A COMPARISON OF ABSOLUTE COUNTING PARTICLES FOR QUANTIFICATION OF CELLULAR SUBSETS BY FLOW CYTOMETRY IN DRUG DEVELOPMENT

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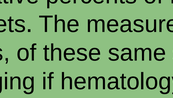
ABSTRACT

Rationale: Flow Cytometry can be used to measure relative percents of many leukocyte subsets. The measurement of absolute counts, of these same subsets, can be challenging if hematology data is not available. Many commercial vendors have developed particles, which when added to whole blood specimens can provide absolute counts, eliminating the need for hematology data. The purpose of this experiment was to compare absolute counting particles from four different vendors to determine if they compare for quantifying counts in the standard T-cells and subsets, B-cells and NK cells. **Experimental Procedures:** Particles from BD, Beckman Couter, Spherotech and Life Technologies were used for this comparison. The manufacturer's method was used as a guide for testing the samples. A commercially available control with known reference ranges for absolute counts was used as the test specimens. **Results:** The results showed that the four particles compared with a %CV of less than 12.0%. In addition, the results were reproducible between days with a relative percent difference of less than 11.0%. All four particles are reliable for measuring absolute counts in whole blood specimens; however, the disadvantages and advantages or each particle should be considered when choosing the appropriate bead for your application.



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INTRODUCTION

Flow Cytometry can be used to measure relative percents of many leukocyte subsets. The measurement of absolute counts, of these same subsets, can be challenging if hematology data is not available. Many commercial vendors have developed particles, which when added to whole blood specimens can provide absolute counts, eliminating the need for hematology data.

The purpose of this experiment was to compare four different vendor absolute counting particles to determine if they compare for quantifying counts in the standard T cells and subsets, B cells and NK cells. Since absolute counts are not known in a normal human blood draw, a commercial control samples will be used as the known sample which contain references ranges for these populations established by the manufacturer.

For these testing, the %CV will be calculated comparing the different particles and is expected to be below 20.0% to show similarity between the products. Also the testing will be conducted on two separate days to determine reproducibility by calculating the relative percent difference.

MATERIALS AND METHODS

A commercial control with established reference ranges, CD Chex Plus®, was used for this comparisons. The control was obtained from Streck (NE) and stored at 2-8°C until use.

Particles used for comparison: BD TruCount™ IVS Absolute Counting Tubes, Beckman Coulter Flow-Count™ IVD Fluorospheres, Sperotech AccuCount Particles and Life Technologies CountBright™ particles.

Procedure: Whole blood (50 µL) was incubated with conjugated antibodies CD3, CD4, CD8, CD16, CD56, CD45, and CD19 for 15 – 20 minutes at room temperature. To lyse red cells 450 µL of 1X BD FACS Lyse was added for 12 – 15 minutes. Flow-Count™, AccuCount and CountBright™ beads were added after the incubation, 50 µL. Samples were acquired on a BD FACSCanto™ flow cytometer .

RESULTS

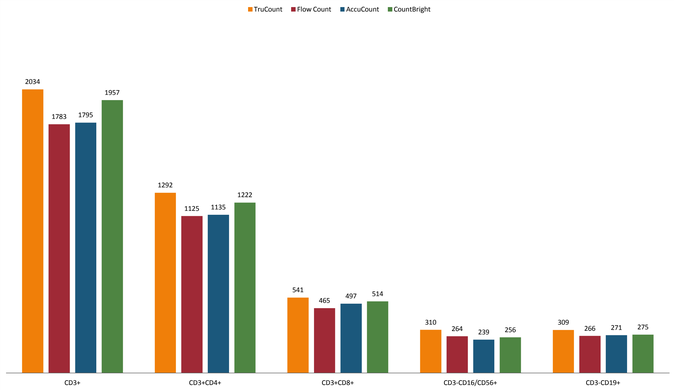

Figure 1: The comparison of 4 different absolute counting particles for measuring leukocyte subsets in a commercially available whole blood specimen.

Marker	Mean (cells/µL)	SD	CV%	Reference Range (cells/µL)
CD3+	1892	123	6.5	1523 - 2223
CD3+CD4+	1194	79	6.6	1034 - 1394
CD3+CD8+	504	32	6.3	396 - 736
CD3-CD16/CD56+	267	30	11.4	122 - 412
CD3-CD19+	280	20	7.0	132 - 432

Table 1: The results showed that when comparing the 4 particles the %CV was less than 12.0%.

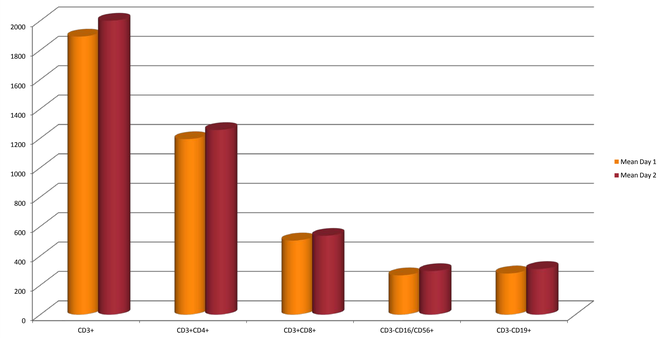

Figure 2: The comparison was performed on two days showed similar results. The relative percent difference was less than 11.0% for all subsets.

RESULTS (cont.)

Advantages/Disadvantages:
-TruCount™ are purchased pre-aliquoted into the tubes so no pipetting of the particles is required.
- TruCount™ particles are very small so increased FSC threshold can eliminate the beads.
- For Flow-Count™, AccuCount and CountBright™ beads the complete mixing of beads is important before adding to the sample. Incomplete mixing can cause erroneous results.
- All methods required precise measurement of whole blood which requires special pipettes or reverse pipetting techniques.
- One method can be used to measure absolute counts and relative percents which requires minimal handling.
- Cost of IVD vs. RUO reagents.

CONCLUSIONS

In this experiment, four commercially available absolute counting particles were compared. These particles can be used for determining absolute counts in whole blood of various lymphocytes subsets by flow cytometry. The results showed the all four particles are similar to each other, but each have advantages and disadvantages that should be carefully considered.

REFERENCES

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ACKNOWLEDGEMENTS

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