Inconsistencies Between Manual and Automated Cell Counting

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ABSTRACT

Aim: Inconsistent cell counting for flow cytometric crossmatching can affect a multitude of downstream processes, such as using the appropriate amount of pronase, to plating the correct cells-to-serum ratio, etc. We wished to examine whether differences between hand counting cells with a hemacytometer gave results similar to an automated cell counter.

Methods: Using isolated node cells, an initial cell count was performed on the automated cell counter(Countess™ 3, Thermo-Fisher Scientific), followed by nine manual cell counts from all technologists in our laboratory, on the same sample utilizing a hemacytometer (Neubauer Hemacytometer, LW Scientific) . Followed by a final count on the automated cell counter. The average, standard deviation, and coefficient of variation of each method were calculated for comparison purposes.

Results: An initial cell count was recorded on the automated system. An acceptable read on the automated system requires the total live cell population to be greater than 80% for the reading to be acceptable, greater than 90% viable cells is ideal. Eight technologists performed nine manual cell counts. The same cell suspension was reloaded one time across the nine counts. The average cell counts with the hemacytometer was ~3.2 x 10/mL with the standard deviation of 2.6 x 10/mL. The coefficient of variation was 8.42% (Table 1).The first automated cell counter result was 9.33 x 10/mL. The second count also gave the result of 9.33 x10/mL. While there was no change to the total cells counted on the automated counter, there was a small change visualized in the living cell population between the first and second count (Table 2). Comparing the manual and automated cell counts, there was a tenfold difference in cells counted with the automated system versus the manual method.

Tech	Hemocytometer Physical Count	Calculated Cells/mL
1	155	3.1x10^5/ml
2	153	3.06x10^5/ml
3	189	3.78x10^5/ml
4	168	3.36x10^5/ml
2 (second count)	159	3.18x10^5/ml
5	156	3.12x10^5/ml
6	140	2.8x10^5/ml
7	164	3.28x10^5/ml
8	154	3.08x10^5/ml
Average	159.7777778	3.196x10^5/ml
Standard Deviation	13.45155918	2.6906x10^4/ml
%CV	8.42	8.42

Table 1: Hemacytometer counts per individual technologist.

	Total Cells	Live Cells
1st Count	9.33x10^6/mL	8.91x10^6/mL
2nd Count	9.33x10^6/mL	8.98x10^6/mL
Average	9.33x10^6	8.945x10^6
Standard Deviation	0	49497.47468
%CV	0	0.005533535

Table 2: Automated cell count for same sample.

Conclusion: After comparison, it became readily apparent that the two methods provided vastly different cell counts. The cell counter maintained much higher precision with little to no change in total and living cell counts, whereas manual cell counting had some tech-to-tech variability but is considered to have acceptable precision. The fact that the automated counting system gave a result that was greater than a log higher than the manual cell count caused us to reconsider using the manual method. It is anticipated that using the automated system will allow for greater reproducibility in our flow cytometric crossmatching

INTRODUCTION:

Accurate and precise cell counting is integral for flow cytometric crossmatch (FXCM), as inaccuracies in cell count can impact pronase concentration, cell-to-serum ratios, and crossmatch results. In this study, cells from peripheral blood, lymph node, and spleen samples were counted, using manual and automated methods, before and after DNase treatment in accordance with the laboratory's standard FCXM procedure.

MATERIALS AND METHODS:

Cells were isolated using the laboratory's existing isolation protocols for each sample source. Pelletized lymphocytes were suspended in 1mL of PBS, and cells were counted on an automated counter (Countess™ 3, Thermo-Fisher Scientific Inc.), before and after, counting on a manual hemacytometer (Neubauer Hemacytometer, LW Scientific). Afterward cells were treated with DNase, washed two times, and suspended in 1mL of flow crossmatch wash buffer. Automated counts were performed immediately before and after manual counting.

The average, standard deviation, and coefficient of variation were calculated for each method for comparison purposes. The physical counts on the hemocytometer were also calculated to compare with the automated cell counter using the formula below:

$$cells/mL = \frac{(Number\ of\ cells\ counter)(Dilution\ factor)}{(Number\ of\ squares\ counted)(area)}\ \text{x}\ 10{,}000$$

RESULTS:

Illustrated in Figure 1, the average cells per milliliter counted using automated and manual methods showed relatively similar cell counts across most samples' pre-DNase treatment. However, after DNase treatment, cells counted using the automated method provided higher cell averages across all sample types in comparison to the manual counting method.

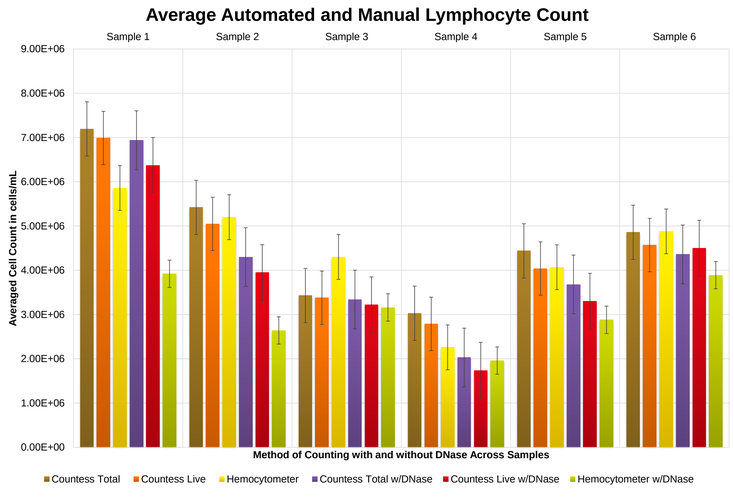


Figure 1: Averaged number of cells per milliliter counted, pre- and post-treatment with DNase, utilizing both automated and manual counting methods.

The coefficient of variation (CV) between manual and automated methods, represented in Figure 2, shows a significant CV shift between the pre- and post-DNase treated cells. The automated method demonstrated a striking decrease in CV among most sample types after cells were treated with DNase. Whereas, the manual hemacytometer method remained relatively unchanged, aside from Samples 1 and 2, which showed an increased CV.

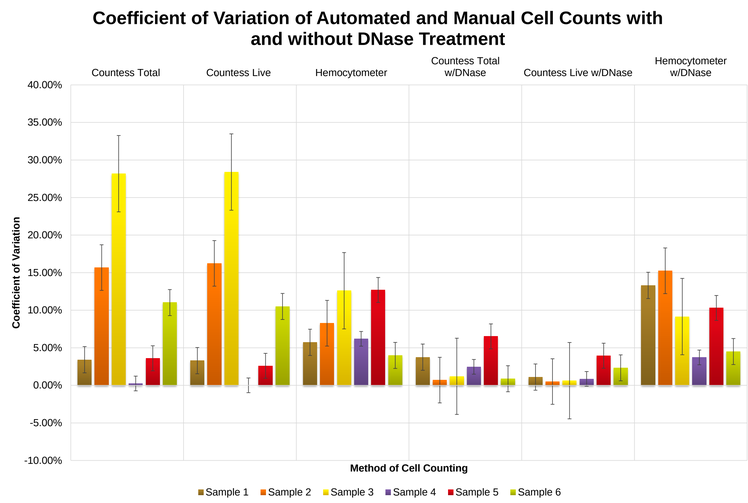


Figure 2: Coefficient of Variation of the average automated and manual counted cell both pre- and post-DNase treatment.

CONCLUSION:

In conclusion, the results between automated and manual cell counts did show some differences, but these differences were not as substantial as previously determined.

Initial cells counts from the hemocytometer and automated cells counter showed relatively few differences, with only a few samples from the hemocytometer showing lower counts and one sample showing a higher count of cells. However, following DNase treatment and wash steps, the automated cell counter proved to have more precise reads than the hemacytometer. There also appeared to be fewer cells observed under manual reading conditions versus automated.

Perhaps most importantly, the automated cell counter removed judgement and potential calculation errors that were observed under manual methods. Thus, the automated method proved to be a better method for our lab, but it did not fully rule out the use of a hemacytometer.

The increased precision of automated counting was merely one factor in our laboratory's decision to move away from a reliance on manual counting methods. Automated counting offered greater standardization, which could not be reliably replicated through manual methods.

In addition, automated counting reduced the time required to process multiple samples, determine cell concentrations, and perform dilutions. When multiple instruments are available, samples can be counted simultaneously, further increasing efficiency and reducing the time spent determining cell concentrations. As an added benefit, automated cell counters help reduce the eye strain commonly associated with frequent microscope use.