Performance Assessment of an Automated Flow Cytometry Sample Preparator for Clinical Testing

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Automated Sample Preparation Should facilitate Error Prone Manual Flow Cytometry Sample Preparation for Clinical Testing

Sample preparation for flow cytometric analysis is a highly complex and mostly manual procedure, and therefore error-prone and time consuming. A commercially available fully automated system preparator having antibody cocktail mixing, lyse, stain, fix, centrifuge and sample mixing (FlowStainer™ FS100, InstruNor A/S) was evaluated as an alternative to manual sample preparation. We assessed whether sample processing by the automated system preparator was meeting precision and accuracy standards for clinical testing using the in-vitro diagnostic (IVD) TBNK testing (T, B cells and NK staining).

Material and Methods

- **Matrix samples:** whole blood from healthy donors
- **Quality Control (QC) Material:** CD Chex Plus normal (CDN) and low (CDL) from Streck Laboratories
- **Assay:** TBNK assay in BD Trucount™ Tubes using the BD Multitest™ 6 color TBNK reagent. Percentages and absolute counts of total CD3+ T cells, CD4+ T cells, CD8+ T cells, B cells and natural killer (NK) cells were assessed.
- **Instrumentation:** BD FACSCanto™ II flow cytometer with BD FACSCanto clinical software version 3.1

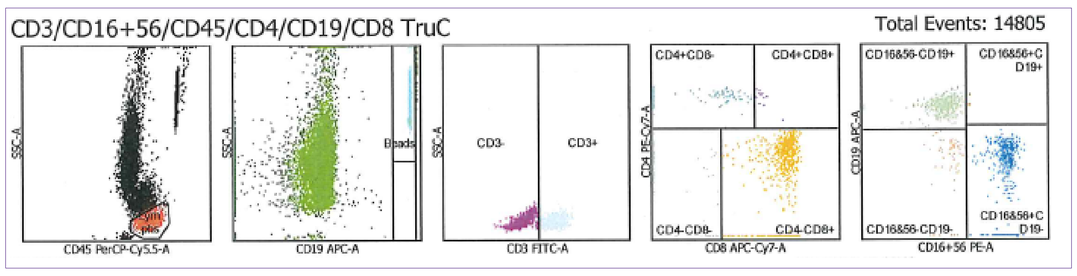


Figure 1. Representative analysis of a TBNK stained CDL replicate analyzed in BD FACSCanto clinical software version 3.1

Optimization of the FlowStainer™ Settings

- The position of the robot arm needle during blood dispension was adjusted to be at the bottom of the Trucount™ tube - to ensure the correct volume of sample was pipetted to assess absolute counts.
- Duration of the needle washing was adjusted to obtain minimal sample carryover

Pipetting Accuracy

- CDN and CDL were processed by FlowStainer™ FS100 equipment in replicates of 5 in four independent runs.
- Percentages and absolute count reportables were compared against vendor's defined target values for the defined reportables.

A

Relative % of CD45 Lymphocytes	CDN Grand Mean	CD Chex CDN target mean*	CD Chex target range*		% Difference of Means
T cells	73.16	75.50	65.50	85.50	3.10
CD8+ T cells	22.57	23.00	17.00	29.00	1.87
CD4+ T cells	48.89	50.60	44.60	56.60	3.38
NK cells	11.02	10.40	5.40	15.40	5.96
B cells	14.35	13.40	8.40	18.40	7.09
absolute counts (cells /µL)	**CDN Grand Mean**	**CD Chex CDN target mean***	**CD Chex target range***		**% Difference of Means**
T cells	1,661.97	1,736.00	1,386.00	2,086.00	4.26
CD8+ T cells	512.94	523.00	353.00	693.00	1.92
CD4+ T cells	1,110.61	1,165.00	985.00	1,345.00	4.67
NK cells	250.64	266.00	121.00	411.00	5.77
B cells	325.81	338.00	188.00	488.00	3.61

**values obtained from CD Chex vendor Streck for LOT 9077*

B

Relative % of CD45 Lymphocytes	CDL Grand Mean	CD Chex CDL target mean*	CD Chex target range*		% Difference of Means
T cells	56.91	59.60	47.60	71.60	4.51
CD8+ T cells	38.94	39.70	30.70	48.70	1.91
CD4+ T cells	14.26	14.80	9.80	19.80	3.65
NK cells	18.55	17.40	9.40	25.40	6.61
B cells	21.87	21.20	13.20	29.20	3.16
absolute counts (cells /µL)	**CDL Grand Mean**	**CD Chex CDL target mean***	**CD Chex target range***		**% Difference of Means**
T cells	651.37	679.00	414.00	944.00	4.07
CD8+ T cells	445.76	450.00	255.00	645.00	0.94
CD4+ T cells	163.21	172.00	62.00	282.00	5.11
NK cells	212.39	221.00	46.00	396.00	3.90
B cells	250.31	252.00	77.00	427.00	0.67

**values obtained from CD Chex vendor Streck for LOT 9077*

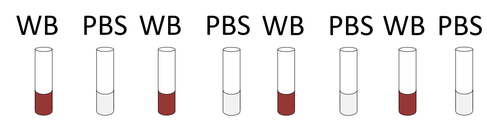
Figure 2. Pipetting Accuracy. Percentages and absolute counts obtained from two levels of QC Material (A, CDN and B, CDL) processed by the FlowStainer™ FS100 were compared to the vendor given target values.

- Obtained absolute counts and percentages were within the vendor given target ranges for all reportables
- Comparing obtained values with the vendor given target mean resulted in a % difference of means below 10% for all reportables

Specimen Carryover

- Tubes with PBS were placed between 4 whole blood (WB) replicates and processed as a sample in the FlowStainer™ FS100 to measure sample carryover
- 'PBS samples' were processed in 5mL regular flow tubes to measure bead carryover

A



B

% Carryover	PBS after S1	PBS after S2	PBS after S3	PBS after S4	Mean
Bead events	0.000	0.000	0.010	0.000	0.003
Lymph events	0.009	0.018	0.005	0.007	0.010
Total events	0.090	0.081	0.085	0.094	0.088

Figure 3. Specimen Carryover in whole processing in the FlowStainer™ FS100. (A) order of samples placed in the FlowStainer™ FS100 for processing. **(B) %** carryover of bead and lymph events in PBS tubes placed between whole blood (WB) samples enumerated as: events in processed PBS samples divided by the events in the processed WB sample.

The resulting percentage carryover is considered acceptable for a high throughput instruments.

Reproducibility with QC Material

- CDN and CDL were processed by FlowStainer™ FS100 equipment in replicates of 5 in four independent runs.
- For each independent run the % CV of the quintuples was calculated. To compare the inter-run repeatability the grand % CV was calculated

Relative % of CD45 Lymphocytes	Mean %CV		Grand Mean %CV	Acceptance Criteria (Target %CV*)
	CDL	CDN		
T cells	1.64	0.85	1.25	≤2.50 (range 1.40-2.50)
CD8+ T cells	1.70	0.86	1.28	≤4.40 (range 4.00-4.40)
CD4+ T cells	2.63	0.68	1.66	≤ 2.80 (range 1.50-2.80)
NK cells	2.65	1.97	2.31	≤5.40 (range 5.00-5.40)
B cells	1.12	2.29	1.71	≤4.90 (range 4.30-4.90)
absolute counts (cells /µL)	**Mean %CV**		**Grand Mean %CV**	**Acceptance Criteria (Target %CV*)**
	CDL	CDN		
T cells	2.30	0.65	1.48	≤5.40 (range 4.80-5.4)
CD8+ T cells	2.35	1.24	1.80	≤7.80 (range 5.70-7.80)
CD4+ T cells	3.24	1.33	2.29	≤5.10 (range 4.90-5.10)
NK cells	2.34	3.28	2.81	≤8.20 (range 7.00-8.20)
B cells	2.01	3.10	2.56	≤5.90 (range 3.20-5.90)

**Acceptance criteria established in initial TBNK assay validation using manual sample preparation*

Figure 4. Reproducibility with QC Material. 2 Levels of QC analyzed in quintuples in 4 independent runs.

- Obtained grand mean % CV were below 10 and within previously established acceptance criteria

Repeatability with Matrix Samples

- 3 replicates of four whole blood samples from four different donors (S1, S2, S3, and S4) processed by the FlowStainer™ FS100 equipment

Relative % of CD45 Lymphocytes	%CV of triplicates				Grand Mean %CV	Acceptance Criteria (Target %CV)*
	S1	S2	S3	S4		
T cells	1.08	1.72	0.20	1.55	1.14	≤2.30 (range 0.70-2.30)
CD8+ T cells	3.07	3.55	1.71	7.06	3.85	≤3.30 (range 0.70-3.30)
CD4+ T cells	1.99	1.35	1.59	0.84	1.44	≤4.80 (range 0.30-4.80)
NK cells	9.65	4.78	4.62	6.34	6.35	≤10.60 (range 3.50-10.60)
B cells	5.60	4.23	1.14	2.43	3.35	≤8.10 (range 1.40-8.10)
absolute counts (cells /µL)	**%CV of triplicates**				**Grand Mean %CV**	**Acceptance Criteria (Target %CV)***
	S1	S2	S3	S4		
T cells	1.00	3.12	5.25	3.69	3.27	≤9.00 (range 2.40-9.00)
CD8+ T cells	3.29	1.35	5.58	9.13	4.84	≤6.80 (range 2.40-6.80)
CD4+ T cells	1.76	3.59	6.40	1.70	3.36	≤10.10 (range 1.70-10.10)
NK cells	9.64	8.71	5.81	4.40	7.14	≤14.10 (range 3.50-14.10)
B cells	5.50	8.75	6.23	3.20	5.92	≤14.20 (range 2.60-14.20)

** Acceptance criteria established in initial TBNK assay validation using manual sample preparation*

Figure 5. Repeatability with Matrix Samples (Whole Blood)

- Achieved % CVs were below 10 and within previously established acceptance criteria

Conclusion

After troubleshooting and adjustment of the instrument settings, accurate and precise values for all TBNK reportables were achieved with the automated sample preparation platform. Comparison of obtained values with the vendor given target values achieved a % difference of Means between 0.94%- 7.09%. Intra-assay precision using whole blood samples achieved a % CV between 1.14-7.14%, while inter-assay precision using QC material was 1.25-2.81% In addition, a 0.01% carryover of lymphocytes was achieved after adjustments of the washing procedure. In conclusion, commercially available automated flow cytometry sample preparators can accurately replace manual sample processing for clinical flow cytometric testing. Nevertheless, every application comes with its own set of precision requirements, that will need to be carefully re-evaluated in the automated sample preparation platform.

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