standard operating procedure (SOTIO SOP 11-KON), and cell count and viability were measured using Bürker chamber (SOTIO SOP 16-KON) and the Vi-CELL counter, in parallel. Two size ranges for DC recognition: 11.5–30 µm and 12–30 µm were tested as possible Vi-CELL setting parameters. **(A)** The number of total DCs per aliquot; and **(B)** number of viable DCs per aliquot. DC viability 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.)

This confirmed the 11.5–30 µm range to be more suitable as a Vi-CELL size parameter for DC counting (Figure 3). DC viability values (%) determined by Vi-CELL were significantly different from those obtained by Bürker chamber counting. This is most probably related to the fact that a different number of cells were analyzed by each method. The Vi-CELL system was counting around 1000 cells per run compared to approximately 100 cells counted in the Bürker chamber, suggesting the automated method to be more precise in percentage determination.

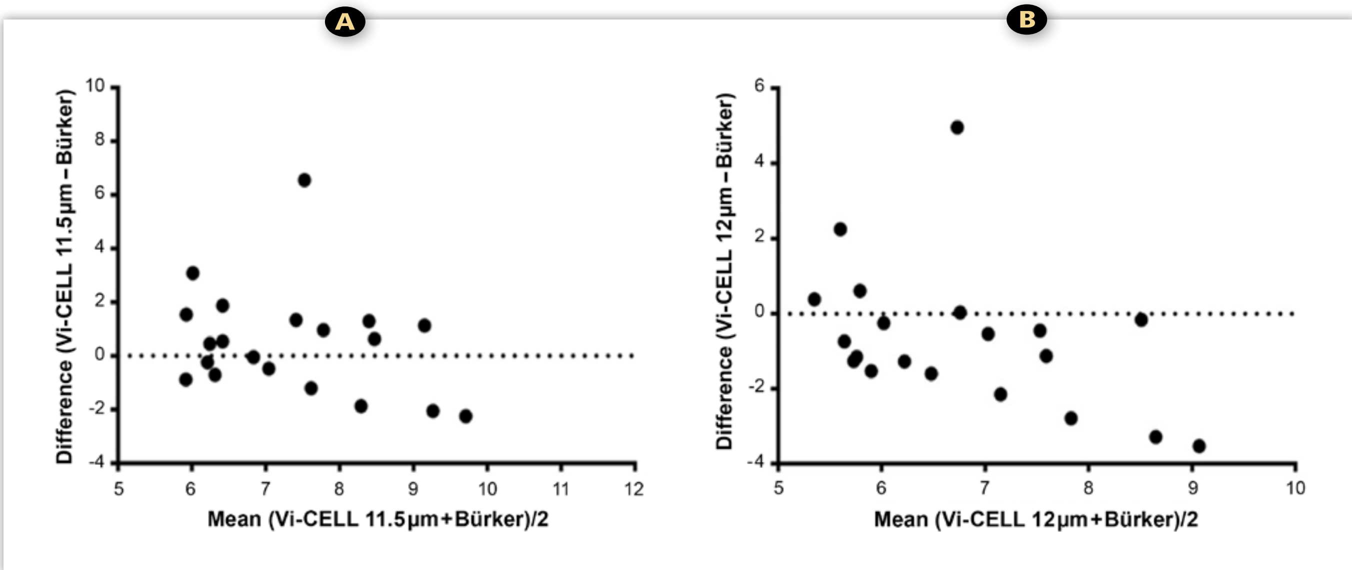


FIGURE 3. XY plot. The difference between a pair of measurements (Vi-CELL value – Bürker value) was plotted against their mean (Vi-CELL value + Bürker value/2) for both size range parameters: 11.5–30 µm **(A)**; and 12–30 µm **(B)**. The level of differences and its position toward the zero was analyzed. The differences were smaller and more centered around zero in the case of 11.5–30 µm.

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 count values obtained by Vi-CELL and the Bürker chamber. Moreover, the size range of 11.5–30 µm is important for DC recognition in the Vi-CELL and shown to be suitable as the DC size setup parameter.

References

[1] Louis KS, Siegel AC, Levy GA. *Comparison of manual versus automated trypan blue dye exclusion method for cell counting*. Application Note – Industrial BioDevelopment Laboratory.

[2] Bottová I. *Dendritic cell counting in Vi-CELL automatic cell counter*. Internal Validation Study Report, SOTIO, a.s.

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