Drift analysis approach to investigate the effect of specimen age and prepared sample age on the stability of lymphocyte subsets.

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INTRODUCTION

The use of flow cytometric analysis of peripheral whole blood to enumerate lymphocyte subsets is commonly used to assess the immunological status of patients in a wide variety of clinical conditions. The testing to enumerate lymphocyte subsets often occurs in specialized reference laboratories remote from the site of blood collection from the patient, often causing a delay between specimen collection and performance of the assay. In addition, once the lab has prepared the specimen for flow cytometry analysis, it is not uncommon for the lab to store this prepared sample prior to analysis or reanalysis. Therefore, it is important to determine the effect of both specimen and prepared sample age on lymphocyte subset enumeration.
A novel statistical approach was used to assess the stability of CD3+/CD4+, CD3+/CD8+, CD3-/CD56+, CD19+ and CD3+ lymphocyte subsets in CYTO-STAT® tetraCHROME™ stained samples and analyzed on FC 500™ or Navios™ flow cytometers.

MATERIALS AND METHODS

Approximately 30 normal and clinical specimens were included in the data analysis for each study. Among them ≥ 50%, had CD4+ absolute counts lower than 500 cells/ul. Specimens were stored at room temperature for the following time periods: fresh (≤8 hours), 24, 48 and 72 hours post-venipuncture. Additionally, samples at each time point, were prepared and stored refrigerated up to 48 hours prior to analysis.
The following time points were tested for specimen age and prepared sample:

Time	Specimen age			
	0	24	48	72
Prepared Sample Age	0	0	0	0
	Not done	24	24	Not done
	Not done	48	48	Not done

Samples were analyzed using automated Navios tetra Algorithm on Navios ™ instrument or Tetra CXP algorithm on Cytomics FC500 ™ instrument.

Data were modeled as a linear function of samples, specimen age, and prepared time for each test case. A mixed model was used to analyze the data. Samples constituted the random component of the model while “age” and “prepared” were used as regressor fixed effects. The PROC MIXED routine of SAS was used for data analysis. Drift at different combinations of “age” and “prepared” was calculated based on the slopes of age and prepared (2- variable regression).

Average drift (δ) at a certain time point was calculated as the difference between the response at that time point and the response at time zero (t_0).

$$\delta = \mu + \beta_1 t_1 + \beta_2 t_2 - \mu - \beta_1 t_0 + \beta_2 t_0$$

Where μ was the intercept, β_1 was the coefficient for specimen age, and β_2 was the coefficient for prepared sample age.
Since $t_0 = 0$, and intercepts cancelling out, the resulting equation for calculating drift was,

$$\delta = \beta_1 t_1 + \beta_2 t_2$$

Standard error (se) of drift σ was calculated based on the standard errors of the two components (σ_1 and σ_1) and their covariance Cov(β_1,β_2).

$$\sigma = (\sigma_1 \beta_1 t_1 + \sigma_2 \beta_2 t_2 + 2\ \mathrm{cov}(\beta_1, \beta_2)\ t_1\ t_2)^{1/2}$$

The upper confidence limit of the drift was calculated based on the standard error of the drift and 95% confidence. Upper limit of the drift represented the worst case scenario of the drift (“95% Upper Limit” in the tables below).

RESULTS

Table below shows Navios tetra and Tetra CXP results for up to 48 hours of both specimen age and prepared sample. It is recommended that samples are prepared with CYTOSTAT tetraCHROME reagents and analyzed with Navios tetra or Tetra CXP from specimens within 24 hours post venipuncture. Prepared samples can be stored to up 24 hours at 2-8C in the dark.

Specimen Age in hours	Prepared Sample age in hours at refrigeration	Parameters	Navios tetra												Tetra CXP											
			Total CD3+ %	Total CD3+ Count	CD3+/C D4+ %	CD3+/C D4+ Count	CD3+/C D8+ %	CD3+/C D8+ Count	Total CD3+ %	Total CD3+ Count	CD3-/CD56+ %	CD3-/CD56+ Count	CD19+ %	CD19+ Count	Total CD3+ %	Total CD3+ Count	CD3+/C D4+ %	CD3+/C D4+ Count	CD3+/C D8+ %	CD3+/C D8+ Count	Total CD3+ %	Total CD3+ Count	CD3-/CD56+ %	CD3-/CD56+ Count	CD19+ %	CD19+ Count
0	0	Mean	74.42	1141	36.8	575	35.83	539	74.57	1259	10.64	171	11.9	206	74.54	1219.5	33.5	537.17	38.71	645.64	74.45	1252.9	9.91	163.97	13.15	220.78
24	0	Mean	75.15	1059	37.16	528	36.25	505	75.23	1171	10.44	156	11.41	182	74.49	1176.72	33.11	510.46	38.93	628.84	74.41	1220.9	10.01	162.81	13.01	214.35
		Drift	1.42	-52.44	1.27	-17.78	0.25	-31.27	1.57	-55.58	-1.15	-26.48	-0.38	-17.1	2	-76.68	1.57	-21.91	0.52	-50.26	1.95	-76.13	-1.24	-29.24	-0.64	-24.31
		Upper Limit	1.56	-64.35	1.4	-23.6	0.34	-38.5	1.71	-68.99	-1.25	-29	-0.45	-19.41	2.18	-87.65	1.72	-27.3	0.62	-56.69	2.14	-86.94	-1.36	-31.68	-0.75	-26.45
	24	Mean	74.63	1135	36.38	561	36.58	548	74.9	1205	10.4	159	11.34	186	74.84	1224.3	33.1	528.6	39.39	658.57	74.95	1221.8	10.04	161.14	12.14	196.62
		Drift	0.89	-17.84	0.67	-5.33	0.36	-9.68	0.98	-61.06	-1.01	-25.01	-0.27	-15.52	1.35	-44.3	0.88	-12.57	0.6	-27.79	1.45	-68.39	-0.94	-24.18	-0.68	-25.03
		Upper Limit	1.09	-34.99	0.86	-13.72	0.49	-20.1	1.19	-80.38	-1.16	-28.65	-0.38	-18.84	1.62	-60.13	1.09	-20.34	0.74	-37.06	1.72	-83.99	-1.12	-27.7	-0.84	-28.11
	48	Mean	74.6	1147	36.36	573	36.62	550	75.07	1220	10.07	159	11.22	187	74.85	1229.7	33.02	532.57	39.48	660.29	75.18	1213.7	9.83	157.43	12.02	191.12
		Drift	0.36	16.76	0.07	7.12	0.47	11.9	0.39	-66.54	-0.87	-23.55	-0.17	-13.93	0.71	-11.93	0.19	-3.22	0.67	-5.33	0.94	-60.64	-0.65	-19.13	-0.72	-25.75
		Upper Limit	0.68	44.23	0.37	20.54	0.67	28.58	0.72	-97.48	-1.1	-29.37	-0.35	-19.24	1.12	-37.26	0.52	-15.67	0.9	-20.17	1.38	-85.62	-0.94	-24.75	-0.97	-30.69
48	0	Mean	76.25	1052	38.56	538	36.1	492	76.56	1193	9.29	136	11.27	178	76.43	1108.79	34.48	494.79	39.61	582.19	76.14	1162.22	8.97	133.03	12.53	193.05
		Drift	2.85	-104.88	2.55	-35.57	0.51	-62.53	3.13	-111.15	-2.31	-52.95	-0.75	-34.21	4	-153.36	3.15	-43.82	1.04	-100.52	3.91	-152.26	-2.47	-58.49	-1.28	-48.62
		Upper Limit	3.12	-128.7	2.81	-47.21	0.68	-76.99	3.42	-137.99	-2.51	-58	-0.9	-38.82	4.37	-175.31	3.43	-54.6	1.24	-113.37	4.28	-173.88	-2.72	-63.36	-1.5	-52.9
	24	Mean	76.07	1085	38.07	548	36.38	513	76.29	1142	8.93	124	11.36	177	76.4	1136.84	34.66	508.61	39.59	597.72	76.35	1114.7	8.11	118.3	12.45	183.82
		Drift	2.32	-70.28	1.95	-23.12	0.62	-40.95	2.54	-116.63	-2.16	-51.49	-0.65	-32.62	3.35	-120.99	2.46	-34.48	1.12	-78.05	3.4	-144.51	-2.18	-53.43	-1.32	-49.34
		Upper Limit	2.62	-97.08	2.24	-36.22	0.81	-57.22	2.87	-146.83	-2.39	-57.17	-0.82	-37.81	3.76	-145.71	2.78	-46.62	1.34	-92.53	3.82	-168.88	-2.46	-58.91	-1.56	-54.16
	48	Mean	75.81	1093	37.75	551	36.54	519	75.96	1112	9.06	125	11.6	175	76.03	1140.86	34.33	510.91	39.55	599.48	76.01	1149.66	8.87	131.9	12.36	187.24
		Drift	1.78	-35.68	1.34	-10.67	0.72	-19.36	1.95	-122.11	-2.02	-50.02	-0.55	-31.03	2.71	-88.61	1.77	-25.13	1.19	-55.59	2.89	-136.77	-1.89	-48.37	-1.36	-50.06
		Upper Limit	2.18	-69.99	1.72	-27.43	0.98	-40.19	2.37	-160.76	-2.31	-57.3	-0.77	-37.67	3.23	-120.26	2.18	-40.68	1.48	-74.13	3.44	-167.97	-2.25	-55.39	-1.67	-56.23
72	0	Mean	78.79	969	40.41	511	36.99	442	79.44	1087	7.44	97	10.24	145	81.32	1002.1	38.97	477.51	40.24	499.91	81.27	1032.6	5.95	76.25	10.5	136.49
		Drift	4.27	-157.33	3.82	-53.35	0.76	-93.8	4.7	-166.73	-3.46	-79.43	-1.13	-51.31	6	-230.05	4.72	-65.73	1.56	-150.78	5.86	-228.38	-3.71	-87.73	-1.92	-72.94
		Upper Limit	4.68	-193.05	4.21	-70.81	1.03	-115.49	5.13	-206.98	-3.76	-87.01	-1.35	-58.23	6.55	-262.96	5.15	-81.89	1.86	-170.06	6.42	-260.82	-4.08	-95.03	-2.25	-79.35

The following graphs illustrate specimen and prepared sample stability drift for samples analyzed on Navios™ flow cytometer: Fig. 1 Specimen Age; Fig. 2 Prepared Sample. The most significant contributor to overall specimen stability was specimen aging in whole blood. In contrast, prepared sample storage had less impact on the cell loss.

Fig. 1 Specimen age

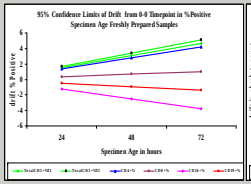
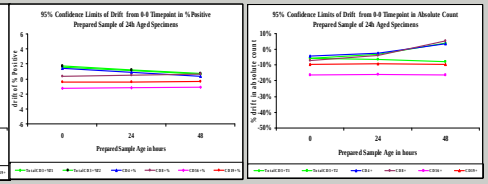


Fig. 2 Prepared sample stability



Derived regression models allow to predict the drift at time points within tested interval of Specimen age and/or Prepared sample stability time. Tables below show prediction of the drift at 36 h for both Navios tetra and Tetra CXP systems.

Navios tetra results

Specimen Age in hours	Prepared Sample age in hours at refrigeration	Parameters	Total CD3+ %	Total CD3+ Count	CD3+/ CD4+ %	CD3+/ CD4+ Count	CD3+/ CD8+ %	CD3+/ CD8+ Count	Total CD3+ %	Total CD3+ Count	CD3-/CD56+ %	CD3-/CD56+ Count	CD19+ %	CD19+ Count
36	24	Drift	1.60	-44.06	1.31	-14.22	0.49	-25.32	1.76	-88.84	-1.59	-38.25	-0.46	-24.07
		Upper Limit	1.85	-65.76	1.55	-24.83	0.65	-38.49	2.02	-113.29	-1.77	-42.85	-0.60	-28.27

Tetra CXP results

Specimen Age in hours	Prepared Sample age in hours at refrigeration	Parameters	Total CD3+ %	Total CD3+ Count	CD3+/C D4+ %	CD3+/C D4+ Count	CD3+/C D8+ %	CD3+/C D8+ Count	Total CD3+ %	Total CD3+ Count	CD3-/CD56+ %	CD3-/CD56+ Count	CD19+ %	CD19+ Count
36	24	Drift	2.35	-82.65	1.67	-23.52	0.86	-52.92	2.42	-106.45	-1.56	-38.81	-1.00	-37.19
		Upper Limit	2.69	-102.66	1.93	-33.35	1.04	-64.65	2.77	-126.18	-1.79	-43.25	-1.20	-41.09

CONCLUSION

The derived models provide a robust method for prediction of the drift at different time points within the tested range.