Counting Murine CAR T Cells Using the Corning® Cell Counter

Application Note

CORNING

Jeff Partridge
Corning Incorporated, Life Sciences
Bedford, MA USA

Introduction

About CAR T cells

Chimeric Antigen Receptor, or CAR T cells, are genetically modified autologous T cells that are currently used for immunotherapy to treat certain cancers[1-3]. These T cells are modified to express CARs, which are proteins that allow T cells to recognize an antigen on targeted tumor cells[4].

Early outcomes from CAR T-cell trials have generated impressive results in patients with refractory or relapsed leukemias and lymphomas[1-2], and research is underway on using CAR T cells in solid tumors[2,4].

Generating CAR T cells

Whole blood is collected from a patient (in this application note, mouse) and PBMC (peripheral blood mononuclear cells) are separated and saved while the remaining blood cells and components are returned to the body (leukapheresis). Next, T cells are enriched from the PBMC population using various methods (e.g., density gradient, size-based fractionation, antibody bead conjugates). The T cells are genetically modified with genes of interest, such that they now express CARs, allowing them to target and destroy tumor cells. CAR T cells are activated and expanded (*ex vivo*), and in production scale there are additional QA and transportation/delivery steps. Finally, CAR T cells are infused back to the patient[3]. This may be indirectly to the peripheral circulation or directly to the affected site depending on the tumor type[5].

Counting of CAR T cells

CAR T cells are often counted using time consuming manual methods such as a hemocytometer. While the hemocytometer has been considered the gold standard for cell counting since the 18th century[6], results are frequently subjective, variable, and small differences in cell diameters cannot easily or reproducibly be discerned.

The Corning Cell Counter offers an alternative method to quickly and accurately count these cells. The counting algorithm was developed to identify and remove debris and account for dead cells, while the functionality of the application allows for the population to be gated to remove unmodified T cells from the final count.

Materials and Methods

Murine CAR T cells obtained just prior to infusion were counted on the Corning Cell Counter, then immediately manually counted and viability determined using a hemocytometer and the trypan blue exclusion method. Representative images of the area counted (3 mm^2) are shown in Figure 1.

The experimental cell suspensions counted were typically heterogeneous samples, each having many dead cells, debris, and non-modified T cells, which are smaller than CAR T cells[7].

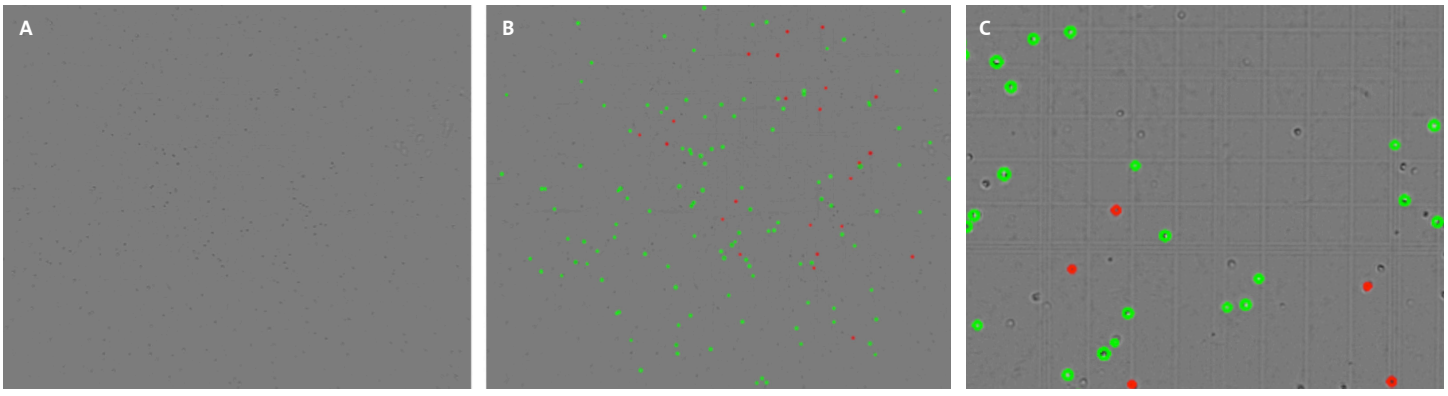

Figure 1. Representative the Corning Cell Counter images after focusing and after counting. (A) Properly focused image before count. (B) Results of count. Live cells are circled green; dead cells are circled red. Small cells and debris may be excluded from counting by the algorithm and by user specified gating (example in Figure 2). Note that a counting chamber having counting grids may be used on the Corning Cell Counter, but grids are not necessary. (C) Magnified view of B panel. Not all cells on screen were counted. **This is normal for this application.** Many of these cells were not modified (i.e., are not CAR-T cells) and should be excluded by size and/or cell morphology[7].

Results and Discussion

Using the *post hoc* histogram capability in the Axion app or Axion cloud, the bottom gate was set at 8 µm (Figure 2) due to the size difference between unmodified T cells and CAR T cells. For the experimental data generated during this evaluation, the results over nine independent data sets are shown in Figures 3 and 4.

When comparing the two counting methods using a Paired t-test, p values for both cell densities and percent viability were greater than 0.05, indicating there were no statistical differences between each data set. These results indicate the suitability of the Corning® Cell Counter for accurate counting of CAR T cells.

Although the human eye is quite sensitive, it may not be able to tell the difference between cells of five to eight microns, while the Corning Cell Counter can. Thus, gating out based on known cell size is a simple process with the Corning Cell Counter but not as easy when manually counting. This functionality allowed for accurate counts of murine CAR T cells by a trained operator.

Conclusions

- The Corning Cell Counter may be used in murine CAR T production where accurate cell counting is required. The Corning Cell Counter is intended as a replacement method for manual counting with a hemocytometer.
- While the Corning Cell Counter is very easy to use, proper focusing techniques need to be observed to avoid subjectivity in counts from different users. Increased familiarity with the Corning Cell Counter improves this accuracy. With every count, users will gain experience and proficiency.
- It is possible to count cell suspensions of a higher density on the Corning Cell Counter than by manual means. Thus the overall cell population will more accurately be represented, and the associated problem of "clicker fatigue" is avoided altogether.

References

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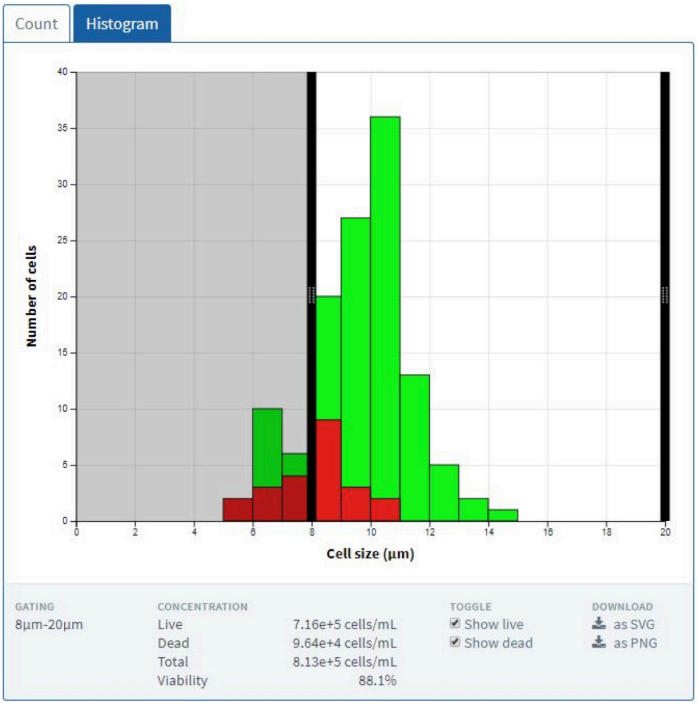

Figure 2. Histogram showing bottom gate set at 8 µm cell diameter. Cells below this threshold were excluded from the count. Dead cells (red bars) and live cells (green bars) can be toggled to independently examine each subpopulation.

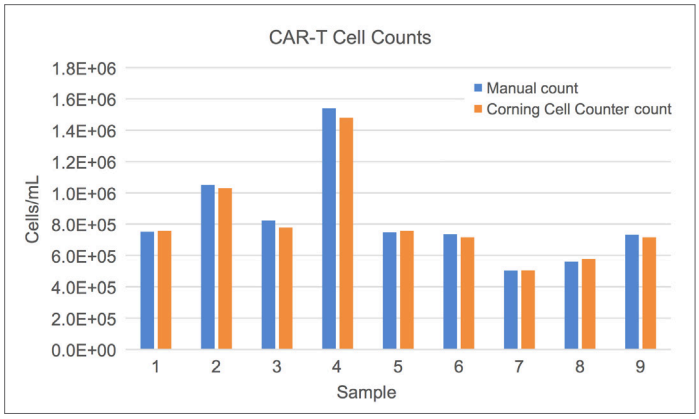

Figure 3. Cell densities by count method. Paired t-test p = 0.135.

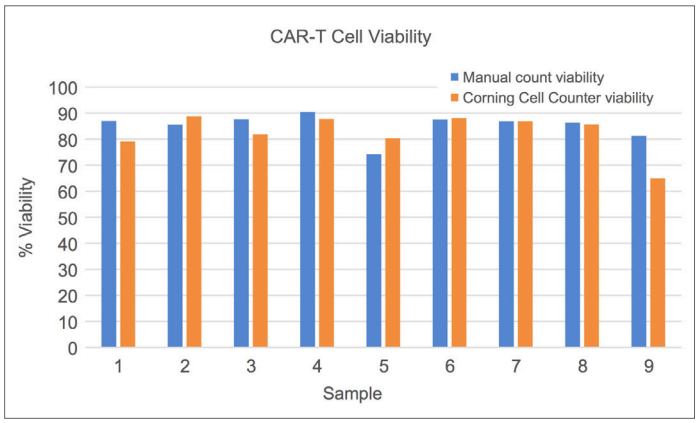

Figure 4. Cell viabilities by count method. Paired t-test p = 0.274.

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EUROPE
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France
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Germany
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The Netherlands
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United Kingdom
t 0800 376 8660
All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400