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Application Note

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Cell Line Development: High-Throughput Viable Cell Density Determination Using the iQue® Advanced Flow Cytometer

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Introduction

The use of clinical biologics is a rapidly growing market, which is underpinned by the efficient development of cell lines to support the manufacture of these therapeutics. From gene cloning and initial clone selection through to final cell evaluation, the continual assessment of cell count and viability is important for determining the best growing and highest producing clones. This information can be used for monitoring proliferation rates, optimizing growth conditions and normalizing cell data for further studies. Traditional methods for measuring cell count and viability are often low throughput, time-consuming and lacking in linearity. Advanced flow cytometry provides a fast, accurate and reproducible solution for cell viability and density quantification, which is essential for efficient biologics development processes.

This application note documents the use of the iQue® Cell Count and Viability Kit in combination with the iQue® Advanced Flow Cytometer to accurately quantify both cell count and viability in 96- or 384-well plates. The data presented here shows that the iQue® Cell Count and Viability Kit alongside the integrated iQue Forecyt® analysis software can be effectively used with various non-adherent cell types for rapid and accurate quantification, requiring small cell volumes. The data also demonstrates how this kit can be easily, rapidly and successfully utilized for a cell line development workflow.

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Method and Workflow

The iQue® Cell Count and Viability Kit is designed for reproducible, quantitative analysis of cell count and viability. The kit provides fast quantification of a variety of non-adherent cell lines (not optimized for adherent cell types) across a large linear range.

The streamlined workflow (Figure 1) guides the user from cell labelling, using a simple no-wash protocol, through to analysis within the iQue Forecyt® software. The kit includes validated reagents and pre-set gating templates to enable simple, automated identification of live cells in both 96- and 384-well plates. Samples can be either cultured cells or freshly thawed cells.

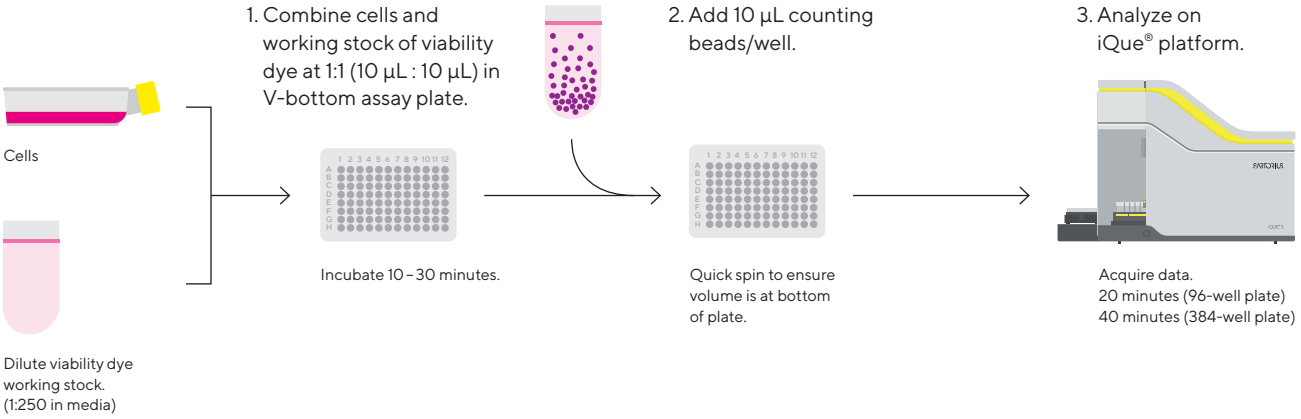

Figure 1: *iQue® Cell Count and Viability Kit Workflow*

Protocol

1. Allow all kit components to come to room temperature prior to assay. To prepare a 2× viability dye working stock, dilute iQue® Live Viability (R/Red) Dye into cell culture medium at a dilution factor of 1:250.

2. Perform Assay
   - 2.1 Combine cell samples and iQue® Live Viability (R/Red) Dye working stock at 1:1 ratio in a V-bottom assay plate (10 µL cells and 10 µL working stock dye).
   - 2.2 Quickly spin (300 × *g*, 5 seconds) and briefly shake (2000 RPM, 20 seconds) the assay plate to ensure samples are thoroughly mixed.
   - 2.3 Incubate samples for 10-30 minutes at room temperature, while protecting from light.
   - 2.4 Add 10 µL iQue® Counting beads to each sample well of the assay plate. The beads should be vortexed prior to use.
   - 2.5 Quickly spin (300 × *g*, 5 seconds) and briefly shake (2000 RPM, 20 seconds) the assay plate to ensure samples are thoroughly mixed.

3. Plate Acquisition and Data Analysis
   - 3.1 Analyze the plate on the iQue® platform using the provided template with pre-set gating, acquisition settings, and analysis settings for the iQue Forecyt® software.
   - 3.2 Use the template to gate Beads, All Cells and Live Cells (see Figure 2).
   - 3.3 Viability (%) is calculated from Live Cell population as a percent of All Cells. Cell density is automatically calculated from the known bead density × (cell count/bead count), which is pre-set in the template.

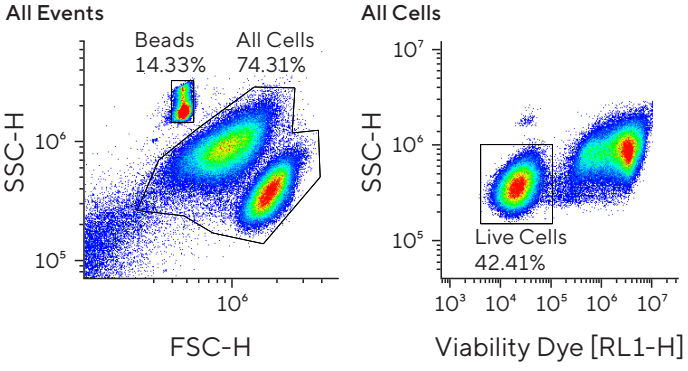

Figure 2: *Example Plots Taken From iQue Forecyt® Using the Templated Pre-set Gating and Analysis for Cell Count and Viability*

Validation of Kit

To validate the accuracy of the kit, it was initially tested across a range of non-adherent cell types to assess the linear range of quantification compared to an alternative quantification method. Jurkat, Raji or Ramos cells were grown in culture and mixed with heat-killed cells at various ratios to provide specific cell viability percentages. For heat killing, cells were heated to > 60°C in a water bath for a minimum of 10 minutes before cooling.

Mixed samples were taken, and cell viability was assessed using the iQue® Cell Count and Viability Kit and an alternative technique based on trypan blue exclusion counting (Figure 3A). The data clearly demonstrates a linear correlation between the two counting techniques ($R^2 > 0.99$) for all cell types across 0 to > 90% viability range. A similar assessment was performed using Jurkat cells at various cell densities (Figure 3B) showing a linear correlation ($R^2 > 0.97$) across cells densities from 8×10^4 to 2×10^7 cells/mL.

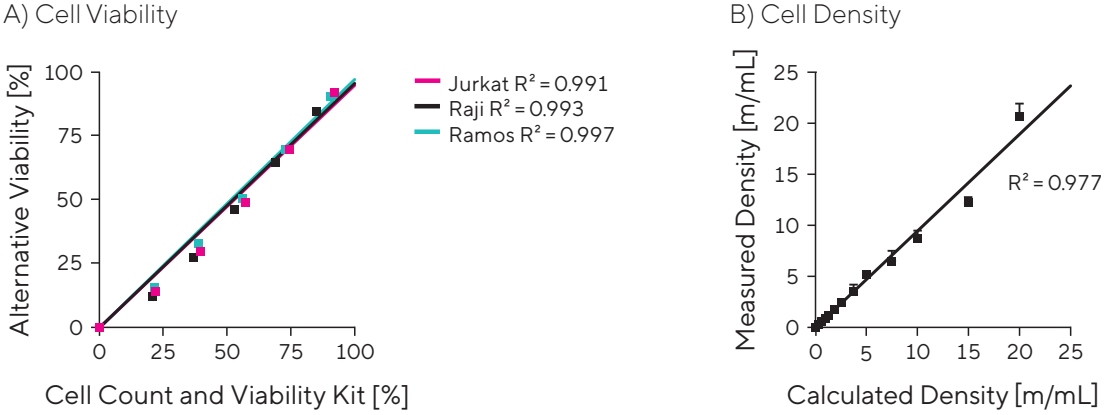

Figure 3: *Linear Assessment of Quantification. Comparison of the iQue® Cell Count and Viability Kit to Alternative Counting Method to Assess the Viability of Various Ratios of Live and Heat-Killed Cells (A) Or Jurkat Cell Density (B). Data Shown as Mean of (A) 12 or (B) 4 Samples ± SD, With Linear Regression Analysis.*

Validation as Part of a Cell Line Development Workflow

Assessment of viable cell density (VCC) is an important quantification during a cell line development strategy. This is of key interest during the phases of pool evaluation, single-cell cloning and then clone evaluation. During these stages, there can be high numbers of samples (> 100 pools and clones) that need assessment at regular intervals. The use of a high throughput, reproducible, fast analysis system is advantageous to ensure the identification of the best clones. This selection process has traditionally been performed using methods such as comparison of titer measurements as the assessment of viable cell count has been too laborious and time consuming.

To validate the use of the iQue® Cell Count and Viability Kit in this workflow it was tested against two alternative counting methods. One method used a tube based, trypan blue exclusion counting method using a high sample volume (200–500 µL), the other an electrical resistance-based technique again using tubes, but using a lower volume of sample (50–100 µL). Clear success criteria were defined for the iQue® solution.

The data needed to be robust, accurate and comparable to traditional methods (maximal ± 20% deviation). It also needed to significantly reduce hands-on time and enable high-throughput VCC determinations using limited sample volumes.

After optimization of iQue® instrument settings, accuracy, and reproducibility of the quantification of VCC was assessed by counting a clone sample across 72 repeats (Figure 4A). The culture was determined by the iQue® to have a mean value of 75.75×10^5 cells/mL (SD ± 3.7×10^5), with an average deviation across samples of -0.17% (SD ± 4.67%). Similar data was determined using precision count beads (Precision Count Beads™ (Biolegend), data not shown) which showed an average deviation of -0.74% (SD ± 4.42%). Accuracy was further assessed against alternative quantification methods for both a mini-pool and clone cultivation sample (Figure 4B and 4C). The iQue® data shows some variation in values (mini-pool 5.35% SD ± 12.79%, clone -2.85% SD ± 22.92%), but within the ± 20% limits and comparable to data from the other methods already in use in the lab.

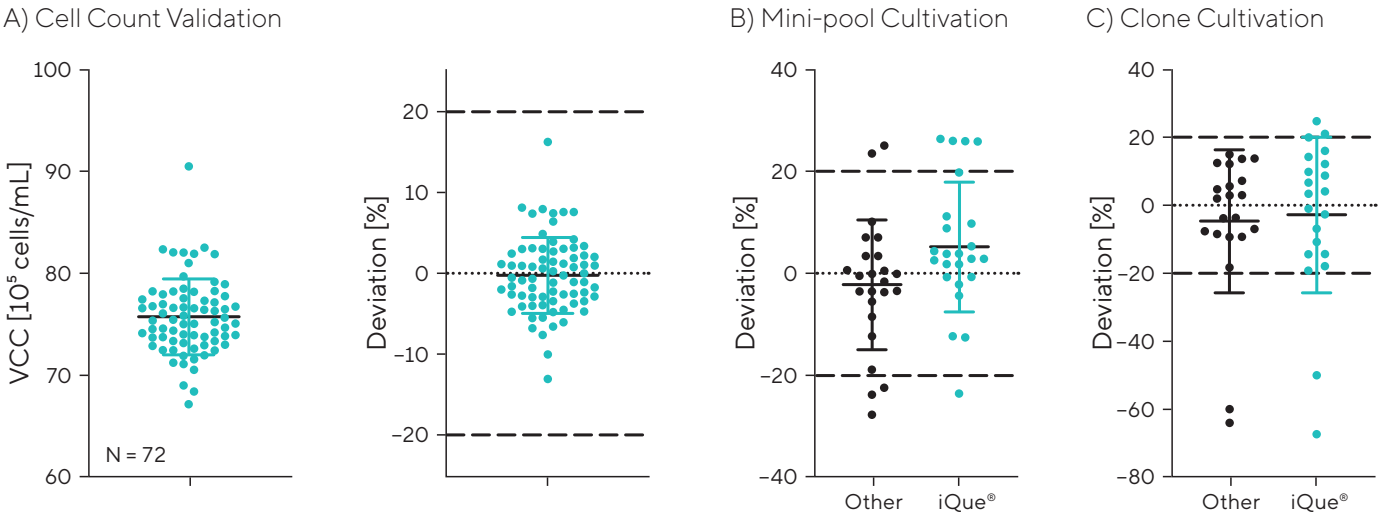

Figure 4: *Accuracy Assessment of Viable Cell Counting With iQue®. (A) VCC and Deviation Data for 72 Repeat Clone Samples Assessed by iQue® Cell Count and Viability Kit. Deviation in Counts for Mini-Pool (B) And Clone Cultivation (C) Samples Assessed by iQue® (Teal) And an Alternative Method (Black). Data Points Show Individual Determinations With Mean (Solid Black Line) ± SD (Teal Line).*

The iQue® Cell Count and Viability Kit was also evaluated during a clonal expansion phase. The goal of these experiments was to simulate the clone expansion phase used during a standard cell line development process and to analyze the reliability of the iQue® workflow to replace an alternative counting method. As with the previous examples, the acceptance criteria defined values were required to be within ± 20% deviation from the alternative count method. Ten clones of the cell line CHO-DG44 were seeded in duplicate to a VCC of 2×10^5 cell/mL in a 25 mL culture volume. Cells were measured and split based on VCC determination by the iQue® and an alternative counting method covering the period of six splits over 25 days in culture.

When the ten clones were assessed across splits (Figure 5A), 63.3% of values were within the ± 20% deviation criteria. In general, the better growing clones were identified by both methods (data not shown). Example data is shown for two of the ten clones (Figure 5B and 5C) showing how the VCC compares over the various splits for the two counting methods. This data confirms that the iQue® Cell Count and Viability Kit in combination with the iQue® Advanced Flow Cytometer can be used as part of a clone expansion phase. In addition, the user sample preparation time was reduced using the iQue® method, meaning multiple replicates could be run for each sample, increasing the accuracy and robustness of the data generated.

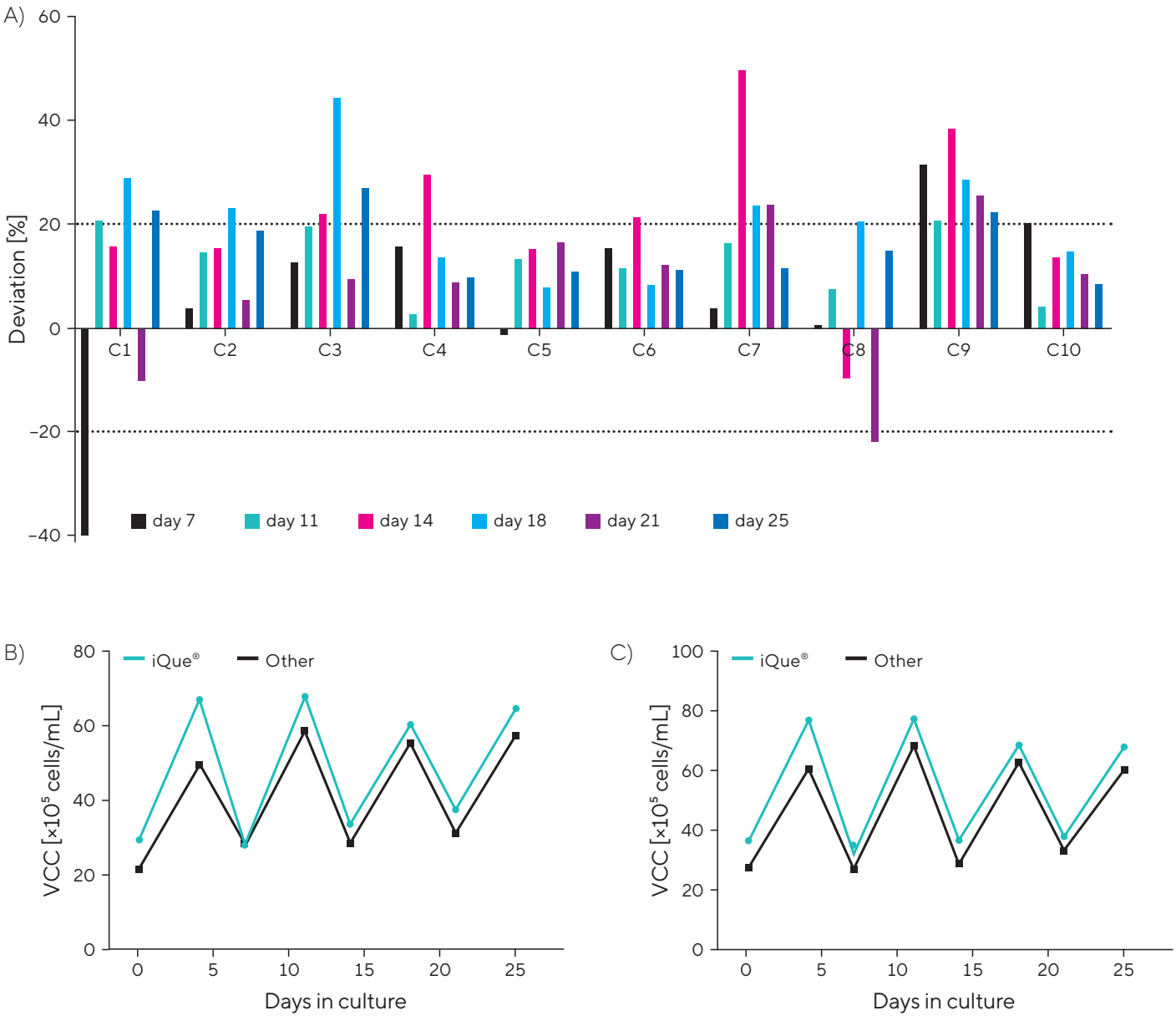

Figure 5: *Use of VCC as Part of Clone Expansion Protocol. The Deviation (%) Between iQue® Workflow and an Alternative Counting Method Was Compared Across Ten Clones (C1–C10) Over 25 Days of Culture (A), Dotted Lines Represent the Acceptance Criteria of ± 20%. Absolute VCC Data for Two Clones Are Shown in Detail (B and C) Over the Various Splits for the iQue® (Teal Circles) And Alternative Counting Method (Black Squares).*

The final validation data directly compares the iQue® workflow to the two alternative counting methods described previously. For this data, samples were taken from 20 different shake flasks of the cells used in the previous experiment and measured using all three counting methods. The same acceptance criteria were applied to this data. The comparison data (Figure 6) shows that the iQue® cell counts are generally in between the two alternative counting methods, but show a reasonable correlation ($R^2 > 0.8$), with values falling within 20% of unity.

As mentioned previously the iQue® values are the means of three determinations per sample enabled by the easy sample handling and high-throughput quantification method. During this experiment it was noted that for one of the alternative counting methods (method_1, electrical resistance-based method) the preparation of the sample before counting made a considerable difference to the absolute counts. This data supports the use of the iQue® workflow as an alternative measuring tool for VCC determinations.

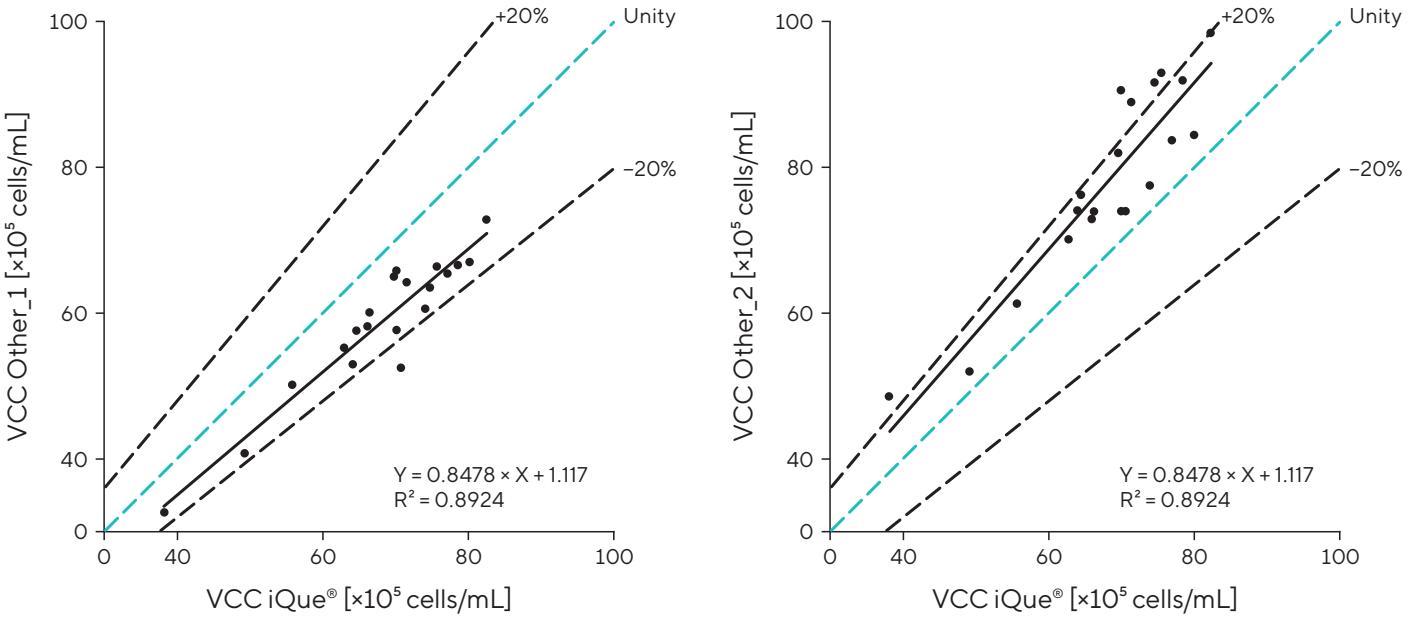

Figure 6: *Direct Comparison of Quantification Using iQue® Workflow to Two Alternative Counting Methods. Correlation Plots Show VCC Data From Twenty Different Shake Flasks at Various Cell Densities. Linear Regression Analysis Shown (Black Line, Equation With R^2 Value), Line of Unity (Teal) And ± 20% (Dashed Black Line).*

Conclusion

The data presented here validates the iQue® Cell Count and Viability Kit for both cell counting and viable cell density quantification of non-adherent cells. The kit components in combination with a simplified sample handling workflow and fully integrated, pre-defined data analysis provides the user with an accurate, high-throughput and rapid cell quantification method. Further validation demonstrates how this workflow can be integrated into a cell line development process to aid the rapid identification of healthy growing clones.

Conclusions from the data collected during the clonal expansion experiments demonstrate comparable quantification of VCC compared to alternative accepted methods. Further significant advantages in time required for experiment and analysis were recorded using the iQue® workflow:

- Significant increase in capacity to quantify VCC samples enabled through use of 96- or 384-well plates, in combination with rapid analysis (96 samples in 10 minutes).
- User-friendly templates to enable plug-and-play measurement and analysis within the iQue Forecyt® software.
- Access to both total cell count and viable cell count data within the template, which saves on analysis time.

The workflow using the iQue® Cell Count and Viability Kit in combination with the iQue® Advanced Flow Cytometry Platform along with the iQue Forecyt® software offers the user a high-throughput method for viable cell count determination for use throughout the cell line development process.

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