

Accurate Counting of Bio-Plex® Magnetic or Polystyrene Beads Using the TC10™ Automated Cell Counter

Philip Chapman and Eli Hefner, Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547

Introduction

The Bio-Plex suspension array system is a powerful tool for measuring analyte concentration. At the core of Bio-Rad's Bio-Plex Pro™ assays are magnetic carboxylated beads, microscopic spheres capable of carrying detection molecules, typically antibodies. While a variety of validated assays are currently available, many Bio-Plex system users are developing custom assays. When developing a custom assay, it is essential to determine bead concentration accurately and consistently to ensure uniform quantities of antibody are used in the bead-antibody conjugation step. Suboptimal conjugation will affect the sensitivity and dynamic range of the assay.

A method commonly used to determine bead concentration is by particle counting using a Coulter-based flow cytometry device such as a Coulter Counter. Flow cytometry-based devices measure particles in a stream using impedance technology. A precisely controlled flow allows volumetric measurements and estimation of bead concentration within the sample. While the technology is capable there are some disadvantages: Coulter Counters are relatively expensive, must be calibrated regularly, are prone to clogging, and do not provide an image to confirm count accuracy.

Another frequently used method for bead counting is manual counting using a hemocytometer. Manual counting suffers from a variety of issues: it is tedious and laborious and results are prone to error due to user subjectivity (Hefner et al. 2010). The advantage is that hemocytometers are readily available, inexpensive, and are used with standard laboratory equipment.

We demonstrate that while the TC10 cell counter was optimized for cell counting (Hefner et al. 2010, Hsuing et al. 2010), the instrument is also well suited to counting microspheres used in the Bio-Plex suspension array system. In addition, the TC10 cell counter offers a variety of advantages over the Coulter Counter and the hemocytometer.

Materials and Methods

Raw polystyrene and magnetic beads were prepared in a range of concentrations from stock solutions of 1.25×10^7 beads/ml. Six dilutions were prepared for each bead type by vortexing the stock tube of beads for 30 sec to fully resuspend the beads, then diluting the bead stock into phosphate buffered saline.

Hemocytometer Counting

Bead counts were performed using C-Chip disposable hemocytometers (INCYTO) and an Olympus IX70 inverted microscope (Olympus America Inc.) fitted with a Retiga EXi CCD camera from QImaging. Counting was repeated three times at each concentration and the number of beads was calculated by using the mean number of beads from the four corner cells of the Neubauer grid.

Beckman Coulter Counting

The beads were counted using a Beckman Coulter Z2 Coulter Counter (Beckman Coulter, Inc.). The counter was gated for 4–8 μm for the polystyrene beads and 5–9 μm for the magnetic beads. The beads were diluted in IsoFlow sheath fluid (Beckman Coulter). Each count was repeated three times using the automated count average function.

TC10 Automated Cell Counting

Bead counts were performed using a TC10 automated cell counter (Bio-Rad Laboratories, Inc.) by loading 10 μl of each bead solution into a dual-chamber TC10 counting slide (Bio-Rad). Each bead solution was counted three times and averaged.

The same person conducted all of the counting using the different techniques and worked from the same tubes of beads for consistency. Each bead preparation was counted three times so that an average and standard deviation could be determined.

Results

Six concentrations from 1.25×10^4 to 9.38×10^5 beads/ml of uncoupled Bio-Plex beads, both polystyrene ($5.6 \mu\text{m} \pm 0.2 \mu\text{m}$) and magnetic ($6.5 \mu\text{m} \pm 0.2 \mu\text{m}$), were measured using a TC10 cell counter, a hemocytometer, and a Coulter Counter. All three methods produced comparable results that matched the expected concentration (Figure 1). Except for the 9.38×10^5 beads/ml concentration of polystyrene beads, the standard deviations for data collected on the TC10 cell counter were equal to or lower than for the data collected using the Coulter Counter and the hemocytometer (Table 1). The bead concentration in Figure 1 represents dilutions from a stock concentration of 1.25×10^7 beads/ml.

The bead stock solution (1.25×10^7 beads/ml) was also counted using a TC10 cell counter and a Coulter Counter. Figure 2 is a representative image captured with a TC10 counter of a high-concentration solution of beads with bead identifier annotation. As for the diluted bead solutions, results were comparable between the two methods with precision favoring the TC10 cell counter (Figure 3). It should be noted that the hemocytometer counts using the undiluted beads were not attempted due to the difficulty associated with counting extremely high concentrations with this method.

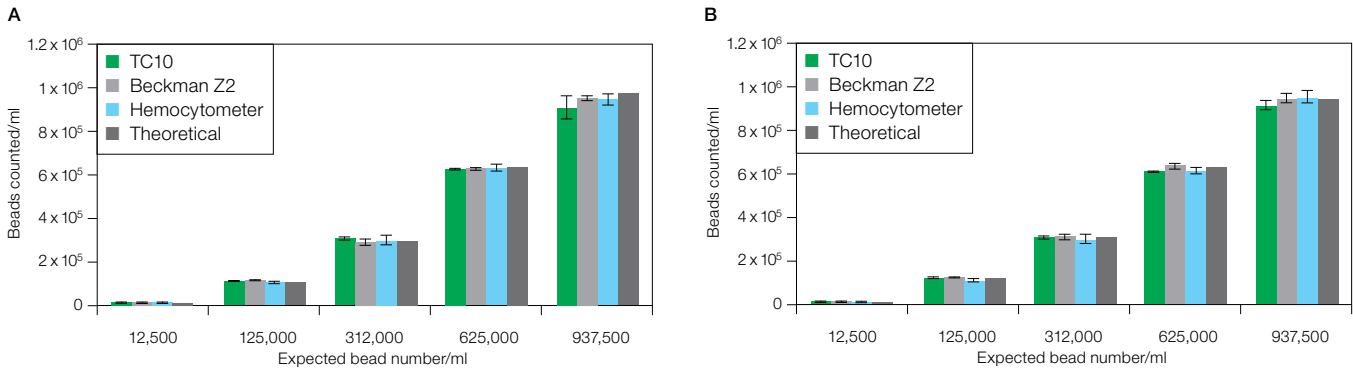


Fig. 1. Comparison of counts of a range of bead concentrations using the various methods. A, polystyrene beads; B, magnetic beads. The theoretical concentration was determined from dilutions of a validated stock solution. Error bars = 1 standard deviation.

Table 1. Bead counts using the three counting methods. Counting data for magnetic and polystyrene beads. **A**, TC10 automated cell counter; **B**, Beckman Z2 Coulter Counter; **C**, hemocytometer.

A		Magnetic Beads			Polystyrene Beads		
Tube	Theoretical Bead Concentration/ml	Average Bead Count (N = 3)	SD	CV, %	Average Bead Count (N = 3)	SD	CV, %
1	1.25×10^4	1.32×10^4	4.16×10^2	0.33	1.30×10^4	2.08×10^2	0.17
2	1.25×10^5	1.25×10^5	1.53×10^3	0.48	1.26×10^5	1.15×10^3	0.37
3	3.12×10^5	3.15×10^5	5.77×10^2	0.09	3.11×10^5	1.53×10^3	0.25
4	6.25×10^5	6.16×10^5	1.53×10^3	0.17	6.19×10^5	2.31×10^3	0.26
5	9.38×10^5	9.07×10^5	2.36×10^4	0.22	8.88×10^5	5.58×10^4	0.48
6	1.25×10^7	1.10×10^7	7.77×10^5	7.08	1.17×10^7	3.51×10^5	3.01

B		Magnetic Beads			Polystyrene Beads		
Tube	Theoretical Bead Concentration/ml	Average Bead Count (N = 3)	SD	CV, %	Average Bead Count (N = 3)	SD	CV, %
1	1.25×10^4	1.33×10^4	4.01×10^2	3.02	1.27×10^4	3.84×10^2	3.03
2	1.25×10^5	1.24×10^5	1.16×10^3	0.93	1.26×10^5	2.28×10^3	1.81
3	3.12×10^5	3.12×10^5	8.35×10^3	2.68	2.90×10^5	1.19×10^4	4.12
4	6.25×10^5	6.41×10^5	1.40×10^4	2.18	6.27×10^5	7.69×10^3	1.23
5	9.38×10^5	9.37×10^5	2.06×10^4	2.20	9.38×10^5	6.54×10^3	0.70
6	1.25×10^7	1.04×10^7	9.69×10^5	9.30	1.04×10^7	6.79×10^5	6.52

C		Magnetic Beads			Polystyrene Beads		
Tube	Theoretical Bead Concentration/ml	Average Bead Count (N = 3)	SD	CV, %	Average Bead Count (N = 3)	SD	CV, %
1	1.25×10^4	1.05×10^4	9.75×10^2	9.30	1.15×10^4	1.74×10^3	15.18
2	1.25×10^5	1.18×10^5	3.88×10^3	3.29	1.21×10^5	6.50×10^3	5.39
3	3.12×10^5	2.98×10^5	1.71×10^4	5.74	3.01×10^5	1.70×10^4	5.65
4	6.25×10^5	6.11×10^5	1.34×10^4	2.19	6.22×10^5	1.41×10^4	2.27
5	9.38×10^5	9.46×10^5	3.40×10^4	3.59	9.24×10^5	2.78×10^4	3.01
6	1.25×10^7	NA	NA	NA	NA	NA	NA

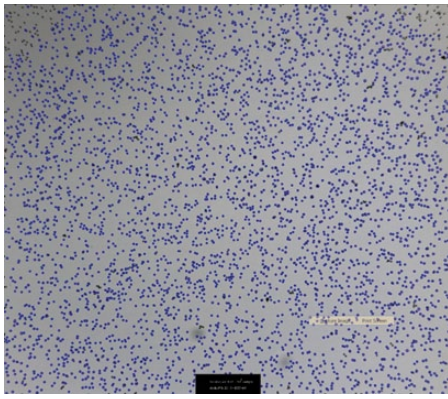


Fig. 2. Representative image of a high-concentration solution of beads. Image was taken using a TC10 automated cell counter. The blue dots indicate beads that were counted.

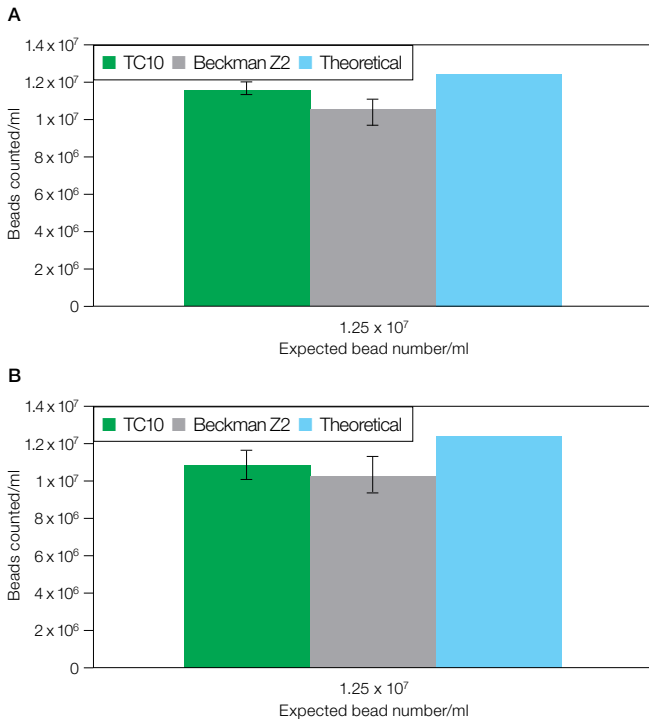


Fig. 3. High-concentration bead counting. Count of a high-concentration bead solution was performed on a TC10 cell counter, a Beckman Z2 Coulter Counter, and a hemocytometer. **A**, polystyrene beads; **B**, magnetic beads. The theoretical concentration was determined from a validated stock solution. Error bars = 1 standard deviation.

Conclusions

The TC10 automated cell counter accurately and precisely counted both polystyrene and magnetic Bio-Plex beads over a wide range of concentrations. Significant advantages of the TC10 cell counter compared to alternative approaches are the speed at which counts can be achieved and ease of use. Both the Coulter Counter and the hemocytometer require significant input from the operator to prepare the samples and then operate the machine. The time to count the beads on the hemocytometer is concentration dependent, with the lowest and highest concentrations taking up to 4–5 min per sample to obtain an accurate count. Also the auto-focus function of the TC10 cell counter makes it quicker and easier to use compared to the hemocytometer. The TC10 cell counter is an excellent option for counting beads used in the Bio-Plex suspension array system.

References

Hefner E et al. (2010). Comparison of count reproducibility, accuracy, and time to results between a hemocytometer and the TC10™ automated cell counter. Bio-Rad Bulletin 6003.

Hsiung F et al. (2010). Multifocal plane analysis is essential for accurate cell viability assessment using an automated cell counter. Bio-Rad Bulletin 6011.

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