APPLICATION NOTE

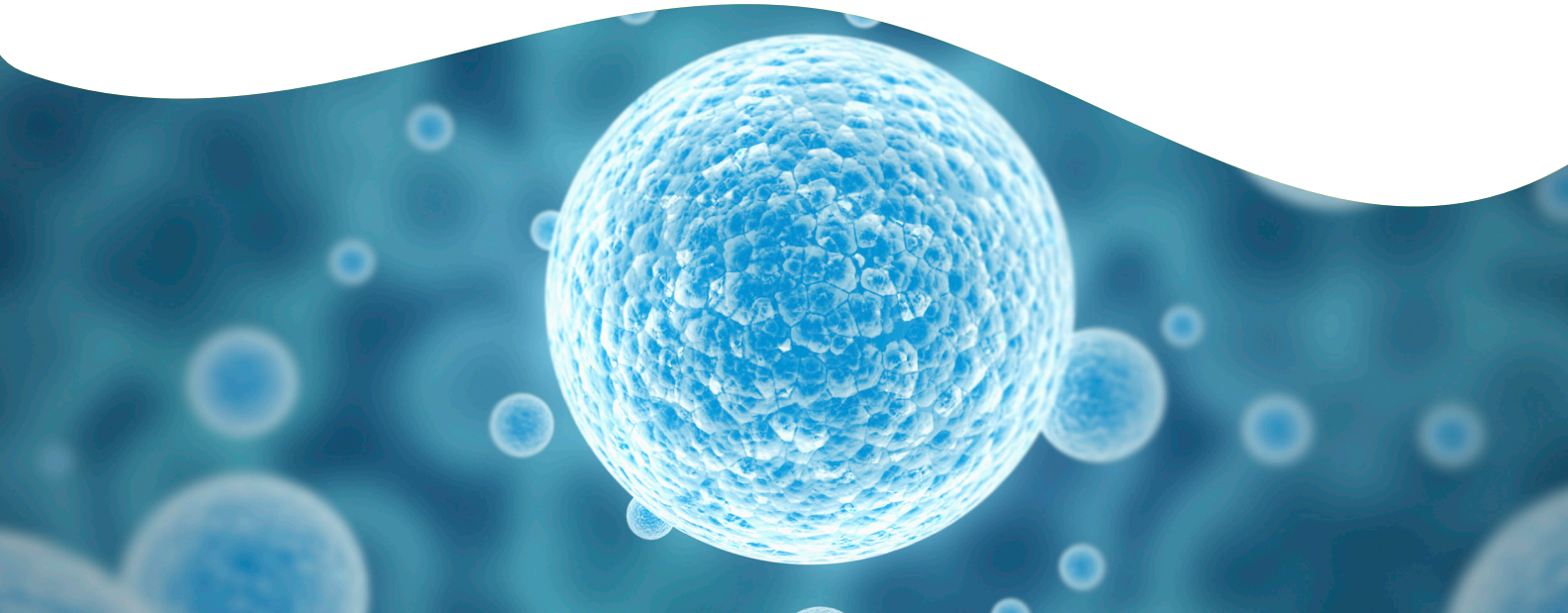
Optimizing the cell counting process with the Cellaca MX high-throughput cell counter and the AssayMate automated liquid handling workstation.

Overview

Identification of cell concentrations and viability is one of the fundamental processes in cell and gene therapy, bioprocessing, and drug discovery. Cell counting is a multi-step process, which involves cell sample preparation, cell counting data collection, data analysis, and data interpretation. As the need for upstream and downstream assays involving cell counting grows, it is imperative to search for solutions to optimize the cell counting processes regarding throughput, consistency, and operation efficiency.

In a single scan, Revvity's Cellaca™ MX high-throughput cell counter has been designed to deliver reliable and repeatable cell concentrations and viability measurements in as low as 3 minutes for 24 samples. While this provides significant time saving in data collection and analysis for multi-sample experiments, manual sample preparation, involving mixing, dilution, staining, and transfer, remains a significant chokepoint that can introduce inconsistency and limit scalability.

This application note demonstrates a streamlined cell counting process utilizing the AssayMate™ liquid handling workstation to automate sample preparation. An evaluation study following a modified version of ISO 20391-2 protocol confirmed that the automated sample preparation process delivered results comparable to manual methods. In this study, the automated process reduced manual pipetting from over 20 steps to just 4, while cutting hands-on time by approximately 70%. The streamlined cell counting process supports the consistency and throughput required for high-volume laboratory environments.



Introduction

Identification of cell counts and viability is one of the fundamental processes in many research translation workflows, which involves cell sample preparation, data collection, and analysis. As the demand for upstream and downstream assays grow, laboratories need to find solutions that optimize throughput of the cell counting process without sacrificing precision or operational efficiency.

Revvity's Cellaca MX high-throughput cell counter helps to address the data collection bottleneck by providing rapid, image-based cell counting for up to 24 samples at a time. However, the preceding preparation steps are often labor-intensive and prone to human error. To address these chokepoints, we evaluated the feasibility of an automated sample preparation process using the AssayMate liquid handling workstation, which features:

- Air displacement pipetting technology and 8-channel gripper for precise handling.
- A reconfigurable deck with 12+4 staging slots to accommodate complex protocols.
- User-friendly, drag-and-drop software for easy integration into existing laboratory workflows.

To verify this automated sample preparation process, linearity and repeatability tests were conducted using beads and various cell lines, including U937, Jurkat, and peripheral blood mononuclear cells (PBMCs). Furthermore, a modified version of the ISO 20391-2 protocol was executed to evaluate and compare the quality of cell counting measurements using both automated process and manual pipetting process. Meanwhile, the same study was used to evaluate the impact of the automation on operation efficiency.

The following data illustrates how the synergy between the AssayMate liquid handling workstation and the Cellaca MX high-throughput cell counter maintains high-quality cell measurements while enhancing laboratory productivity.

Instrumentation

This study utilizes the **Cellaca MX high-throughput cell counter** for rapid image-based cell count and viability measurement and the **AssayMate automated liquid handling workstation** to streamline cell sample preparation, together optimizing throughput and consistency.

Cellaca MX high-throughput cell counter *(Part no. MX-AOPI)*

- Batch samples – run up to 24 samples at a time
- Sample volume: 25 µL to 200 µL
- Brightfield + Green/Red Fluorescence
- Autofocus
- Automated data analysis and customizable report
- 21 CFR part 11 ready
- Application: Cell count, viability, apoptosis, GFP

AssayMate automated liquid handler *(Part no. yas8g01)*

- 8 channels + gripper
- Air displacement pipetting technology
- Partial tip loading capability
- Reconfigurable deck with 12 + 4 staging slots.
- Pipette volume range: up to 1000 µL
- User-friendly software with drag-and-drop interface
- Compact size and budget friendly
- Common applications: no-wash immunoassays such as Alpha, HTRF™ and LANCE™

Process verification and workflow

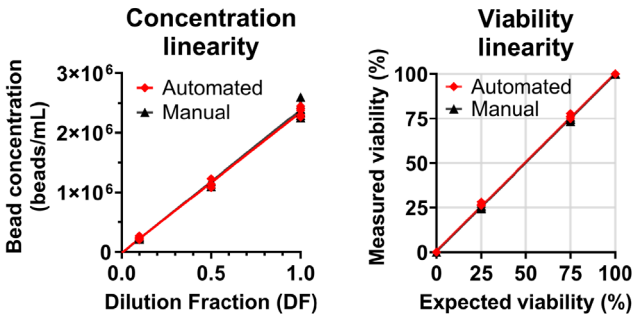
Concentration and viability linearity with beads

The performance of the automated sample preparation process was first verified using beads, which demonstrated linear trends in both bead concentration and bead viability that were consistent with the manual pipetting process.

For concentration linearity study, three concentrations of beads at the target dilution fractions of 0.1, 0.5 and 1.0 were used. For viability linearity study, four mixtures of green and red fluorescent beads at the target "viability" fractions of 0, 25%, 75% and 100% were used. All the stocks of beads were prepared prior to the verification study. 400 µL of beads were transferred from each of the thoroughly mixed bead stock solutions to an individual well of a 96-well storage plate (Revvity, part number: 6008290). Two storage plates of beads were prepared for automated sample preparation process and manual pipetting process, respectively.

To perform sample preparation, 50 µL of beads from one bead condition were transferred from the storage plate to one loading well of the Revvity's Ergonomic 8x3 high-throughput cell counting plates (part number: CHM24-B100-099) using either automated or manual process. Repeat the transfer step six times to create six replicate samples for one bead condition and repeat the whole transfer process for the rest of the bead conditions.

Then, the cell counting plates were scanned by the Cellaca MX high-throughput cell counter for bead concentration and viability measurements. Measured bead concentrations for concentration linearity study were plotted against target dilution fractions and fitted with a linear model.



	Concentration linearity		Viability linearity	
	Automated	Manual	Automated	Manual
Slope	2.35E+06	2.40E+06	0.9975	0.9971
R^2	0.9963	0.9928	0.9994	0.9997

Measured bead viabilities for viability linearity study were plotted against target viability fractions and fitted with a linear model as well. Slopes and coefficients of determination (R^2) of the linear fits were reported as the final results.

Overlapping linear trends were observed in concentration and viability measurements of beads (n = 6) using the AssayMate workstation's automated sample preparation process and the manual pipetting process, with comparable slope values reported from the concentration and viability linearity fits between two processes.

Coefficients of determinations (R^2) were >0.99 for both concentration and viability linearity fits for the automated and manual processes.

Repeatability across different cell lines

Side-by-side comparisons of the cell counting repeatability between the AssayMate workstation's automated sample preparation process and manual pipetting process were conducted using U937, Jurkat, and PBMC cell lines.

The storage plates containing U937, Jurkat and PBMC cell samples in individual wells and the reservoirs (12-column reservoir, Revvity, part number 6008700) containing acridine orange/propidium iodide solution (AO/PI, Revvity, part number CS2-0106-5mL) in a column were prepared prior to the repeatability study.

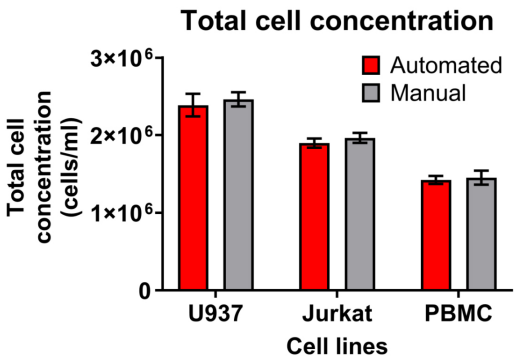
The cell sample of each cell type was thoroughly mixed before 200 µL of cells were transferred to a new well ("mixing well") and stained with an equal volume of the AO/PI solution (1:1 ratio).

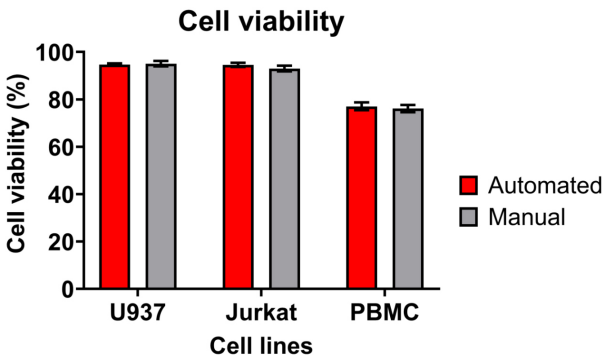
After staining, the cell sample of one cell type was mixed 10 times. Then, 50 µL of the stained cells were transferred to one loading well of the Ergonomic cell counting plates. Repeat the transfer step six times to create six replicate samples for each cell type and repeat the whole transfer process for the rest of cell samples of different cell types. The sample preparation process was performed using either automated or manual process.

The cell counting plates containing AO/PI stained cells of all three cell lines were scanned by the Cellaca MX high-throughput cell counter. Average total cell concentrations, cell viabilities, and the precision of both measurands were calculated. The differences between automated and manual processes were reported.

Results showed that the AssayMate workstation's automated sample preparation process was in line with the manual process with U937, Jurkat, and peripheral blood mononuclear cells (PBMCs).

- Less than 4% differences in total cell concentration and cell viability were observed for all three cell lines tested.
- Precisions, quantified using the coefficient of variation (CV), were comparable between the two processes.





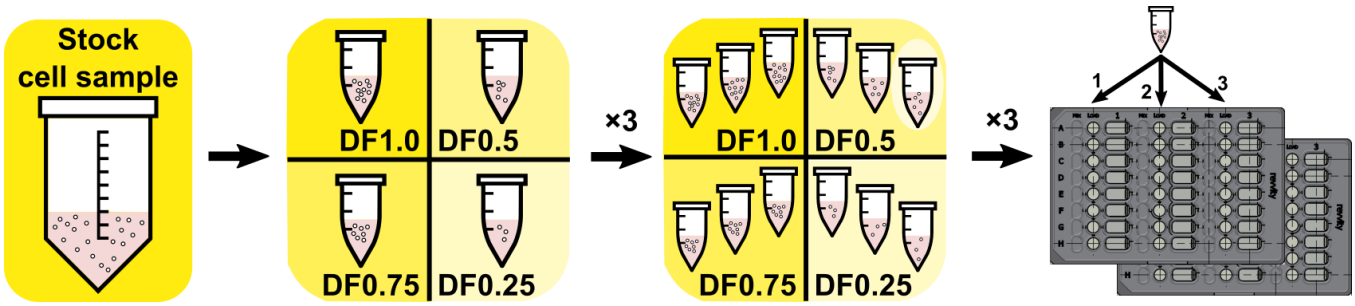
		Total cell concentration					Cell viability				
		Automated		Manual			Automated		Manual		
Cell type	N	Average (cells/mL)	CV (%)	Average (cells/mL)	CV (%)	diff. (%)	Average (%)	CV (%)	Average (%)	CV (%)	diff. (%)
U937	6	2.39E+06	6.1	2.46E+06	3.7	-3.1%	94.7	0.5	95.0	1.2	-0.3%
Jurkat	6	1.90E+06	3.1	1.97E+06	3.3	-3.5%	94.6	0.9	93.0	1.3	1.6%
PBMC	6	1.42E+06	3.6	1.45E+06	6.3	-2.1%	77.0	2.2	76.1	2.0	0.9%

Practical application: modified ISO 20391-2 protocol

A practical application of the system following a modified version of ISO 20391-2 protocol showed that the AssayMate workstation's automated sample preparation process delivered comparable quality of cell counting and cell viability results as compared to the manual pipetting process.

In this application, the AssayMate workstation's automated sample preparation process reduced manual pipetting from over 20 steps to just 4, while cutting hands-on time by approximately 70%.

Modified ISO 20391-2 cell counting protocol



Jurkat cells stock concentration: ~2e6 cells/mL

4 dilution fractions (DF)

3 replicates per DF

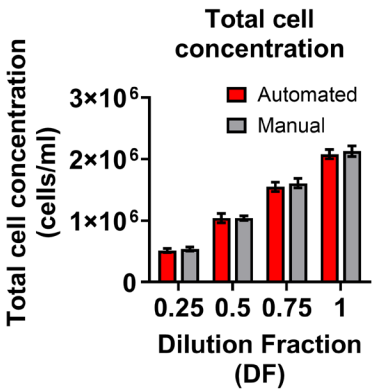
3 observations per replicate in one row of Ergonomic 8×3 plate
36 observations total

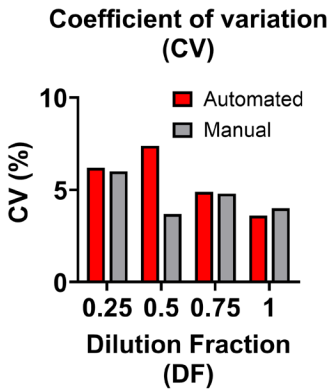
- A stock of Jurkat cell samples, cell culture media, and acridine orange/propidium iodide (AO/PI) solution were loaded into a 12-column reservoir. Two reservoirs were prepared for both automated sample preparation process and manual pipetting process, respectively.
- 12 biological replicates, with four dilution fractions (DFs) and three biological replicates per DF, were independently prepared in different wells of a 96-well storage plate. Each biological replicate was created by mixing Jurkat cell samples and cell culture media at a target mixture ratio.
- Each biological replicate was stained with the AO/PI solution at 1:1 ratio and mixed 10 times.
- After mixing, three replicate observations of samples were transferred immediately to one row of the Ergonomic cell counting plate. In total, 36 observations of samples were loaded in two Ergonomic cell counting plates.
- The sample preparation was performed by either automated or manual process. After the sample preparation, these cell counting plates were scanned by the Cellaca MX high-throughput cell counter.
- Average total cell concentrations, cell viability, and their precisions were calculated for each DF.
- Proportionality plot and the results of the coefficient of determination (R^2) and proportionality index (PI), derived from the statistical model described in ISO 20391-2 standard, were reported.

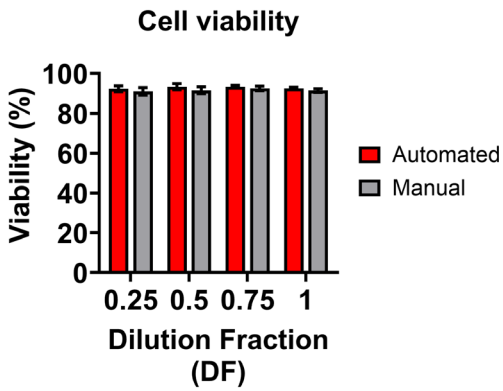
Averaged total cell concentration and cell viability

Averaged total cell concentrations and cell viabilities at different DFs were comparable between the AssayMate workstation's automated sample preparation process and the manual pipetting process.

Coefficients of variation (CVs) of all tested samples were under 10% for both processes.



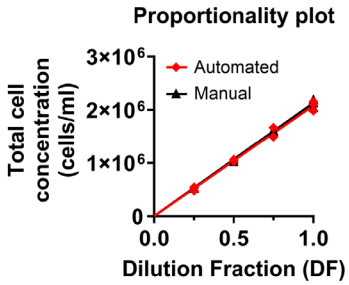




		Total cell concentration				Viability			
		Automated		Manual		Automated		Manual	
Dilution Fraction (DF)	N	AVG (cells/mL)	CV (%)	AVG (cells/mL)	CV (%)	AVG (%)	CV (%)	AVG (%)	CV (%)
0.25	9	5.15e+05	6.2%	5.38e+05	6.0%	92.4%	1.6%	91.0%	2.1%
0.5	9	1.04e+06	7.4%	1.04e+06	3.7%	93.4%	1.7%	91.6%	1.9%
0.75	9	1.55e+06	4.9%	1.61e+06	4.8%	93.4%	0.8%	92.5%	1.3%
1	9	2.08e+06	3.6%	2.13e+06	4.0%	92.6%	0.5%	91.5%	1.0%

Proportionality plot

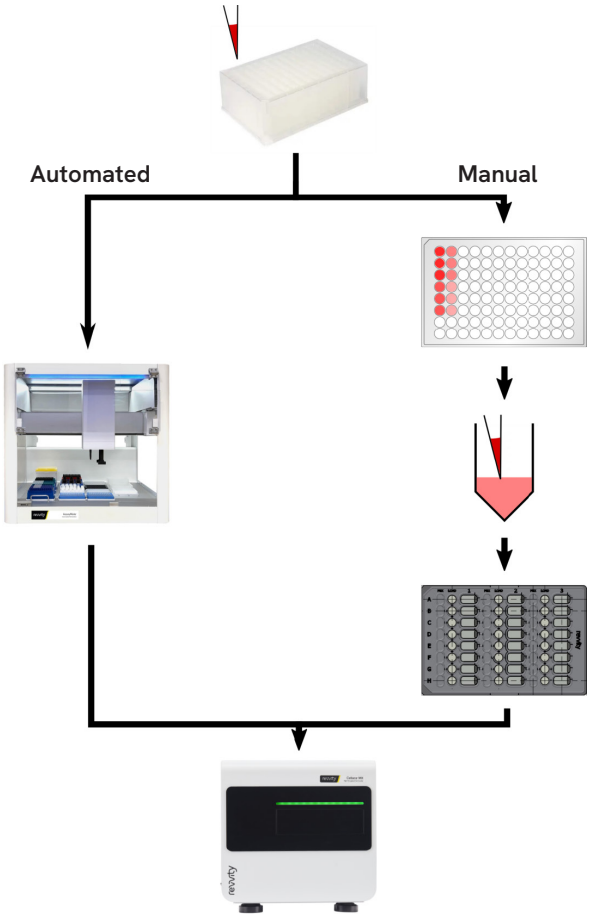
Results of the coefficient of determination (R^2) and the proportionality index (PI), derived from the proportionality model, were comparable between the two processes.



	Automated	Manual
Slope	2.08e+06	2.13e+06
Coefficient of determination (R^2)	0.998	0.999
Proportionality Index (PI)	0.06	0.12

Practicality measurements

Number of manual pipetting & mixing steps versus automated for this study was counted for each step of the sample preparation process.



Load reservoir

Pipette: 3

Mix: 1

Dilute

Pipette: 21 (Single)
7 (Multi-channel)

Mix: 4

Stain

Pipette: 12 (Single)
2 (Multi-channel)

Mix: 12 (Single)
2 (Multi-channel)

Transfer

Pipette: 36 (Single)
6 (Multi-channel)

Scan each cell counting plate by the Cellaca MX high-throughput cell counter

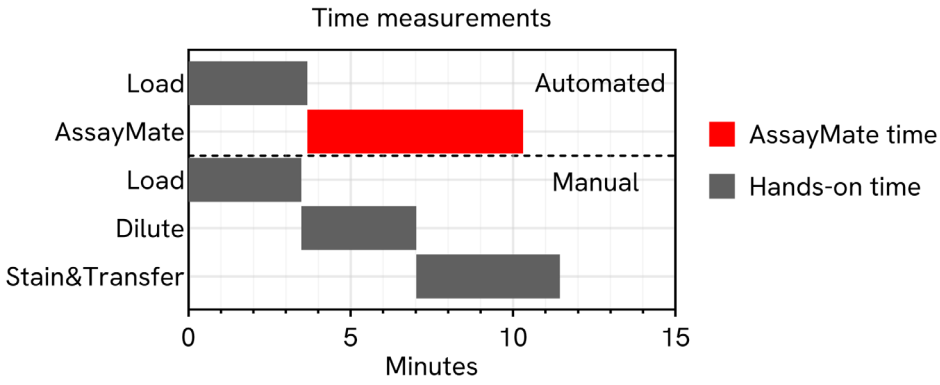
Total steps for the automated process: 4.

Total steps for the manual process: 89 (Single), 25 (Multi-channel).

Time Measurements

Measurements of elapsed time were conducted for each step using a timer.

In this study, hands-on time could be reduced by ~70% using AssayMate workstation's automated sample preparation process.



Summary

The advanced cell counting process, powered by the AssayMate automated liquid handling workstation and the Cellaca MX high-throughput cell counter, showed:

- Comparable concentration and viability results from beads and different cell lines.
- Similar quality of cell measurements, e.g., precision (CV) and linearity/proportionality (R^2 and PI).
- Reduced manual pipetting and mixing steps and hands-on time in a complicated experiment, e.g., modified ISO 20391-2 cell counting protocol
- This advanced cell counting process could be a solution for the modern laboratories with high-volume cell counting needs.

Reference

1. ISO 20391-1:2018 Biotechnology — Cell counting — Part 1: General guidance on cell counting methods. International Organization for Standardization.
2. ISO 20391-2:2019 Biotechnology — Cell counting — Part 2: Experimental design and statistical analysis to quantify counting method performance. International Organization for Standardization.
3. Huang Y, Bell J, Kuksin D, Sarkar S, Pierce LT, Newton D et al (2021), Practical application of cell counting method performance evaluation and comparison derived from the ISO Cell Counting Standards Part 1 and 2. Cell and Gene Therapy Insights, 7(9):937–960.