Miltenyi Biotec

Measuring absolute count with absolute certainty

Measurement assurance and harmonization using the MACSQuant® Analyzer in cell counting applications

Since it is often a key tool for providing critical information on proceeding actions in clinical and cell manufacturing environments, it comes as no surprise that numerous options are available for cell counting methodologies. These span multiple technologies, including fluidic-based systems or immobilized slide-based techniques, and range from manual to fully automated counting, depending on the device. What is surprising – and concerning – is how often consistency and reproducibility fall short. [1,2]

Given that there are diverse approaches to cell counting, each bringing its own strengths and limitations, a prudent laboratory will explore a technology's fitness for a specific application before selecting it. For example, a situation where the concentration varies greatly, or where sample debris is high, might exclude the use of certain methodologies. To add additional consideration to the evaluation, laboratories will also enlist control material/standards which bring documented levels of variability to the measurement. In the end this addition can bring greater variability or bias to a counting application.

I. What's the problem?

The challenge arises from the perennial struggle between accuracy and efficiency. Coupling this with human error, you can begin to appreciate the innate tension between accuracy and reproducibility. Given the importance of reproducibility in research settings, Miltenyi Biotec's instruments are developed to maximize consistency of results, then further optimized to offer greater speeds and efficiency.

In doing so, the following design prerequisites are essential:

1. Precision:
   Will multiple measurements of the same sample yield identical results?
2. Linearity:
   a. Will varying concentrations yield sensible counts (e.g. double the concentration, double the count)?
   b. In addition, will varying volumes yield sensible counts (e.g. double the volume, double the count)?

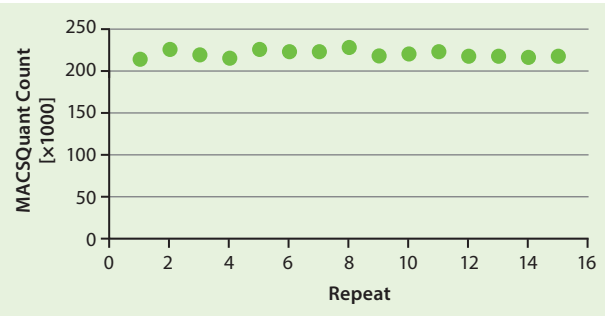

Figure 1: Count of a PBMC sample measured 15 times sequentially in standard measure-mode and 25 µL uptake volume. The CV of this data-set is ~1.5%.

Both during development and at the quality control stage, MACSQuant® Analyzers are extensively tested against these criteria. As an example of stability assurance, Figure 1 shows 15 sequential counts of a single peripheral blood mononuclear cell (PBMC) sample using the MACSQuant Analyzer. The stability and low CV of the method can clearly be seen, meeting the precision criterion described above.

Testing of the cell concentration aspect of the linearity criterion was conducted by repeatedly diluting a stock-solution of cells by a factor of 1:2. Figure 2A shows the results of a MACSQuant Analyzer on a log-log scale. For comparison, Figure 2B shows the results of testing the same material with a well-established impedance-based cell counter, which will be used as a reference method further below.

Cell count plotted against sample volume is displayed in Figure 3A, with concentration (count/volume) becoming constant over a wide range of analyzed volumes (Fig. 3B), thus satisfying the volume linearity criterion above.

As these graphs demonstrate, while the MACSQuant Analyzer utilizes a highly robust counting behavior, absolute count measured by different technical solutions (e.g. a coulter principle analyzer, hemocytometer, or flow-cytometer) can differ. This is a function of their respective intrinsic

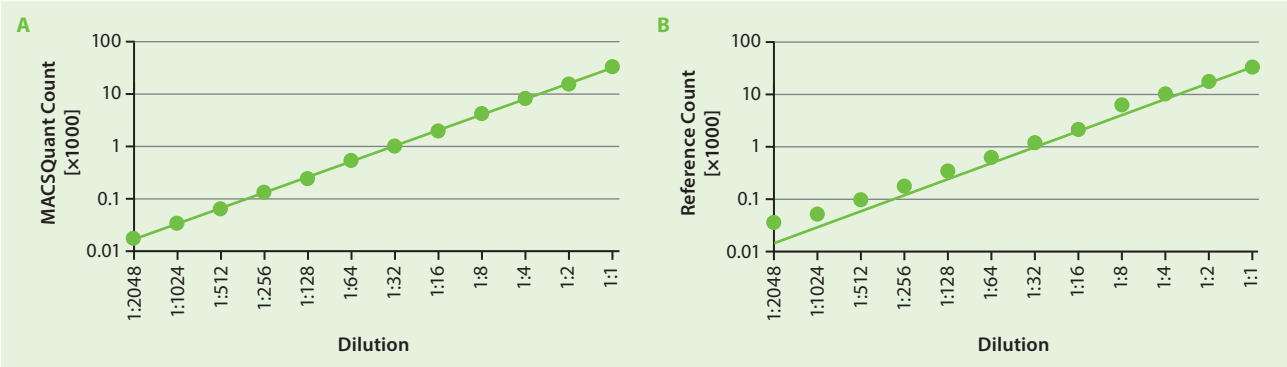

Figure 2: (A) Measured cell count on a MACSQuant Analyzer as a function of dilution factor on a log-log scale. For comparison, (B) shows the same samples on an impedance-based cell-counter, displaying deviations at low concentrations.

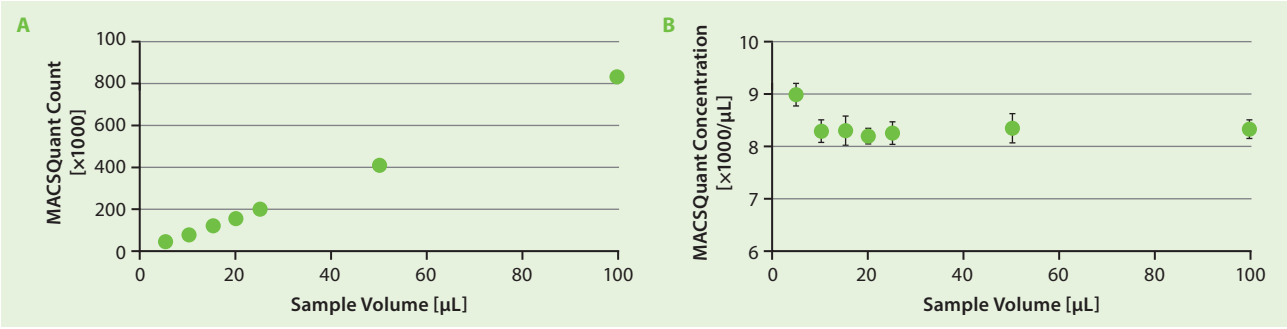

Figure 3: (A) Cell count measured as a function of uptake volume. (B) The same data, but normalized to the measurement volume.

methodologies and material-specific properties, but can easily be remedied so long as both methods being compared fulfill the above-mentioned pre-requisites of absolute counting.

II. What's the solution?

In order to map one type of absolute count to another, for example if your laboratory has an absolute count method that is different to the MACSQuant Analyzer's, a transfer-factor can be established straightforwardly:

- Prepare a concentration series of the sample of interest that is suitable for both methods
- Measure the count of each concentration with both methods
- Plot the MACSQuant Analyzer–count on the x-axis and the count of the reference method on the y-axis
- The slope of a linear regression is the factor between the two methods

As an example, Figure 4 shows the combination of data from Figures 2(A) and 2(B), with the results of the impedance based cell-counter measurement representing the reference methodology. A transfer factor of 1.0849 was obtained, which means that the MACSQuant Analyzer–count has to be multiplied by this number to obtain the corresponding reference-count.

If preparation and counting of a concentration series is not possible or practical, a single concentration can instead be used; this concentration should then correspond to the designated concentration window for regular application. In this simplified scheme, the transfer factor is the ratio of reference count to the count of the MACSQuant Analyzer.

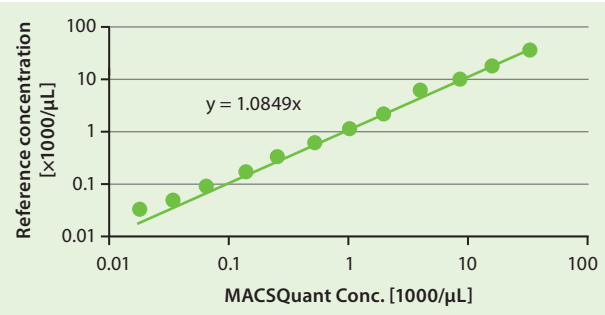

Figure 4: Correlation of MACSQuant and impedance-based cell-counter data on a log-log scale. Linear regression gives a transfer-factor of 1.0849.

To recap: In order to obtain a meaningful absolute count, whichever analysis method you choose must be reproducible, as well as stable in both varying concentrations and volumes. The MACSQuant Analyzer meets these conditions. It can be easily correlated to any other cell-counting methodology a laboratory might employ, so long as it too fulfils these criteria.

References

1. Salinas, M. *et al.* (1997) Ann. Rheum. Dis. 56(10): 622–626.
2. Saraiva, L. *et al.* (2019) Cytometry Part B 2019; 9999B: 1–6.

Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | **www.miltenyibiotec.com**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

V. 01