Robust Automated Cell Counter and Viability Measurement for Engineered T Cell Product Release

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Abstract and Background

Cell viability assessment is a critical step in cellular product testing. A minimum release criterion for viability must be established for cell products administered under Investigational New Drug applications (INDs) and approved cellular therapies. Historically, viability release testing of cryopreserved cellular products manufactured in our facility was performed manually by two technicians via hemocytometer, light microscope, and Trypan Blue (TB) dye exclusion. However, automated cell counters (ACC) equipped with dual fluorescence optics capable of combining nucleic acid binding fluorescence dyes such as Acridine Orange (AO) and Propidium Iodide (PI) can provide more accurate and objective cell viability assessments and minimize user-to-user variation due to the inherent subjectivity of manual viability check thus offering clear advantages to the cellular therapy field. We selected and validated an ACC candidate with an AO/PI staining protocol that fit our GMP needs of device quality and features and performed an end-user validation testing the device performance, comparability of data to our current method and protocol optimization.

Results

Part 1. Validation of Device Performance

Figure 1: Linearity of the ACC

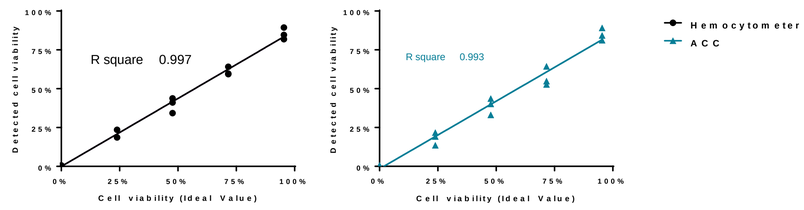


Figure 1. Heat kill assay was performed using purified primary T cells. % Cell viability was evaluated in triplicates for each viability point using Hemocytometer and ACC. R square was calculated for each method separately using linear regression and resulted in 0.997 for Hemocytometer and 0.993 for the ACC.

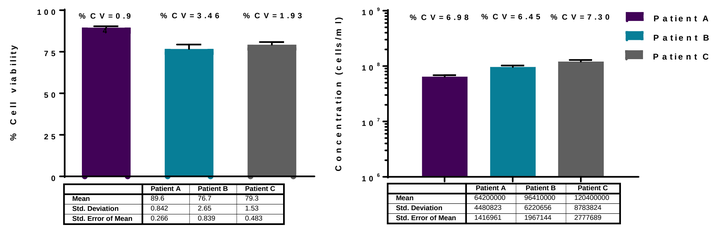
Table 1: Accuracy of the ACC.

% Viability by Hemocytometer	% Viability by ACC	Mean	SD	%CV
0.48	0.5	0.49	0.01	**2.04**
21.9	18.1	20	1.9	**9.50**
39.7	39	39.35	0.35	**0.89**
61.1	57.3	59.2	1.9	**3.21**
85.3	84.9	85.1	0.2	**0.24**

Table 2: ACC Lower Limit Of Detection (LOD).

% Viability of Heat Kill sample (0% expected viability)	SD	Linear regression Slope	LOD [(3.3xSD of 0)/LR Slope]
0.4	0.14	0.875	(3.3 x 0.14)/0.875= **0.528**
0.4			
0.7			

Figure 2: Device Precision- ACC validated for repeatability for both cell viability and cell concentration measurements.



	Patient A	Patient B	Patient C
Mean	89.6	76.7	79.3
Std. Deviation	0.842	2.65	1.53
Std. Error of Mean	0.266	0.839	0.483

	Patient A	Patient B	Patient C
Mean	64200000	95410000	120400000
Std. Deviation	4480823	6220656	8783824
Std. Error of Mean	1416961	1967144	2777689

Figure 2. Cell viability and concentration measurements of purified primary T cells from three patients were evaluated using the ACC. A total of 10 viability and concentration measurements were done for each sample (n=10).

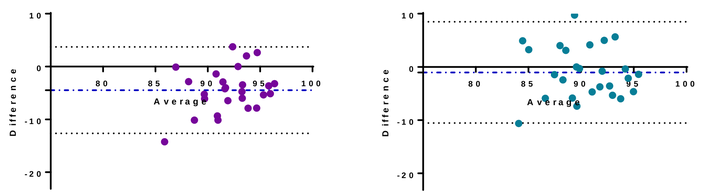
The ACC met all acceptance criteria for device performance, demonstrating repeatability, linearity and accuracy when compared with Hemocytometer throughout all levels of viability and lower limit of detection <1%.

Part 2. Method Validation and optimization

Table 3: Differences in ACC protocol settings.

Parameter	Device range	Protocol A (Default)	Protocol B (Optimized)
Cell size Range	1-60 µm	6-60 µm	5-60 µm
Green threshold (Live cell detection)	1-10	5	4
Red threshold (Dead cell detection)	1-10	5	3

Figure 3: Method Optimization- Viability.



Bias	-4.47
SD of bias	4.17
95% Limits of Agreement	
From	-12.6
To	3.71

Bias	-1.04
SD of bias	4.86
95% Limits of Agreement	
From	-10.6
To	8.48

Figure 3. ACC protocol optimization for viability detection. Bland Altman test was done on cell viability measurements from 26 purified primary T cell samples evaluated using Hemocytometer and two different ACC protocols (protocol A in purple, protocol B in teal). Bias, SD of bias and 95% limits of agreement were calculated and resulted in Bias<10 and with no systematic difference for both of the ACC protocols.

Figure 4: Method Optimization- Cell concentration.

	Protocol A	Protocol B
Number of values	26	26
Minimum	0.153	0.292
25% Percentile	4.76	3.09
Median	7.72	5.27
75% Percentile	16.2	11.5
Maximum	28.9	17.2
Mean	10.4	7.2
Std. Deviation	7.83	5.19
Std. Error of Mean	1.54	1.02

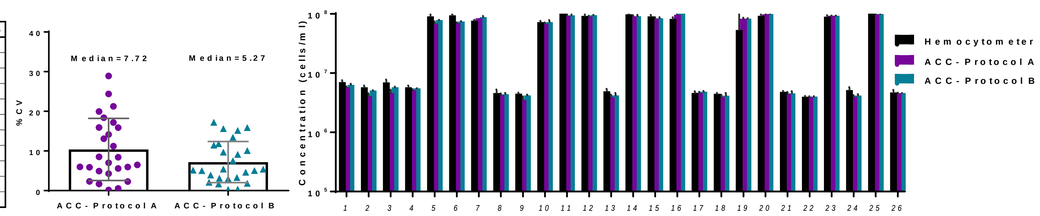
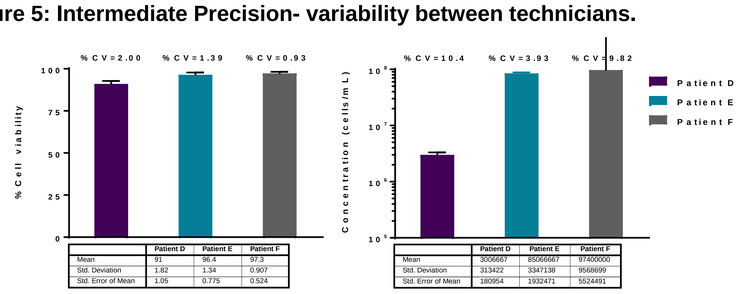


Figure 4. Cell concentration measurements from 26 purified primary T cells samples evaluated using Hemocytometer and two different ACC protocols. %CV was calculated for each sample and resulted in Median %CV of <10% for both of the ACC protocols.

Figure 5: Intermediate Precision- variability between technicians.



	Patient D	Patient E	Patient F
Mean	91	96.4	97.3
Std. Deviation	1.82	1.34	0.907
Std. Error of Mean	1.05	0.775	0.524

	Patient D	Patient E	Patient F
Mean	3006667	85066667	97400000
Std. Deviation	313422	3347138	9568699
Std. Error of Mean	180954	1932471	5524491

Figure 5. Cell viability and concentration measurements of purified primary T cells from three patients were evaluated using the ACC. Samples were counted by three technicians in triplicates. %CV was calculated for the viability and concentration measurements from each sample (n=3).

The new viability detection method using the ACC met all acceptance criteria for method comparability and intermediate precision and was optimized for the specific end-user testing needs.

Conclusions

✓The ACC met all acceptance criteria set in our validation

Parameter	Acceptance Criteria	Validation Results	Passes Criteria?
Intermediate Precision-variability between Technicians-Viability	% CV of replicates ≤10%	%CV= 0.93–2.00	☑Yes
Accuracy, linearity and LLD	r^2 ≥0.90	r^2 = 0.993	☑Yes
Precision- repeatability within methods-Viability	% CV of replicates ≤10%	%CV= 0.94-3.46	☑Yes
Repeatability within methods-Cell count	% CV of replicates ≤10%	%CV= 6.45–7.30	☑Yes
Comparability between methods-Viability	Bias ≤10	Bias= -1.04	☑Yes
Comparability between methods-Cell count	Total %CV (Median) ≤10%	Median %CV=5.40	☑Yes

✓The device successfully analyzed cell viabilities across the tested viability range.

✓The optimized ACC protocol proved to be comparable to the manual viability detection method and was approved for viability release testing of clinical products.

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