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Aquios CL flow cytometer performance in the automated quantification of lymphocyte subpopulations

Desempenho do citômetro de fluxo Aquios CL na quantificação automatizada de subpopulações linfocitárias

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ABSTRACT

Flow cytometry (FC) is an essential tool for diagnosis, prognosis and therapeutic follow-up of several hematologic malignancies. In addition, it performs the quantification of lymphocytes subpopulations for diagnosis and monitoring of primary and acquired immunodeficiencies through the antigenic expressions of CD19 and CD20 for B lymphocytes; CD2, CD3, CD4, CD8 for T lymphocytes; and CD56 and CD16 for the identification of natural killer (NK) cells. The cytometry technique has revolutionized the way that the cells are identified, and over the years this platform has progressed with several advances in hardware and software that aim to improve workflow resulting in higher productivity, quality and cost savings. The Aquios CL – Beckman Coulter (BC) is an example of this advance because it is a complete automation instrument in flow cytometry called "Load & Go flow cytometer" for quantification of lymphocyte subpopulations in the routine diagnosis. In this study, the Aquios CL was validated, and quantification in frequency and absolute numbers of the lymphocyte subpopulations had an excellent correlation with the results obtained by the dual platform quantification performed in the Cytomics FC500 (BC) and automated Sysmex XE-2100 cell analyzer.

Key words: Aquios CL; flow cytometry; subpopulation of lymphocytes; CD4/CD8.

RESUMO

A citometria de fluxo (CF) é uma ferramenta importante para diagnóstico, prognóstico e acompanhamento terapêutico de diversas neoplasias hematológicas. Além disso, possibilita a quantificação das subpopulações linfocitárias (SPL) para assistência diagnóstica e monitoramento das imunodeficiências primárias e adquiridas por meio das expressões antigênicas de CD19 e CD20 para linfócitos B; CD2, CD3, CD4 e CD8 para linfócitos T; e CD56 e CD16 para identificação de células natural killer *(NK). A técnica de CF revolucionou a maneira como as células são identificadas e, ao longo dos anos, essa plataforma tem progredido com diversos avanços em* hardware *e* software *que visam melhorar o fluxo de trabalho, resultando em maior produtividade, qualidade e redução de custos. O Aquios CL – Beckman Coulter (BC) é um exemplo desse avanço, pois é um equipamento de automação completa em CF (denominado* Load & Go flow cytometer*) para quantificação de SPL na rotina diagnóstica. Neste estudo, o Aquios CL foi validado, e a quantificação em números relativos e absolutos das subpopulações linfocitárias teve uma excelente correlação com os resultados obtidos pela quantificação em plataforma dupla realizada no Cytomics FC500 (BC) e no analisador automatizado de células Sysmex XE-2100.*

Unitermos: *Aquios CL; citometria de fluxo; subpopulação linfocitária; CD4/CD8.*

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RESUMEN

La citometría de flujo (CF) es una herramienta importante para diagnóstico, pronóstico y seguimiento terapéutico de diversas neoplasias hematológicas. Además, posibilita la cuantificación de las subpoblaciones linfocitarias (SPL) para asistencia diagnóstica y monitoreo de las inmunodeficiencias primarias y adquiridas a través de las expresiones antigénicas de CD19 y CD20 para linfocitos B; CD2, CD3, CD4 y CD8 para linfocitos T; y CD56 y CD16 para identificación de células natural killer *(NK). La CF ha revolucionado la manera como las células son identificadas y, a lo largo de los años, esa plataforma ha progresado con diversos avances en* hardware *y* software *que aspiran a mejorar el flujo de trabajo resultando en mayor productividad, calidad y reducción de costos. El Aquios CL Beckman Coulter (BC) es un ejemplo de ese avance, pues es un instrumento de automación completa en CF (llamado* Load & Go flow cytometer*) para cuantificación de SPL en la rutina diagnóstica. En este estudio el Aquios CL fue validado, y la cuantificación en números relativos y absolutos de las subpoblaciones linfocitarias tuvo una excelente correlación con los resultados obtenidos por la cuantificación en plataforma dupla realizada en el Cytomics FC500 (BC) y en el analizador automatizado de células Sysmex XE-2100.*

Palabras clave: *Aquios CL; citometría de flujo; subpoblación linfocitaria; CD4/CD8.*

INTRODUCTION

Flow cytometry (FC) is an essential tool for diagnosis, prognosis and therapeutic follow-up of several hematological malignancies, as well as for primary or acquired immunodeficiencies[1-3].

Immunophenotyping by FC quantifies lymphocyte populations and subpopulations, evaluating, basically, antigenic expressions of CD19 and CD20 for B lymphocytes; CD2, CD3, CD4 and CD8 for T lymphocytes; and CD56 and CD16 for identification of natural killer (NK) cells[2, 4]. Quantification of these populations helps monitor immunosuppressing and immunomodulatory therapies and the diagnosis and monitoring of primary immunodeficiencies, besides contributing to the evaluation of post-transplant lymphocytic populations[4-6]. Moreover, quantification of CD4 T lymphocytes in the peripheral blood of patients with the acquired immunodeficiency syndrome (Aids) is important to evaluate disease progression and response to treatment[7-9].

The increased number of carriers of the human immunodeficiency virus (HIV) has provided a considerable advance in FC. The search for services equipped with the technology of CD4 T lymphocyte quantification has become more and more frequent, and the use of tools that add speed, efficacy and quality to diagnosis and to monitoring of immunodeficiencies has been made essential in clinical practice[8, 10].

The FC practice revolutionized the way cells are characterized and identified and, over the years, this platform has progressed with several improvements in hardware and software that aim at enhancing work flow, resulting in higher productivity, quality, and cost reduction. However, FC is still a laborious technique that involves several technical steps of washing, labeling, acquisition, and sample analysis, what increases significantly error chances during the process[11].

The Aquios CL instrument – Beckman Coulter (BC) is a complete automation tool in FC. It is identified as "Load & Go flow cytometer" for quantification of lymphocyte subpopulations in diagnostic routine, representing a real and significant improvement in the area of laboratory diagnosis[11, 12]. This work was aimed at validating and comparing quantification in frequency and absolute numbers of lymphocyte subpopulations performed at Aquios CL with the result obtained by the double platform performed at Cytomics FC500 (BC) and in the Sysmex XE-2100 (Sysmex) automated cell analyzer. Besides, we will discuss the actual benefits of this instrument in diagnostic routine.

MATERIALS AND METHODS

Samples

Seventy-two samples of peripheral blood were analyzed. CD3, CD4 and CD8 T lymphocyte quantification was determined in all samples; quantification of CD19+ B lymphocytes and CD16+ CD56+ NK cells, in 28 of the 72 samples. These were collected in a tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA), and processed within 24 h after collection. The patients presented age median of 47 years (age group 1-99); 37 were males.

Cytomics FC500 and Sysmex XE-2100

Leukocyte count was done in the automated cell counter Sysmex XE-2100 by the impedance technique and optical reading. The leukocyte differential was automatically given by the counter and, when necessary, a blood smear was prepared for microscopic evaluation and accurate quantification of total lymphocytes.

For the characterization and quantification of subpopulations of CD3, CD4, and CD8 T lymphocytes, the samples were labeled in a single tube with the monoclonal antibodies described in **Table 1**. In order to identify the population of NK cells and B lymphocytes, we used three labeled tubes with the monoclonal antibodies described in **Tables 1** and **2**. After labeling and incubation of 20 minutes, the lysis of red cells and fixation of leukocytes were performed by the automated no-wash Coulter TQ-Prep system (Beckman Coulter, SN: AV26068). Then, samples were acquired in the flow cytometer Cytomics FC500, and data analysis was performed in the software CXP Cytometer 2.2 and at Kaluza (Beckman Coulter).

Aquios CL

Aquios CL is a flow cytometer, a single-platform system and automated cell counter that has the advantage of using impedance for absolute quantification of leukocytes, and not the conventional FC strategy by means of fluorescent particles (beads) of known concentration.

For evaluation and quantification of CD3, CD3/CD and CD3/CD8 T lymphocytes subpopulations, the mix of monoclonal antibodies described in **Table 3** was used, available in the ready-to-use format called Tetra Panel 1. In the analysis of populations of B lymphocytes and NK cells, the system uses the mix of monoclonal antibodies Tetra Panel 2, described in **Table 4**. The use of Tetra Panel 1 and 2 for complete quantification of T, B and NK subpopulations of lymphocytes is called Aquios Tetra Combo.

In the Aquios CL, sample labeling was done in a 96-well microplate in an automated manner; red cell lysis, by means of the no-wash technique. Data analysis was carried out by the equipment itself using the gating automatic strategy. However, the software permits the user to make fine gate adjustment, when necessary.

Statistical analysis

The results for each method were described with the use of mean and standard deviation (SD), and the agreement between the methods for each parameter was verified with the use of the intraclass correlation coefficient (ICC) and 95% confidence intervals (CI). The difference between the methods was estimated by calculating the repeatability for each parameter. The statistical analysis was performed with the software SPSS, version 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The comparison between the different platforms of lymphocyte quantification took into consideration results in frequency and absolute numbers and the final test interpretation.

TABLE 1 – Monoclonal antibodies used for identification of CD3/CD4/CD8 T lymphocyte and B subpopulations at Cytomics FC500

	Markers		Clone		
Fluorochromes	CD3/CD4/CD8 T lymphocyte subpopulation	B lymphocyte subpopulation	CD3/CD4/CD8 T lymphocyte subpopulation	B lymphocyte subpopulation	Manufacturer
FITC	CD8	CD19	SFCI21Thy2D3	89B	Beckman Coulter
PE	CD4	CD2	SFCI12T4D11	SFCI3Pt2H9	Beckman Coulter
ECD	CD45	CD45	J33	J33	Beckman Coulter
PC5	CD3	CD3	UCHT1	UCHT1	Beckman Coulter

FITC: fluorescein isothiocyanate; PE: phycoerythrin; ECD: phycoerythrin-Texas Red-X.

TABLE 2 – Monoclonal antibodies used for identification of NK lymphocyte subpopulations at Cytomics FC500

Fluorochromes	Markers	Clone	Manufacturer
FITC	CD3	UCHT1	Beckman Coulter
PE	CD56/16	N901/3G8	Beckman Coulter
ECD	CD45	J33	Beckman Coulter
PC5	CD2	39C1.5	Beckman Coulter

FITC: fluorescein isothiocyanate; PE: phycoerythrin; ECD: phycoerythrin-Texas Red-X.

TABLE 3 – Cocktail of monoclonal antibodies used for characterization of subpopulations of T lymphocytes in the Aquios CL

Fluorochromes	Tetra 1 – Panel	Clone	Manufacturer
FITC	CD45	B3821F4A	Beckman Coulter
RD1	CD4	SFCI12T4D11	Beckman Coulter
ECD	CD8	SFCI21Thy2D3	Beckman Coulter
PC5	CD3	UCHT1	Beckman Coulter

FITC: fluorescein isothiocyanate; ECD: phycoerythrin-Texas Red-X.

TABLE 4 – Cocktail of monoclonal antibodies used for characterization of subpopulations of B and NK lymphocytes in the Aquios CL

Fluorochromes	Tetra 2 – Panel	Clone	Manufacturer
FITC	CD45	B3821F4A	Beckman Coulter
RD1	CD16/CD56	N901/NKH-1, 3G8	Beckman Coulter
ECD	CD19	J3-119	Beckman Coulter
PC5	CD3	UCHT1	Beckman Coulter

FITC: fluorescein isothiocyanate; ECD: phycoerythrin-Texas Red-X.

The analysis of 72 samples demonstrated statistically acceptable correlation between the panels used in the Aquios CL and the panel used in the Cytomics FC500. For the percentage identification of population of CD3, CD4 and CD8 T lymphocytes, the ICC was 0.989, 0.959 and 0.977, respectively. The parameter of the relation CD4/CD8 presented acceptable correlation of 0.74, and the result interpretation was the same for both platforms.

In the percentage analysis of the population of B lymphocytes, ICC was 0.997, while for NK cells, the obtained correlation was 0.98 (**Table 5**).

The gating strategies used to identify and quantify the frequencies of B and NK T lymphocytes for the Aquios CL are represented in **Figures 1** and **2**; for Cytomics FC500/software Kaluza, in **Figure 3**.

TABLE 5 – ICC obtained in the comparison of FC500 with Aquios CL

Variable	FC500			Aquios			ICC	CI (95%)		Repeatability
	Mean	SD	*n*	Mean	SD	*n*		Lower	Upper	
CD3 %	72.7	16.8	72	72.1	15.9	72	0.989	0.982	0.993	1.7
CD3/mm³	1,661.1	1,249.3	72	1,397.6	948.7	72	0.883	0.751	0.938	337.7
CD4 %	41.7	14.8	72	41.1	14.5	72	0.959	0.936	0.974	3
CD4/mm³	945.3	962.6	72	799.1	728.1	72	0.91	0.836	0.948	237.5
CD8 %	27.4	13.7	72	28.7	13.1	72	0.977	0.95	0.988	1.8
CD8/mm³	587.5	451.8	72	552	375.8	72	0.874	0.809	0.918	146.5
CD19 %	18.5	21.1	28	19.2	20.5	28	0.997	0.992	0.999	1.1
CD19/mm³	698.04	1,537	28	638.07	1401.9	28	0.99	0.978	0.995	144.1
CelNK %	8.3	8.3	28	9.7	8.1	28	0.98	0.616	0.995	0.7
CelNK/mm³	198.6	134.3	28	213.4	122.6	28	0.865	0.732	0.935	47
Relação Ratio CD4/CD8	2	1.5	72	1.8	1	72	0.74	0.603	0.832	0.6

ICC: intraclass correlation coefficient; SD: standard deviation; CI: confidence interval.

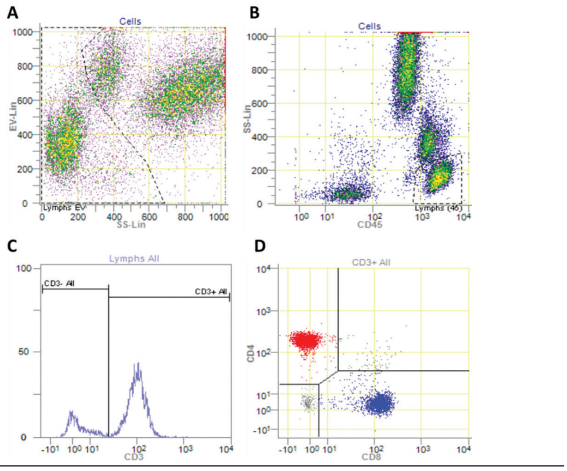

FIGURE 1 – *Gating strategy used in the Aquios CL with the Tetra Panel 1*

The population of lymphocytes is selected by means of EV-Lin × side scatter (A) and SS-Lin × CD45 gates (B). This population is combined at a gate called Lymphs All (C) and in the population CD3+ we can identify the CD4 and CD8 T lymphocytes (D).

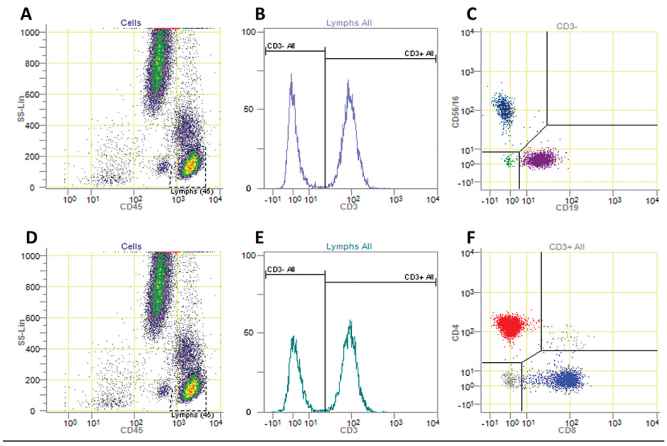

FIGURE 2 – *Gating strategy used in the Aquios CL with the Tetra Panel 2*

A) and D) the population of lymphocytes is selected by means of SS-Lin × CD45; B and E) this population is combined at a gate called Lymphs All; C) inside the population CD3+ we can identify the CD4 and CD8 T lymphocytes; F) inside the population CD3 we can identify the CD45+/CD16+ NK lymphocytes and the CD19+B lymphocytes.

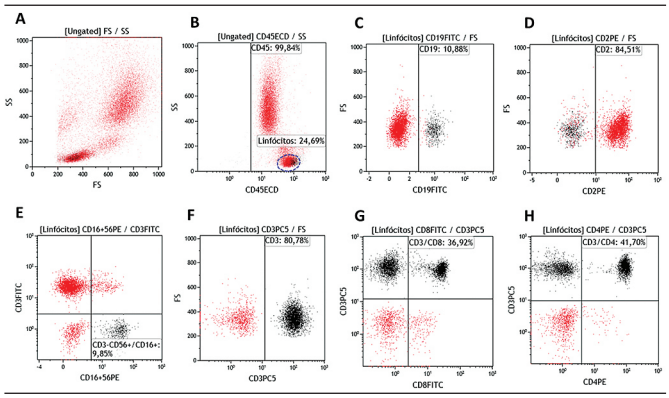

FIGURE 3 – *Gating strategy used in the FC500 cytometer and Kaluza analysis software*

A) selection of the viable population by means of side scatter × forward scatter; B) side scatter × CD45 with gate in the region of total lymphocytes; C, D, E) inside the gate of lymphocytes, we identified populations of T, B, and NK lymphocytes; F, G, H) still inside the gate of lymphocytes, we identified a population of CD3 T lymphocytes and the CD4 and CD8 subpopulations.

DISCUSSION

The enhancement in the work flow of FC is a real necessity in clinical laboratories that search to improve productivity, quality and cost reduction. The Aquios CL instrument meets this demand, because it brings improvement in technical standardization; reduces pre-analytical errors; avoid sample changes, as all pipetting is automated and reagents/samples are monitored with bar codes; improves workers training; eliminates subjectivity from analysis; monitors quality control; reduces result releasing time. Besides, as it is a closed system, the equipment provides the operator a safe manipulation of the sample, avoiding biological contamination.

Our results demonstrate statistical significance to compare quantification of lymphocyte subpopulations between Aquios CL and Cytomics FC500, what corroborates data already published. Gossez *et al*. (2011)[11] showed that Aquios CL obtained good correlation in the quantification of CD4 T lymphocytes when compared with FC500. Besides, it can be used in the diagnostic routine and also in proficiency tests.

Grossi *et al*. (2018)[12] demonstrated good correlation between Aquios CL and the bead-based assay of the equipment BD FACSCanto II. That study also demonstrates an important decrease in turnaround time (TAT) because it is an automated instrument.

At a work recently published by Degandt *et al*. (2018)[4], the performance of Aquios CL in relation to double platform Sysmex XE-5000 and BD FACSCanto II was acceptable; however, around a third of the samples needed manual adjustment in the gate of lymphocytes. An advantage of the software of Aquios CL is the flagging system, which signals the need to review gate.

The system "Load & Go flow cytometer" of Aquios provides sample preparation along with fluorescence analysis; it generates results of frequency and absolute numbers in a single instrument (single platform); has the possibility of interface of results with computerized systems in clinical laboratories[10, 11].

Cost evaluation was not part of the scope of our study, but Aquios CL reagents are known to be more expensive than those used in conventional FC. However, if we take into account all the technical benefits already described for Aquios, principally the significant reduction of technical workforce due to complete automation, it is possible to ensure that this instrument generates economic viability important to clinical laboratories with great volume of samples.

Automation in FC adds new perspectives for the technique and provides improvement in quality and productivity of the process, eliminating chances of error almost completely.

By the automation of CD4 cells process of quantification, it is possible not to depend on formation and expertise of the technical staff, making the process more simplified and independent from specialized workforce, but the interpretation and levels of review continue to be fundamental in the post-analytical process to trigger a flag in the clinical area.

CONCLUSION

The instrument Aquios C presented excellent performance in the quantification of lymphocyte subpopulations. Because it is an instrument with complete automation, it promoted standardization, quality, and reduction in the time of technical procedure, becoming an adequate and viable tool in clinical laboratories with a high sampling flow.

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