Characterization and comparison of acridine orange/propidium iodide and acridine/DAPI viability detection methods for cell and gene therapy development

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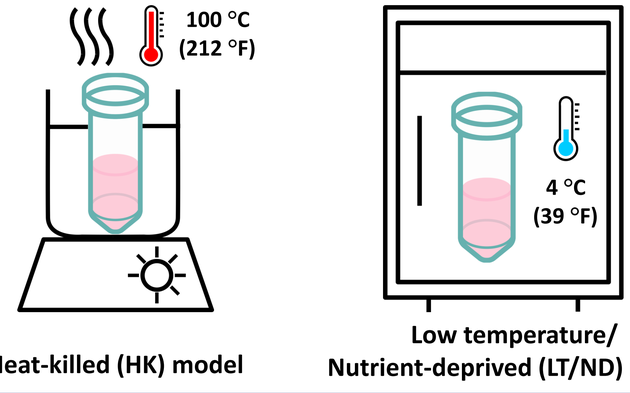
1. ABSTRACT

Cellular therapy development and manufacturing has focused on providing novel therapeutic cell-based products for various diseases. Recently, the International Organization for Standardization (ISO) has provided guidance on critical quality attributes (CQAs) that shall be considered when testing and release cellular therapeutic products, including identity, cell count, purity/impurity, potency/relevant biological activity, viability, sterling, stability, and maturation profile. Specifically, cell count and viability measurement are two of the main CQAs that are assessed during development, manufacturing, testing, and product release. **Acridine orange (AO)/propidium iodide (PI)** and **Acridine orange/4',6-diamidino-2-phenylindole (DAPI)** are two common dual-fluorescence membrane integrity dyes to assess cell viability. It has been shown in previous publications that AO/PI and AO/DAPI demonstrated comparable cell viability measurements using heat-killed Jurkat cell samples as the cell death model for characterization and comparison. However, it has been empirically reported that 10 – 30% viability measurement differences have been observed between AO/PI and AO/DAPI with cell samples used in cellular therapy bioprocesses. In this work, we demonstrate strategies for the characterization and comparison of AO/PI and AO/DAPI staining methods using the heat-killed and low temperature/nutrient deprivation cell death models to determine the differences in cell viability measurements and identify potential causes. In general, the AO/DAPI method provided higher viability results than AO/PI, with the measured viability difference between these two methods ranging from 0% to 24%. To identify the sources of the measured viability difference, we tested a list of factors including cell dying process, staining time and initial viability levels. First, we observed a larger viability measurement difference from low temperature/nutrient deprived (LT/ND) Jurkat cells compared to the heat-killed cells, suggesting that the differences might depend on the cell dying process. Second, we found that AO/PI provided more consistent cell count and viability results than AO/DAPI during the first 30 min of staining time. Finally, the measured viability differences might be larger for low-viability, LT/ND Jurkat cells, as compared to healthy samples. The characterization results may support the selection of viability methods that are fit-for-purpose for analyzing cellular therapeutic products.

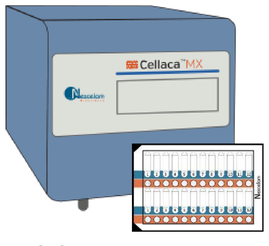
2. CELL DEATH MODELS



Heat-killed (HK) model

Low temperature/ Nutrient-deprived (LT/ND) model

3. CELLACA® MX HIGH-THROUGHPUT CELL COUNTER AND VIABILITY ASSAYS

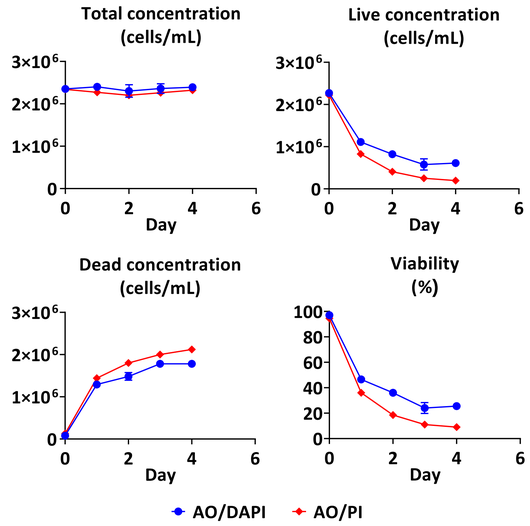


1. Cellaca® MX high-throughput cell counter utilizes a transmission and epifluorescence optical system for brightfield (BF) and fluorescence (FL) image cytometric analysis
2. Four excitation (EX) and five emission (EM) filters for different fluorescence assays.
3. Up to 24 samples at a time with Nexcelom counting plates

Viability assays:

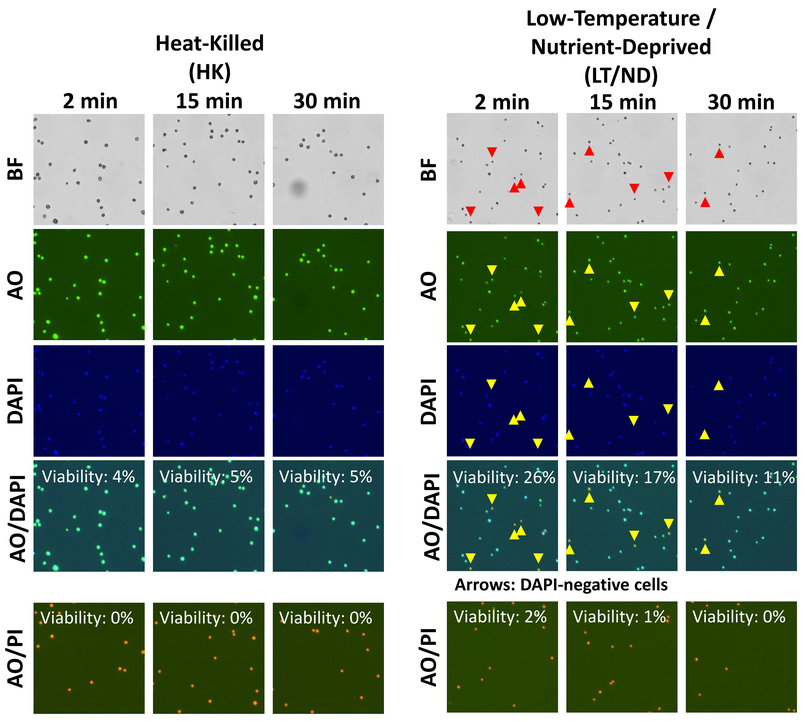
Assay	F1 (Ex/Em, exposure time)	F2 (Ex/Em, exposure time)	Viability equation
AO/PI	AO (475/534 nm, 150 ms)	PI (527/655 nm, 650ms)	F1/(F1+F2) × 100%
AO/DAPI		DAPI (370/452 nm, 6000ms)	(F1-F2)/F1 × 100%

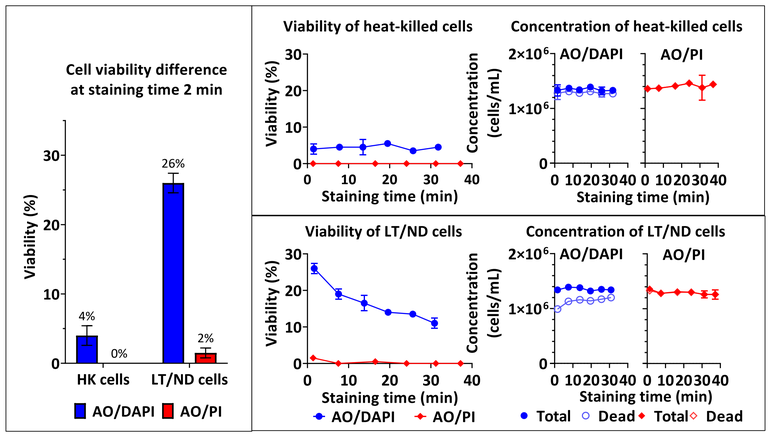
4. MONITOR VIABILITY OF LT/ND CELLS FOR 4 DAYS



- 0 – 20% of viability measurement differences between AO/PI and AO/DAPI were observed in low-temperature / nutrient-deprived (LT/ND) cells
- Viabilities were closer at higher viability and further at lower viability

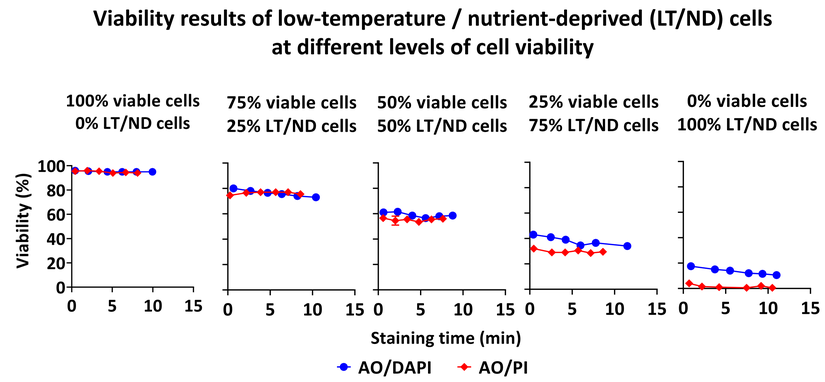
5. STAINING TIME-DEPENDENT AO/PI and AO/DAPI VIABILITY RESULTS





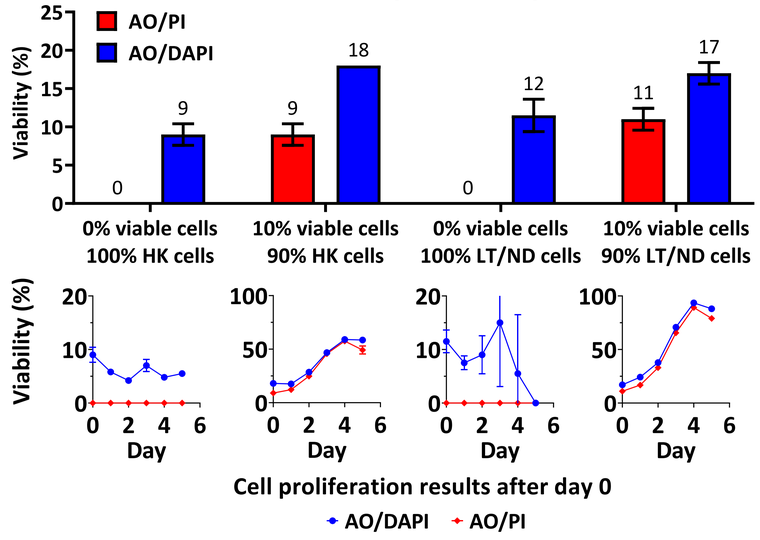
- Heat-killed model: Consistent total, live, dead cell concentrations and viability from both AO/PI and AO/DAPI; a small viability difference.
- LT/ND model: Time-dependent AO/DAPI staining results with increasing DAPI+ cells and decreasing cell viability; consistent AO/PI staining results; viability difference from 24% to 10%.

6. VIABILITY LINEARITY CHARACTERIZATION



- For the 100, 75, and 50% mixtures, the viability measurements were comparable between AO/DAPI and AO/PI staining methods.
- As the viability mixtures decreased to 25 and 0%, the viability measurement differences increased noticeably (~10 – 20%).

7. CELL PROLIFERATION ASSAY VALIDATED CELL VIABILITY MEASUREMENTS



- Both 100% heat-killed (HK) and low-temperature/nutrient-deprived (LT/ND) Jurkat cells showed no sign of cell proliferation, confirming they were completely dead.
- AO/PI staining showed consistent ~0% viability for both dead cell models.
- AO/DAPI staining showed 0 – 15% viability for both dead cell models.
- Both 10% fresh Jurkat-spiked samples showed significant increase in viability over time.

8. SUMMARY OF AO/PI AND AO/DAPI VIABILITY ASSAYS COMPARISON FOR 2 CELL DEATH MODELS

- The AO/DAPI method provided higher viability results than AO/PI
  1. The measured viability difference ranged from 0% to 24%
  2. A larger viability difference was observed from low temperature/nutrient deprived (LT/ND) Jurkat cells compared to the heat-killed (HK) cells
  3. AO/PI provided more consistent cell count and viability than AO/DAPI during the first 30 min of staining time
  4. the measured viability differences may be larger for low-viability LT/ND cells, as compared to healthy cells.
  5. Cell proliferation assay validated that AO/PI staining method accurately identified the viability of cell samples that were considered completely dead.