Validation of the Sysmex sp-1000i automated slide preparer-stainer in a clinical laboratory

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Background: The speed and quality of information have become essential items in the release of laboratory reports. The Sysmex®SP1000-I device has been developed to prepare and stain smear slides. However, for a device to be cleared for use in the laboratory routine it must pass through a validation process.

Objective: To evaluate the performance and reliability of the Sysmex® SP-1000i slide preparer-stainer incorporated into the routine of a hospital laboratory in Porto Alegre.

Methods: Peripheral blood samples of patients attending the laboratory for ambulatory exams with leukocyte counts between 7000/μL and 12,000/μL were evaluated, independent of gender and age. Two slides were prepared for each sample using the Sysmex® SP-1000i equipment; one of the slides was used to perform quality control tests using the CellaVision® DM96 device, and the other slide was used to compare pre-classification by the same device and the classification performed by a pharmacist-biochemist.

Results: The results of all the slides used as controls were acceptable according to the quality control test as established by the manufacturer of the device. In the comparison between the automated pre-classification and the classification made by the professional, there was an acceptable variation in the differential counts of leukocytes for 90% of the analyzed slides. Pearson correlation coefficient showed a strong correlation for band neutrophils (r = 0.802; p-value < 0.001), segmented neutrophils (r = 0.963; p-value < 0.001), eosinophils (r = 0.958; p-value < 0.001), lymphocytes (r = 0.985; p-value < 0.001) and atypical lymphocytes (r = 0.866; p-value < 0.001) using both methods. The red blood cell analysis was adequate for all slides analyzed by the equipment and by the professional.

Conclusion: The new Sysmex®SP1000-i methodology was found to be reliable, fast and safe for the routines of medium and large laboratories, improving the quality of microscopic analysis in complete blood counts.

Keywords: Blood cell count/methods; Diagnostic equipment; Flow cytometry; Validation studies; Automation, laboratory

Introduction

Nowadays, the speed and quality of information have become essential items in the release of laboratory reports and without the advancement of technology, clinical laboratories could not perform a large volume of tests and provide reliable results in a useful time[1-3]. In recent years, automated blood cell counters have undergone major upgrades with the introduction of new methodological principles with progressive evolution of cell analysis and progressive evolution of software[4,5].

Among the numerous advantages of using automated equipment are the reduce time to release results, high sensitivity, greater accuracy with reduced coefficient of variation, better reproducibility and higher productivity in laboratory testing[2,6]. However, for a device to be cleared for use in the laboratory routine it must pass through a validation process. Equipment validation in the clinical laboratory is of great importance in establishing reliable and documented evidence that the process was carried out in accordance with pre-determined specifications and necessary quality requirements[6,7].

The complete blood count (CBC) is the most used tool in the diagnosis of hematologic diseases, and also serves as a complement exam in the diagnosis of other diseases; it is thus one of the most requested tests in clinical investigations[3,8]. Conventional microscopic evaluations of blood cells is of great importance, since the observation of specific cellular changes may be clinically relevant, contributing to the patient's diagnosis[2,9,10]. However morphological and differential analysis of blood cells is subjective and depends on factors such as the experience of the observer and the quality of the blood smear and staining[3,8-10].

The Sysmex® SP-1000i and CellaVision® DM-96 devices developed by Sysmex Corporation® and CellaVision AB, respectively are intended to minimize this subjectivity, increasing the quality and accuracy in hematological analysis.

The Sysmex® SP-1000i device is an automated slide preparer-stainer that can be used to prepare standard slides for reading in the CellaVision® DM-96 with uniform and high quality blood smears, regardless of the hematocrit value of the patient[11]. These systems aim to improve the technical efficiency and consistency of results, increasing the speed, quality, specificity and sensitivity of hematological analysis and contributing to early and accurate diagnoses of diseases.

Some studies have shown that the automated CellaVision® DM96 digital system is an efficient tool to perform leukocyte count and differential[8,12,13], a morphological analysis of erythrocytes[14-16] and platelet counts[17] providing high-definition images of blood cells. To perform the microscopic analysis of slides with this equipment it is necessary that the blood smears present with a gradual transition in thickness, with square or straight edges, showing no ripples, bubbles or spaces; these requirements can only be achieved by automated blood slide preparation[8,18,19].

The objective of this study is to evaluate the performance and reliability of the Sysmex® SP-1000i device incorporated into the routine of a hospital laboratory in the city of Porto Alegre.

Methods

Samples

Eighty peripheral blood samples, with leukocyte counts between 7000/μL and 12,000/μL, from patients of both genders and independent of age were submitted for hematologic evaluation at the Central Laboratory of the Análises clínicas da Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Rio Grande do Sul, from October to November 2011. The samples were collected and stored in 4-mL tubes with ethylenediaminetetraacetic acid (EDTA)-K3 (Vacuette ®, Greiner bio-one).

This study was approved by the Research Ethics Committee of ISCMPA (no. 008939/2011).

Preparation of Blood Smears

The Sysmex® SP-1000i equipment prepares blood smears according to the hematocrit value of each sample, adjusting the level of the blood smear by the angle of inclination and the speed of the spreader slide (Table 1)[11]. Ten samples for each level of blood smear performed by the equipment were selected.

For each patient two slides were prepared by the Sysmex® SP-1000i device, using May-Grunwald-Giemsa stain. The first slide was designed to control the quality, using the CellaVision® DM96 equipment to evaluate the quality of staining and the percentage of artifacts in the blood smear through a cell localization test. This test checks the ability of the equipment to focus and distinguish leukocytes, erythrocytes and artifacts in blood smears; the quality control test is only considered adequate when cell localization is greater than 97% and artifacts are less than 30%.

The second slide was used to perform the differential analysis and qualitative assessments of the white and red blood cells (RBC) and platelets in the CellaVision® DM96 device. The pre-classification test by the equipment was evaluated by a pharmacists-biochemist and in cases of disagreement between the results, the cells were re-classified.

Statistical Analysis

The sample size calculation was performed using the program OpenEpi, version 2, considering an error of 5% and a power of 80%. Statistical analysis was performed with the aid of the Statistical Package for Social Sciences (SPSS), version 15.0, and GraphPad Prism 5. Numerical variables are described as means and standard deviations and categorical variables are described as proportions. Correlations were made by the Pearson correlation test and for all comparisons a level of 5% (p-value ≤ 0.05) was considered statistically significant.

The comparative analysis between the assessments made by the CellaVision® DM96 and the classification by the professional was made using the Variability Statistics Table for manual leukocyte counts[20,21]. The cell count performed by the professional was used as the reference in the comparisons.

Results

The quality of the slides produced by the Sysmex® SP-1000i device was assessed by the CellaVision® DM 96 device taking into account the quality of the staining, the cell localization test and the percentage of artifacts in the blood smear for each hematocrit level (Table 2). All slides evaluated had a quality considered appropriate, following the technical specifications for quality of the CellaVision® DM96 equipment.

The pre-classification of the leukocyte count obtained by the CellaVision® DM96 device was compared with the professional classification after agreement or the re-classification of the leukocytes. This demonstrated that the variation was acceptable in 90% of the slides. One or more leukocytes were incorrectly classified for only eight (10%) slides, with the band neutrophil being the most common wrongly classified cell (Table 3).

Qualitative analysis of the RBC proved to be adequate for the integrity of cells, uniform distribution on the slide, morphology and staining for all of the slides produced by the Sysmex® SP-1000i device. The RBC analysis provided by CellaVision® DM96 was not used for comparison, leaving the qualitative evaluation of the RBC as well as qualitative changes in leukocytes to the professional. It was also possible to perform an estimation of the platelet count and to assess the presence of platelet aggregates and fibrin.

A good correlation was obtained between the automated pre-classification and the professional classification for segmented neutrophils, eosinophils and lymphocytes (Table 4). The correlation coefficient (r2) between the two evaluations was determined for the leukocyte count (Figure 1). The

Table 1 - Settings for blood smear performed by the Sysmex® SP-1000i equipment calculated from the hematocrit of the patient

	Hematocrit (%)	Angle (°)	Speed (mm/s)
Level 1	< 20	31	180
Level 2	20-25	30	175
Level 3	25-30	29	165
Level 4	30-35	29	160
Level 5	35-40	29	155
Level 6	40-45	29	150
Level 7	45-50	29	120
Level 8	> 50	29	100

Table 2 - Evaluation of the quality of slides produced by the Sysmex® SP-1000i, using quality control by the CellaVision® DM96 for each hematocrit level

	n	Hematocrit (%)	Leukocyte count (x10³/µL)	Cell Localization Test (%)	Artifacts (%)
Level 1	10	16.68 ± 1.87	9.9 ± 1.3	99.85 ± 0.24	15.24 ± 5.53
Level 2	10	22.96 ± 1.13	10.1 ± 2.1	100	15.72 ± 11.42
Level 3	10	26.96 ± 1.39	9.4 ± 1.5	99.85 ± 0.24	6 ± 3.68
Level 4	10	32.49 ± 1.38	9.6 ± 1.8	99.81 ± 0.32	13.25 ± 11.21
Level 5	10	37.2 ± 1.47	8.7 ± 1.5	99.9 ± 0.21	8.35 ± 4.81
Level 6	10	41.16 ± 0.78	9.6 ± 1.1	100	10.67 ± 6.15
Level 7	10	46.44 ± 1.12	9.1 ± 1.4	100	15.64 ± 13.62
Level 8	10	52.27 ± 1.95	9.5 ± 1.5	100	14.2 ± 7.75

Table 3 - Number of slides and leukocytes improperly classified by the CellaVision® DM96 using slides prepared by the Sysmex® SP-1000i

Hematocrit level	Samples (n)	Inadequate slides (n)	Inadequate leukocytes		
			Band neutrophils	Segmented neutrophils	Monocytes
Level 1	10	0	0	0	0
Level 2	10	1	1	0	0
Level 3	10	1	1	0	1
Level 4	10	2	2	1	0
Level 5	10	1	1	0	0
Level 6	10	1	1	0	0
Level 7	10	0	0	0	0
Level 8	10	2	2	1	1
Total	80	8 (10.0%)	8 (10.0%)	2 (2.5%)	2 (2.5%)

Table 4 - Correlation for the pre-classification of cells performed by the CellaVision® DM96 and the classification by the professional.

Leukocytes	Correlation (r)	p-value
Band neutrophils	0.802	< 0.001
Segmented neutrophils	0.963	< 0.001
Eosinophils	0.958	< 0.001
Basophils	0.568	< 0.001
Lymphocytes	0.985	< 0.001
Monocytes	0.791	< 0.001
Myelocytes	0.465	< 0.001
Metamyelocytes	0.456	< 0.001
Plasmocytes	0.373	< 0.001
Atypical Lymphocytes	0.866	< 0.001
Erythroblasts	0.708	< 0.001

metamyelocytes, myelocytes and promyelocytes were grouped for analysis because of the low frequency in the sample.

Discussion

The task to change or introduce new technologies into the clinical laboratory is the responsibility of the professionals working in the technical department along with the managers in order to ensure high standards in the new service being provided. Even if a method or device has already been tested and validated by the manufacturer, it is important that its performance is verified in the laboratory to check whether the new equipment works as expected thereby providing adequate results[21].

The present study demonstrates the validation process for the implementation of the Sysmex® SP-1000i equipment in the laboratory routine, where the quality of blood smears and slide staining were evaluated using the CellaVision® DM96 equipment which had previously been validated in the laboratory routine.

The preparation and staining of the blood smear on a microscope slide are fundamental steps to perform a qualified microscopic examination, however the manual preparation of CBC slides, despite being traditional and satisfying, has certain limitations that can directly influence the differential count and morphological analysis of cells.

The ideal blood smear for a qualified CBC analysis should be uniform, free of bubbles and flaws and with well-stained cells that enable their correct identification and classification. These requirements in the preparation of a blood smear are achieved by automated equipment.

The Sysmex® SP-1000i equipment automates the preparation and staining of slides to standardize the preparation of blood smears using the hematocrit value of the sample, regulating the amount of blood, the velocity and the angle to improve the quality of the smear. In this study the smear and staining of slides prepared in the Sysmex® SP-1000i proved to be of good quality as all of the slides provided acceptable results in the cell localization and artifacts tests when processed for quality control in the CellaVision® DM96 equipment.

The pre-classification of leukocyte differential counts by the CellaVision® DM96 device showed that the cells were correctly
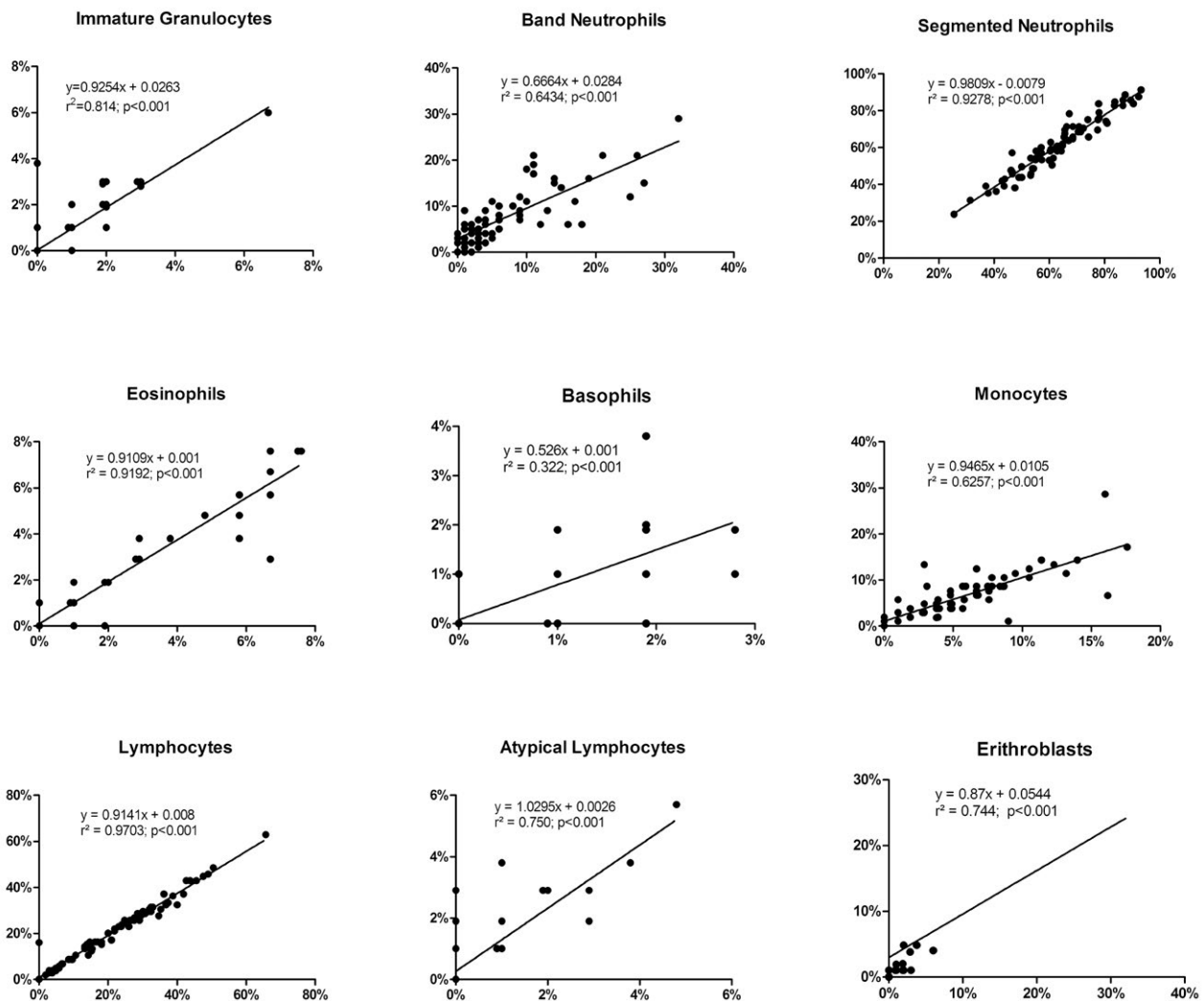
Figure 1 - Linear regression and scatter plots for band neutrophils, segmented neutrophils, eosinophils, monocytes, lymphocytes, atypical lymphocytes, immature granulocytes and erythroblasts

identified in 90% of slides. The low percentage of incorrectly classified slides demonstrates the quality of the slides.

Band neutrophils were the cell class most often pre-classified erroneously by the device. This was also observed by Kratz et al. who reported that the percentage of correct pre-classifications made by this equipment was low for immature and abnormal cells, demonstrating the difficulty of automation to differentiate band neutrophils and segmented neutrophils[8].

All slides were adequate in the evaluation of qualitative changes of white blood cells, RBC and platelets however for samples presenting platelet aggregates, conventional microscopy is essential as the CellaVision® DM96 device selects a fixed area on the slide which cannot be modified by the user.

The correlations between automated pre-classification and the professional's classification were statistically significant, demonstrating the quality of the blood smears.

Our findings demonstrated a good correlation coefficient (r2 > 0.90) between CellaVision® DM96 and the professional's

classifications for segmented neutrophils, eosinophils and lymphocytes as was also observed by Lee et al. for segmented neutrophils and lymphocytes[22]. On the other hand, a weak correlation was observed between the equipment and professional classifications for immature granulocytes, band neutrophils, basophils, monocytes, atypical lymphocytes and erythroblasts. Some studies have also demonstrated a weak correlation between the pre-classification by CellaVision® DM96 and the analysis made by a professional for monocytes[8,14,22], basophils[14,22] and band neutrophils[22], which demonstrates a tendency of this equipment to incorrectly classify these cells in other cell classes.

The differential count of peripheral blood cells is an important diagnostic tool which requires trained personnel and access to an automated microscopy in the hematology laboratory. The use of three technologies: hematology analyzer, automated slide preparer and automated slide analysis can optimize the routine flow, providing rapid and accurate results with excellent cost-benefit.

A limitation of this study was its restricted sample size to validate the equipment, as well as not performing tests on leukopenic and leukocytosis samples.

Conclusion

Choosing new equipment for the laboratory involves the analysis of numerous factors such as the needs of the laboratory, quality control, cost-benefit, ease of handling by professionals, accessible software, as well as the success of the validation process.

The results obtained in this study show the quality of the Sysmex® SP-1000i device in a laboratory; it provides benefits due to its reliability and speed to prepare blood smears thus it is an important ally in the release of quality CBC reports in clinically useful times.

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