WHITE PAPER

ENUMERATION OF T, B, AND NK SUBPOPULATIONS IN AGED WHOLE BLOOD SAMPLES USING THE AQUIOS CL INSTRUMENT WITH TETRA APPLICATION

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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, which can cause delays 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.

Previous studies have shown that the scatter and fluorescence properties of various cell subsets of whole blood stored in EDTA change as a specimen or prepared sample ages. The general observations regarding specimen age have been that the relative proportion of T-cells increases, while the number of detectable B-cells decreases (Nicholson, et. al 1993, Ekong, et al 1993, Jalla, et. al. 2004).

In this study we demonstrate that the new AQUIOS-Tetra method developed for the enumeration of T, B and NK lymphocyte subsets provides accurate results for specimens stored in EDTA for up to 24 hours post venipuncture at room temperature.

Specimen Age and Prepared Sample Stability Analysis Method

The AQUIOS CL instrument with Tetra application is an integrated system of sample preparation and sample analysis; it analyzes samples within 3 minutes after the lysing reagent is added to the sample stained with antibodies. The software controls sample preparation and analysis to limit the overlysing impact. In this study, the whole blood samples were tested at 24 hours of post venipuncture and after a 3 minute incubation time as prepared samples. Each specimen was tested in duplicate at each time point.

Table 1. Time Points for each specimen

SPECIMEN AGE (HOURS)	PREPARED SAMPLE AGE	
	TO	T3MIN
0	X (0-0)	
24	X (24-0)	X (24-3)
32	X (32-0)	

NOTE: The results from at least three time points testing were required to perform regression analysis.

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Seventy-three (73) specimens, including normal and clinical (HIV+) donors were tested for data analysis. The drift (change) in absolute count and percent positive for CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/ CD19+ and CD3-/ CD56+16+ lymphocyte subsets for all replicates were assessed between the reference point 0-0 and other time points (24-0, 24-3). A 95% confidence limit of the drift was also calculated.

AQUIOS drift analysis for Specimen Age

Data was modeled as a linear function of specimen using a mixed model including all the data collection at each time point (0-0, 24-0, and 32-0). Samples constituted the random component of the model while "age" was used as a linear regression fixed effect. The PROC MIXED routine of SAS 9.3 was used for data analysis.

Drift (δ_{Age}) 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).

AQUIOS drift analysis for Prepared Sample

Drift of prepared samples ($\delta_{Prepared}$) was calculated as the difference between the average response of samples aged for 24 hours and prepared for three minutes (24-3) and the average response of samples aged 24 hours tested immediately (24-0). $\delta_{Prepared} = X_{24hrs + 3\ min} - X_{24hrs}$

Where $X_{24hrs + 3\ min}$ and X_{24hrs} were the respective averages. Standard error of this drift ($\sigma_{Prepared}$) was calculated as the standard error of the difference between these two time points.

AQUIOS total drift analysis

Drift at stability claim (24 hours aged and 3 minutes prepared) was calculated as the sum of the specimen age component and the prepared sample component as:

$\delta_{Total} = \delta_{Age} + \delta_{Prepared}$

The confidence limits of the drift were calculated based on the standard error of the drift and 95% confidence.

Specimen Age and Prepared Sample Data Analysis Results

Table 2. Mean values of the recovered drift at each time point

PANEL	MARKER	UNIT	0-0	24-0	24-3	32-0
AQUIOS Tetra-1 (CD45/4/8/3)	Total CD3+	%	73.66	74.00	74.19	73.75
	CD3+/CD4+		29.73	29.89	29.61	29.68
	CD3+/CD8+		41.43	41.60	42.07	41.59
	CD45+ Low SS		35.41	35.72	36.18	36.32
	Total CD3+	cells/µL	1327.20	1320.19	1310.22	1287.28
	CD3+/CD4+		530.02	527.13	517.85	518.14
	CD3+/CD8+		755.06	751.49	750.80	729.38
	CD45+		5380.35	5266.47	5161.18	5097.48
	CD45+ Low SS		1811.50	1795.67	1778.31	1756.16
AQUIOS Tetra-2+ (CD45/56+16/19/3)	Total CD3+	%	73.82	73.95	74.12	73.88
	CD3-/CD19+		13.57	13.29	13.33	13.42
	CD3-/CD56+CD16+		11.36	11.60	11.46	11.46
	Total CD3+	cells/µL	1325.34	1332.01	1328.27	1306.19
	CD3-/CD19+		253.70	251.25	250.68	249.36
	CD3-/CD56+CD16+		202.70	207.37	203.77	201.65
	CD45+ Low SS		1803.83	1811.39	1802.78	1778.75

Table 3. Specimen and Prepared Sample Stability Results

Panel	Marker	Unit	Total Drift (includes 0-0 to 24-0 and 24-0 to 24-3)	95% confidence limits	
				Lower	Upper
AQUIOS Tetra-1 (CD45/4/8/3)	Total CD3+	%	0.24	0.08	0.40
	CD3+/CD4+		0.02	-0.09	0.13
	CD3+/CD8+		0.21	0.08	0.35
	CD45+ Low SS		0.83	0.67	0.99
	Total CD3+	cells/µL	-35.82	-43.79	-27.85
	CD3+/CD4+		-12.09	-15.90	-8.29
	CD3+/CD8+		-22.50	-27.67	-17.33
	CD45+		-275.04	-308.92	-241.16
	CD45+ Low SS		-52.88	-63.20	-42.55
AQUIOS Tetra-2+ (CD45/56+16/19/3)	Total CD3+	%	0.14	-0.02	0.30
	CD3-/CD19+		-0.12	-0.22	-0.03
	CD3-/CD56+CD16+		0.08	-0.03	0.19
	Total CD3+	cells/µL	-13.61	-21.83	-5.40
	CD3-/CD19+		-3.64	-5.89	-1.39
	CD3-/CD56+CD16+		-1.55	-3.83	0.72
	CD45+ Low SS		-20.14	-30.13	-10.14

Conclusions

The AQUIOS CL instrument with Tetra application provides accurate results for recovery of the T, B and NK lymphocyte subsets in clinical and normal specimens collected into the EDTA K3 tubes when stored at room temperature for up to 24 hours.

AQUIOS CL is a Class I Laser Product

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