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ORIGINAL ARTICLE

Establishing Conditions for Blood Smear Drying and Staining on Sysmex SP-50 for Leukocyte Differential Count

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SUMMARY

Background: **The purpose of this study was to determine the staining conditions and appropriate fan1 start time (FAN1ST) for Sysmex SP-50 to produce blood smears (BS) that reflect the true lymphocyte morphology of patient samples.**
Methods: **Using different start times of fan1, we obtained a set of 84 blood smear slides from 21 blood samples and measured 10,920 lymphocyte areas, which were then converted to compare lymphocyte sizes. We also performed a leukocyte differential count using Sysmex DI-60 on 202 blood smear slides prepared before and after the change in staining conditions and compared the results.**
Results: **The mean lymphocyte sizes at FAN1ST 0 second, 5 seconds, 10 seconds, and 30 seconds were 12.55 µm, 12.14 µm, 11.27 µm, and 10.50 µm, respectively. The mean differences in the pre-classification of neutrophils, lymphocytes, monocytes, eosinophils, and basophils in DI-60, according to the SP-50 staining conditions, were 0.88, -1.58, -0.24, 0.37, and 0.07, respectively.**
Conclusions: **Wright-Giemsa staining of blood smears prepared on the SP-50 showed that changing the pH of the concentrated phosphate buffer to 6.6 and adjusting the staining time did not affect the results of the leukocyte differential count. However, since fan1 was used to dry the blood smear on the SP-50 and the lymphocyte size gradually decreased as the start time was delayed, it was necessary to set a start time for fan1 that did not affect the lymphocyte size.**
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Supplementary Data

Table S1. Frequency of lymphocytes in each group according to FAN1ST settings.

Group	FAN1ST	n	Mean	SD	95% CI of the mean	Min	Max
< 11 µm	0 second	21	25.76	11.05	20.73 - 30.79	3	53
	5 seconds	21	37.38	13.47	31.25 - 43.51	4	54
	10 seconds	21	59.57	18.75	51.04 - 68.11	30	92
	30 seconds	21	84.86	18.71	76.34 - 93.38	43	116
11 - 16 µm	0 second	21	102.05	9.80	97.59 - 106.51	77	116
	5 seconds	21	91.86	13.29	85.81 - 97.91	75	124
	10 seconds	21	70.10	18.58	61.64 - 78.55	38	100
	30 seconds	21	45.10	18.60	36.63 - 53.56	14	86
> 16 µm	0 second	21	2.19	2.77	0.93 - 3.45	0	11
	5 seconds	21	0.76	1.04	0.29 - 1.24	0	4
	10 seconds	21	0.33	0.58	0.07 - 0.6	0	2
	30 seconds	21	0.05	0.22	-0.05 - 0.15	0	1

Abbreviations: FAN1ST - fan1 start time, SD - standard deviation, CI - confidence interval.

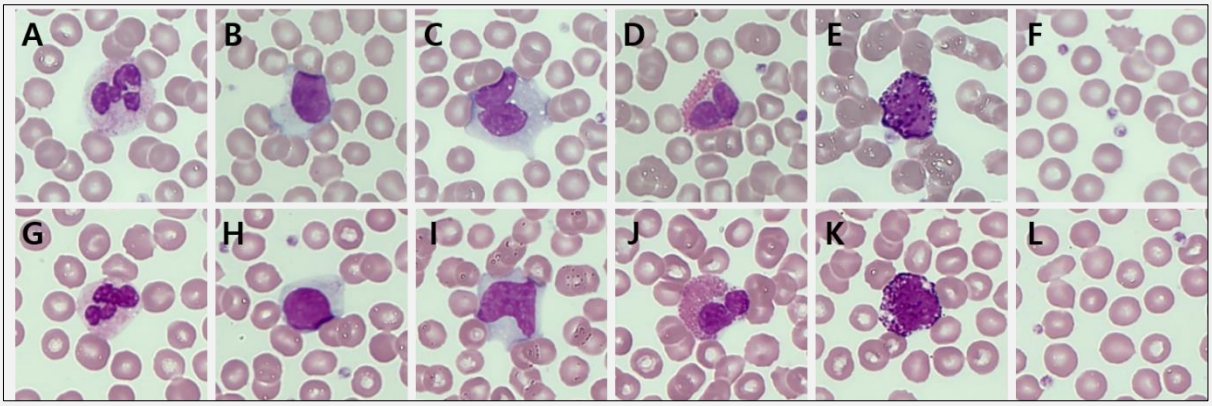

Figure S1. Comparison of blood cells stained with two staining conditions on Sysmex SP-50 (A to F: ISC, G to L: LCSC).

Compared to A to F, G to L were relatively darker stained and appeared to have brighter erythrocytes. A, G - neutrophil, B, H - lymphocyte, C, I - monocyte, D, J - eosinophil, E, K - basophil, F, L - platelets (Wright-Giemsa stain, x 400).
Abbreviation: ISC - initial staining conditions, LCSC - laboratory-customized staining conditions.