Improved Inter-Instrument Consistency and High Precision for CHO Cell Bioprocessing Using the Cellaca™ MX High-Throughput Cell Counter

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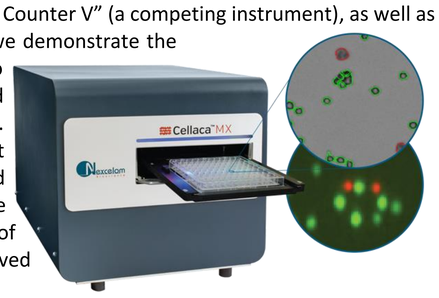
1. INTRODUCTION

CHO cell bioprocessing is a common application for producing biologics, antibodies, and proteins for therapeutic products. One of the most important factors in the CHO bioprocess is the characterization of a cell culture's concentration and viability to ensure that the cells are in optimal condition for production. Traditionally, CHO cells have been measured using a manual hemocytometer or automated cell counter with trypan blue staining. However, these methods have limitations in throughput and instrument-to-instrument consistency.

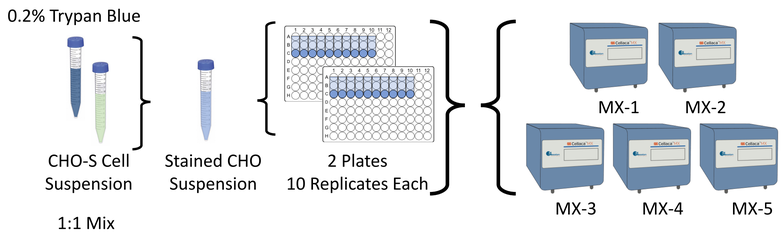
Numerous automated cell counting methods have been introduced. To properly compare new cell counting methodologies for introduction into CHO cell bioprocessing, we utilized the recently published ISO cell counting standards (ISO 20391-1:2018 and 20391-2:2019). Under the ISO guidance, since there are no live cell reference standards, metrics other than accuracy may be used to evaluate cell counting methods. These may include linearity, proportionality, precision, and limits of detection. If the performance is fit-for-purpose, factors such as speed, cost, and ease of use may be prioritized.

Here, we evaluate the performance of the Cellaca™ MX high-throughput cell counter for implementation into the CHO cell bioprocess. We investigate the precision, instrument-to-instrument consistency, linearity, and proportionality following ISO cell counting standard 20391-2:2019. We demonstrate close agreement between multiple Cellaca™ MX instruments using both CHO cells with Trypan Blue (5 instruments) and beads (32 instruments). We also report system-wide precision, which includes variation between multiple counts, consumables, instruments, and days (in the case of beads). Furthermore, we include the results of several comparison experiments in which samples were counted using Cellaca™ MX, hemocytometer, "Cell Counter V" (a competing instrument), as well as the Celigo® Imaging Cytometer. Finally, we demonstrate the use of the ISO cell counting standards to evaluate the linearity, precision, and proportionality index of the Cellaca™ MX. These results show Cellaca™ MX can count trypan blue-stained CHO cells in brightfield in less than 1 min for 24 samples, and the consistency, comparability, and precision of the Cellaca™ MX are significantly improved over the traditional methods.



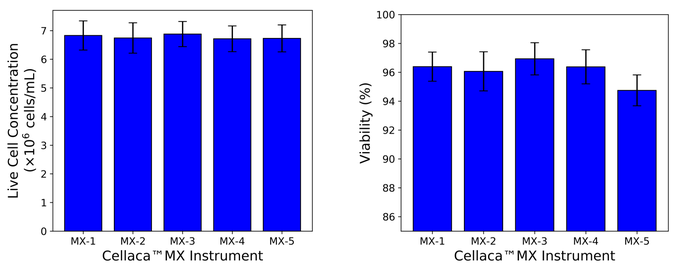
2. HIGH MULTI-INSTRUMENT CONSISTENCY AND PRECISION FOR CHO CELLS

Experimental Protocol



- A sample of healthy CHO cells was gently mixed and stained 1:1 with 0.2% Trypan blue.
- The stained CHO cells were pipetted into 20 Cellaca™ MX counting chambers (10 on each of the 2 plates) .
- Both plates were then scanned on 5 Cellaca™ MX instruments using the same default settings for CHO cells with Trypan blue.

Results



- The 5 instruments showed a maximum variation of less than 2.4% for live cell concentration and less than 2.3% for viability.

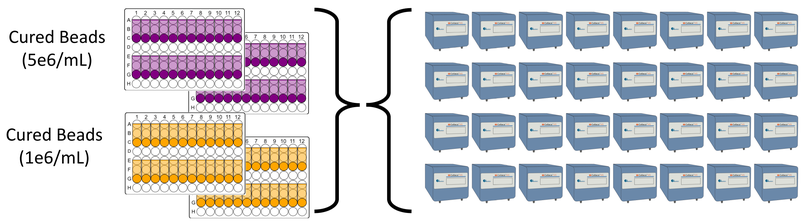
Precision Level	CHO Total Conc. (CV)	CHO Live Conc. (CV)	CHO Viability (CV)
*Count-to-Count	5.5%	5.7%	0.9%
Plate-to-Plate	3.4%	3.2%	0.3%
Instrument-to-Instrument	1.7%	2.0%	0.7%
*System-Wide	7.0%	7.3%	1.3%

*Count-to-Count and System-Wide variation include random error and sample preparation error.

- Sources of variability are broken down above.
- The overall CV (StDev/Mean) for the entire method (100 counts, 20 chambers, 2 plates, 5 instruments) was 7.3% for live cell concentration, 7.0% for total concentration, and 1.3% for viability.

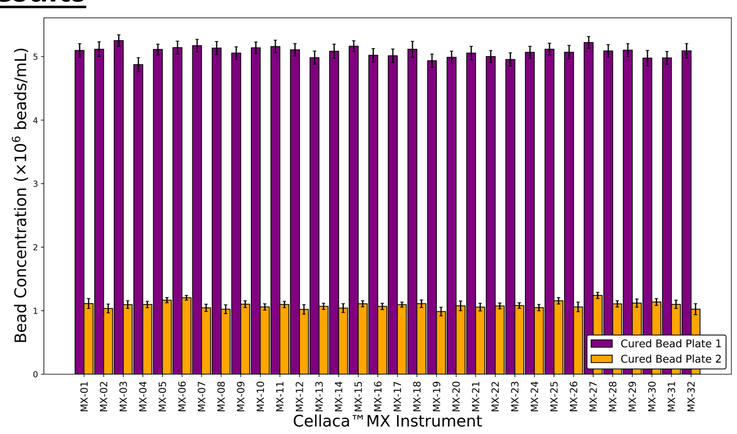
3. HIGH MULTI-INSTRUMENT CONSISTENCY AND PRECISION FOR BEADS

Experimental Protocol



- Prepared four plates of 5-µm beads locked in clear UV-cured polymer (2 plates at 1e6 beads/mL, 2 plates at 5e6 beads/mL).
- Using the same default brightfield settings, the beads in the cured plates were counted on 32 Cellaca™ MX instruments over the course of 1 year.

Results



- The CV of the method as whole (~3000 counts, 96 wells, 4 plates, 32 instruments) was 5.7%.

Precision Level	Beads Total Conc. (CV)	
	5 x 10^6 beads/mL	1 x 10^6 beads/mL
Analysis-to-Analysis	0.0%	0.0%
Scan-to-Scan	1.0%	0.5%
*Count-to-Count	2.2%	5.6%
Plate-to-Plate	0.04%	3.3%
Instrument-to-Instrument	1.6%	4.9%
*System-wide	2.6%	7.6%

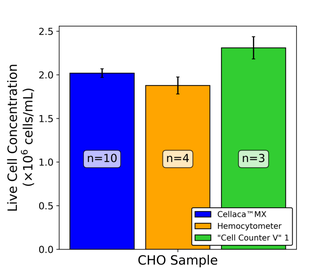
*Count-to-Count and System-Wide variation include random error and sample preparation error.

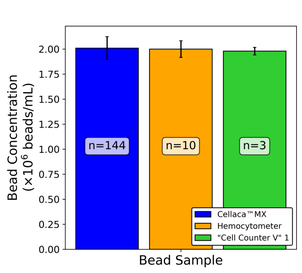
4. CELLACA™ MX CELL COUNTING RESULTS ARE COMPARABLE TO MANUAL HEMOCYTOMETER

Experimental Protocol

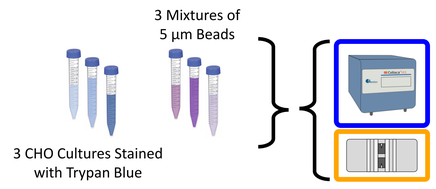
- We measured 1 CHO sample and 1 bead sample using the Cellaca™ MX, a hemocytometer, and a "Cell Counter V".

Results



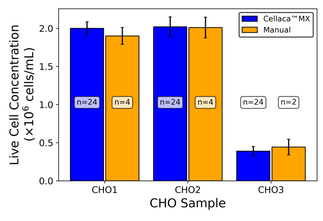


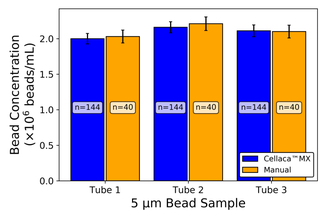
Experimental Protocol



- We measured 3 CHO samples and 3 bead samples using the Cellaca™ MX and a hemocytometer.

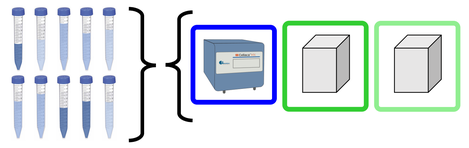
Results





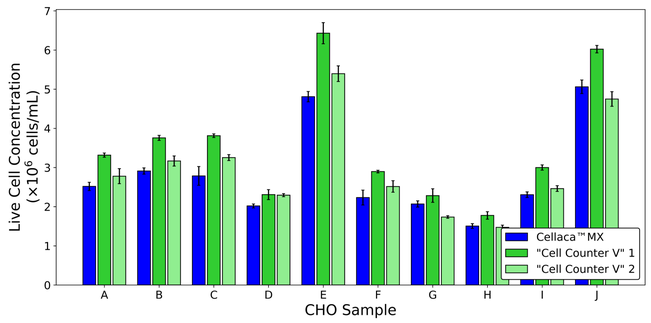
5. HIGH VARIABILITY OBSERVED BETWEEN "CELL COUNTER V" INSTRUMENTS

Experimental Protocol



- We measured 10 healthy CHO cultures on Cellaca™ MX and 2 "Cell Counter V" instruments.
- 10 counts were made on Cellaca™ MX, and 3 on each "Cell Counter V", with 50 images used for each "Cell Counter V" count.

Results



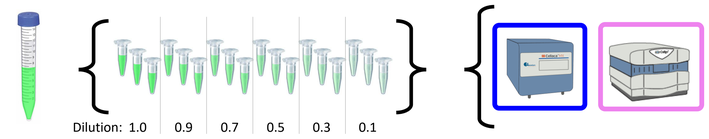
"Cell Counter V" Precision	CHO Total Conc (CV)	CHO Live Conc (CV)	CHO Viability (CV)
Cup to Cup	3.9%	4.0%	0.4%
Instrument to Instrument	9.4%	9.2%	0.3%
System-Wide Precision	10.2%	10.0%	0.5%

- System-wide precision for the "Cell Counter V" was measured at 10.0% for live concentration, 0.5% for viability, and 10.2% for total cell concentration.
- The maximum difference observed between the two "Cell Counter V" instruments for a single sample was 27%.
- Cellaca™ MX was comparable to one of the tested "Cell Counter V" instruments.

6. CELLACA™ MX METHOD EVALUATION FOLLOWING THE ISO 20391-II STANDARDS

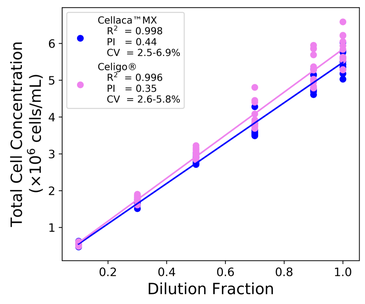
- FDA, NIST, ISO, and other industry partners published the ISO Cell Counting Standards Part I and II for guiding the selection and evaluation of cell counting methods
- Revvity is a contributing member to the ISO standards
- We utilized the ISO 20391-II protocol to evaluate the performance of Cellaca™ MX for proportionality index, precision, and linearity

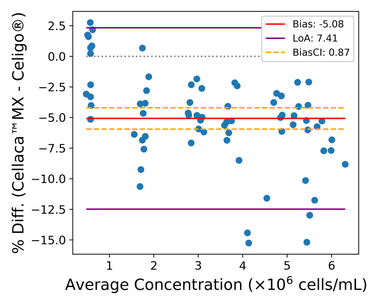
Experimental Protocol



- A single tube of CHO cells was used to create 18 independent dilutions in 6 concentrations.
- The 18 samples were each mixed with Acridine Orange and counted in fluorescence mode on both the Cellaca™ MX and the Celigo® imaging cytometer (4 measurements per sample.

Results





- Both methods show similar proportionality. We measure a proportionality index of 0.44 for Cellaca™ MX, and 0.35 for Celigo®.
- A Bland-Altman plot comparing the two methods reveals a bias of 5.1%, with Celigo® counting higher.

7. CONCLUSIONS

- The Cellaca™ MX shows improvement in system-wide precision compared to the "Cell Counter V".
- The Cellaca™ MX shows high instrument-to-instrument consistency, with inter-instrument CVs of 2.0% for live cell counts and 0.7% for Trypan blue viability.
- Cell counts made on the Cellaca™ MX are comparable to other methods used in bioprocessing applications.

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