Improved Performance of the Automated Slide Preparation Unit, Sysmex SP-100

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Morphologic evaluation of blood films remains the cornerstone for most hematologic malignancies. In our hands, the quality of slides generated by the newly installed Sysmex automated slide preparation unit SP-100 was less than that of manually prepared and stained slides using the default settings on installation. Artifacts in white blood cell morphology, and an increase of damaged cells hampered correct interpretation. Moreover, morphology on the second slide from the duplicate slide option was consistently poorer than the first. To improve on this, a series of studies was performed. It appeared that adaptation of the angle and speed of the spreader glass adjusted for the hematocrit (Hct) very much improved the quality of the cells. The most important improvement, however, was caused by a prolongation of the pre-fix time period to 60 seconds. The default settings for smear angles (°) related to the Hct (<25, 25-35, 35-45, 45-55 and >55) were changed to smaller cohorts (<30, 30-35, 35-40, 40-45 and >45) according to the prevalence of Hct levels in our laboratory, thus enabling easier adaptations at smaller Hct variations. In contrast, variations in the temperature of the May-Grünwald stain, variations in fixation by replacing May-Grünwald for Methanol, variations in the type of slides used or prolongation of the storage time of blood samples (up to 3 hours) did not have any influence on the quality of the blood films.

When CLL patients were tested-known for the high percentage of smudge cells-it appeared, however, that the percentage of smudge cells was always much higher in the SP-100 generated smears than in the manually prepared smears.

Following these adaptations, the option 'duplicate smears' was re-tested and compared with singlely prepared slides. No differences were then found between the differentials and the quality of cellular morphology.

In conclusion, systematically performed adaptations using patients' material can largely improve the quality of SP-100 generated blood films.

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Key Words WBC Differential, SP-100, Automated Slide Preparation Unit

INTRODUCTION

The Sysmex automated slide preparation unit SP-100 was introduced in 1996. This module generates stained blood smears from EDTA anti-coagulated whole blood samples. To achieve this, blood from the specimen container is aspirated by cap-piercing, a small volume is dispensed on to an unstained slide after which a spreader glass prepares the smear using varying angles and speeds adjusted to the hematocrit of the blood sample. Sample information is printed on the slide after which it is dried and placed into a single cassette. In the cassette staining solutions are dispensed and drained after a preset time period. After rinsing, the blood film is dried and ready for analysis. The time and duration of the fixation and staining procedures can be adapted to the requirements of the individual laboratory. The option of duplicate slide preparation is present.

The quality of such an automatically prepared and stained blood smear depends on many factors. Moreover, requirements regarding this quality level differ amongst laboratories. Only the best quality is acceptable for laboratories responsible for patients with hematological diseases but what constitutes 'best quality' varies from laboratory to laboratory. For this reason the setting on the SP-100 are user selectable.

The Central Clinical Hematology Laboratory of the Leiden University Medical Center obtained the SP-100 as part of the Sysmex HST-430 system (Hematalogy Sample Transportation; HST). In spite of several adaptations, the technicians felt that the quality of the blood smears prepared by the SP-100 was worse than those prepared and stained manually. Blood smears generated by the SP-100 frequently showed artifacts in white cell morphology, contained too many damaged cells and repeatedly showed abnormalities in the form (polygonal instead of round) and outline (ruffled membranes) of the white blood cells. Aiming at improvement of the SP-100 derived quality, a series of adaptations were tested which will be described below.

MATERIALS AND METHODS

Blood samples

Routinely processed EDTA-anticoagulated whole blood

samples were used for all experiments. All samples were selected according to the hematocrit or ESR levels, or the specific diagnosis of the patient.

SP-100

The Sysmex (Sysmex Corporation, Kobe, Japan) SP-100 automated slide preparation unit is part of the HST 430, which was installed in the Leiden Central Clinical Hematology Laboratory in 1999. During the experiments, a new version with improved slide shift was installed in May 2000. For staining, the double stain method was used, consisting of May-Grünwald stain followed by Giemsa stain. System specifications and detergents/rinse solutions are specified in the Sysmex SP-100 Operator's Manual, Revised Version August 1996.

Manual blood film preparation

EDTA anti-coagulated blood was carefully mixed for at least 2 minutes and a small drop placed on a glass slide. A spreader glass placed at an angle of 30-45° in front of the drop was then drawn back into it. Next, the spreader gently advances resulting in a thin film over the slide. The smear was considered satisfactory if the end (tail) of the smear was straight; two-third of the glass slide was covered; Newton's rings should be visible at the tail of the slide; ridges were absent; the film had to be of appropriate thickness with a rather light color [1,2].

Staining

Manually prepared and SP-100 prepared slides were stained in the Shandon Varistain 4 automated staining unit (Life Sciences International (Benelux) BV, Veldhoven, The Netherlands). All slides were dried for at least 10 minutes, fixed for 3 minutes in Methanol, stained for 3 minutes in May-Grünwald and for 17 minutes in Giemsa and rinsed for 4 minutes in H_2O unless stated otherwise.

Microscope analysis of blood smears

For visual differentials, a microscope (Carl Zeiss BV, Axioscope 20, Weesp, The Netherlands) was used equipped with a 16 ×, 40 × (Plan Neofluar) and 100 × (Achroplan) objective. Two technicians (JWB and GJ) performed all tests and analyzed all blood smears. Two hundred or 400 cells were analyzed (2 × 100 or 2 × 200 in duplicate). If the duplicate result showed more than 10% difference, a third analysis was done.

RESULTS AND DISCUSSION

Performance of various SP-100 units in The Netherlands

Routinely prepared slides by the SP-100 from 60 different patients were collected from 6 laboratories in The Netherlands, including the CKHL. The smears were analyzed for white cell morphology and artifacts. Data are presented in ***Table 1***. It appeared that the mean percentage of damaged cells varied between 4.1% and 7.8%; the median percentages of damaged cells ranged between 2% and 7%. Damage consisted of severe abnormalities in the shape of the cells almost approaching the smudge cell appearance. The non-damaged cells, however, were in the large majority considered to be abnormal as well. The shape of the cells was changed (polygonal or further malformed instead of round). Normal lymphocytes could frequently not be discerned from abnormal ones, because of changes in the nuclear morphology (finer dispersed chromatin structure) with cytoplasmic membranes showing villi. These abnormalities are outlined in ***Fig. 1*** showing abnormal compared to normal cellular morphology. From the series of laboratories tested, the cellular morphology of the white blood cells from only one laboratory (Lab. B) was considered to be normal with round cells and cytoplasmic membranes without ruffled borders. All other samples showed abnormalities (***Table 1***).

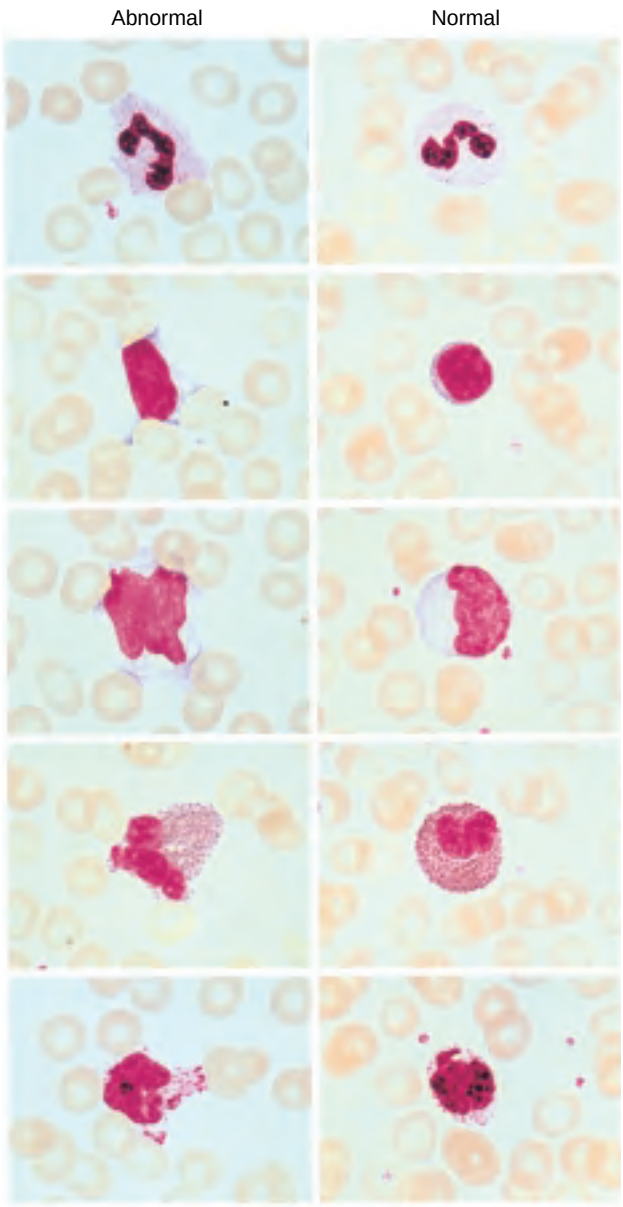

Fig. 1 *Assembly of photographs of WBC showing various artifacts Left panel, abnormal WBC with SP-100 artifacts; right panel normal morphology obtained after a series of adaptations (see text). From up to down: granulocyte; lymphocyte; monocyte; eosinophil, basophil.*

Table 1 *Performance of 6 different SP-100 Units*
% damaged WBC from 60 unselected different patients, in comparison with manually prepared slides in 2000 and 1996.

	B	C	D	E	F	LUMC SP100*	LUMC** Manual*	LUMC** Manual 1996
% damaged cells per 100 WBC	0	8	8	6	5	2	3	2
	10	10	3	7	2	7	2	0
	6	8	10	2	5	19	11	0
	5	4	14	0	3	4	3	1
	2	7	5	2	1	1	1	2
	26	4	8	19	1	8	4	3
	0	5	2	5	2	10	2	5
	3	2	5	6	2	12	3	12
	1	3	1	5	17	9	3	2
	0	11	2	4	3	6	2	3
% median (range)	2.5 (0-26)	6 (2-11)	5 (1-14)	5 (0-19)	2.3 (1-17)	7.5 (2-12)	3 (1-4)	2 (0-12)
% mean	5.3	6.2	5.8	5.6	4.1	7.8	3.4	3.2
WBC morphology	Normal	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Normal	Normal

* Serial slides from the same 10 samples, paired values
**Manually prepared, and automatically stained in the Shandon Varistain 4 staining unit

Comparison between manually prepared slides, archived slides and the SP-100

From 10 patients, slides were prepared manually, stained in the Shandon Varistain staining unit, and compared with SP-100 derived slides from the same patients. To exclude the possibility that the manually prepared slides during these experiments were made with more care and skill than in the past, 10 manually prepared archived slides from 1996 were analyzed as well. The results are shown in ***Table 1***. In almost all paired combinations, the manually prepared smears yielded better morphology.

Variation in batch of slides

Ten different samples were tested in duplicate using Menzol Superfrost slides (batch 4713089) and Lames/Sysmex slides. No difference in the percentage of damaged cells was seen: mean 3.0%, median 3%, range 0-5% in the Superfrost and mean 3.2%, median 3%, range 1-7% in the Lames/Sysmex specimen.

Influence storage time

Ten fresh samples with varying hematocrit levels were tested in duplicate between 09.30 and 14.00 hrs. Every 30 minutes two slides were prepared both in the SP-100 unit and by manual techniques. It appeared that up to 3 hours the quality of the smears remained satisfactory. After 3.5 hours, EDTA-damage became visible. In all experiments, the SP-100 prepared slides again yielded more damaged cells with suboptimal membrane outlines than did the manually prepared slides (data not shown).

Variation in the angle and speed of the smear preparations

Five samples with Hct values between 0.39 to 0.41 were subjected to various adaptations in the way the spreader glass was used to prepare the blood film on the slide. First, the spreader glass angle was altered to various settings between 20° to 50°. As far as cellular morphology was concerned, an angle of 30° yielded the best results (***Table 2***). Next, at an angle of 30°, the speed was adjusted between 2,500 and 5,000 pulses per second (pps). Although all smears were of poorer quality than in the previous experiment, a speed of 4,000 (pps) yielded the best results (***Table 3***). At low speed, the smear showed ridges. At high speed, the length of the smear decreased and thus became too thick, unless the amount of blood deposited on the slide was increased.

Adaptation of hematocrit (Hct) cohorts for smear levels

The default settings of the SP-100 for five different smear angles related to the hematocrit levels cover a wide range: <25%, 25-35%, 35-45%, 45-55% and >55%. We analyzed the prevalence of Hct levels in our laboratory during two days and observed that 90% of all Hct values covered two cohorts only, namely between 25-35% and 35-45%. Only 6% of samples reached a level below 25%, 4% reached a level above 45%, and no samples were seen above 55%. To obtain a better distribution, we adapted the Hct cohorts accordingly: <30% (covering 24% of all samples), 30-35% (28% of the samples), 35-40% (28% of the samples), 40-45% (16% of the samples) and >45% (4% of the samples). With this change, more

detailed adaptations could be made in the sample volume, angle and speed at smaller variations in the Hct.

Prolongation of pre-fix period

The staining procedure of the SP-100 differs from the automated staining performed in the Shandon Varistain unit. Especially, the fixation procedure is much shorter in the SP-100, which might have been responsible for the poor morphology of the white blood cells. To prove this, we tested the influence of different pre-fix periods using the previously defined optimal angle (30°) and speed (4000 pps) settings. Changes in the pre-fix period appeared very important: prolongation to 60 seconds resulted in slides of an almost perfect morphology of white blood cells (***Table 4***, ***Fig. 1***). Immature white blood cells (metamyelocytes and myelocytes) and monocytes, especially, benefited from longer prefixation.

Variation in fixation techniques

We changed the temperature of the May-Grünwald solution from room temperature to 4 °C and performed duplicate slides from 10 different samples at both temperatures using the optimal angle, speed and prefix period. Under these conditions, no differences were seen in the white blood cell morphology. Similarly, replacing May-Grünwald by Methanol (as is the standard fixative in the Shandon Varistain 4) did not improve the results further when visualized by phase contrast microscopy (data not shown).

CLL patients with smudge cells

After having optimized the performance of the SP-100, we analyzed a group of patients known to have very high

Table 2 *Changes in the angle of the spreader glass*

Sample	Hct	Speed (pps)	Level	Angle (°)				Manually prepared smear
				20	30	40	50	
1	0.392	4000	3	no data	3	10	8	1
2	0.393	4000	3	1	0	0	2	2
3	0.394	4000	3	1	2	3	1	2
4	0.411	4000	3	1	0	4	0	0
5	0.396	4000	3	4	0	4	2	5
Mean % damaged cells				1.75	1.0	4.2	2.6	2.0

Table 3 *Changes in the speed of the spreader glass*

Sample	Angle(°)	Speed adjustment						Manually prepared smear
		2500 (pps)	3000 (pps)	3500 (pps)	4000 (pps)	4500 (pps)	5000 (pps)	
1	30	16	4	5	7	7	3	5
2	30	7	2	2	2	8	5	4
3	30	4	3	6	4	2	1	4
4	30	2	1	12	6	3	4	4
5	30	3	3	4	5	4	5	4
Mean % damaged cells		6.4	2.6	5.8	4.8	4.8	3.6	4.2
Additional comments		Uneven smear	Uneven smear	Sub-optimal	Best	Sub-optimal	Smear too thick	

Table 4 *Variation in pre-fix period on cellular morphology compared to methanol fixation*

Sample	Pre-fix 15 seconds	Pre-fix 30 seconds	Pre-fix 60 seconds	Methanol 60 seconds
1	80	–	76	–
2	84	–	95	–
3	61	–	97	–
4	80	–	97	–
5	76	–	90	–
6	–	87	–	97
7	–	90	–	98
8	–	93	–	95
9	–	95	–	97
10*	–	76	–	92
Mean % cells with normal round cellular borders	76	88	91	97

* sample with high % monocytes

percentages of smudge cells, namely patients with classical chronic lymphocytic leukemia (CLL). It appeared that in all 10 patients tested, the percentage of smudge cells (Gumprechtse Schollen) was consistently higher in blood smears generated by the SP-100 than in the manually prepared smears. The difference could be very large, up to a three-fold increase in smudge cells (***Table 5***).

One or two slides?

The SP-100 offers the possibility to prepare automatically more than one slide from a single blood sample. The aspirated sample is dispensed on the first slide, which is subsequently subjected to the printing, drying, loading and staining operation flow. Any delay after the deposit of the first drop causes a delay in the deposit of the next drop on the next slide. The delay between deposits will be at least 30 seconds. After adaptations in the pre-fix time (see before), the delay increased further even up to 60 seconds. During the delay, the blood remains in the aspiration tube. We expected that this would result in sedimentation of red and white cells causing a change in the distribution of the white cells. It is known that such a delay is important if multiple slides are prepared manually from a single pipette. In such cases, care is taken to displace the blood drops on a series of slides within seconds.

Initially, we observed that the quality of the second slide was consistently poorer than the first, not only microscopically, but also macroscopically (***Fig. 2***). After prolongation of the pre-fix time with adaptation of angle and speed of the smear preparation, the quality of the first and second slide did not differ anymore macroscopically. To further test this, we compared 30 different samples: 10 from normal controls, 10 from patients with abnormalities (an elevated ESR, elevated WBC, left shift, elevated % lymphocytes), and 10 from patients with hematological diseases. In all paired experiments, duplicate slides (prepared from one aspirated sample, series D1 and D2) were compared with two single, separately aspirated slides (series S1 and S2). The duplicate series performed as well as both singlely prepared slides (***Fig. 3***) with excellent correlation coefficients as far as the morphology and 3-differentials were concerned (R = 0.99 for % neutrophils and lymphocytes; R = 0.95 and 0.96 for % monocytes). An explanation for this could be found in the method of sample storage between slide preparations: the blood remaining in the aspiration tube being horizontal not *vertical*. The series tested is, however, small given the large variation in patients and diseases and therefore categorical assurance that the quality of duplicate slides is equal under all circumstances can never be given. From a theoretical point, it might be better to aim

Table 5 *Percentage (per 100 WBC) smudge cells from CLL patients in manually prepared blood smears and SP-100 generated smears.*

CLL Patient	WBC×10^9/L	Lymph (%)	Smudge cells (%)	
			Manual	SP-100
1	91.9	98	41	72
2	21.0	47	29	30
3	13.5	9	67	125
4	92.1	93	336	441
5	14.4	60	10	12
6	25.4	80	61	127
7	11.9	64	18	29
8	10.0	41	39	100
9	9.2	97	51	163
10	16.8	27	124	185

Fig. 2 *Sequentially prepared duplicate smears before angle and speed adaptation: large differences can be seen between the length and outline contours of the smears.*

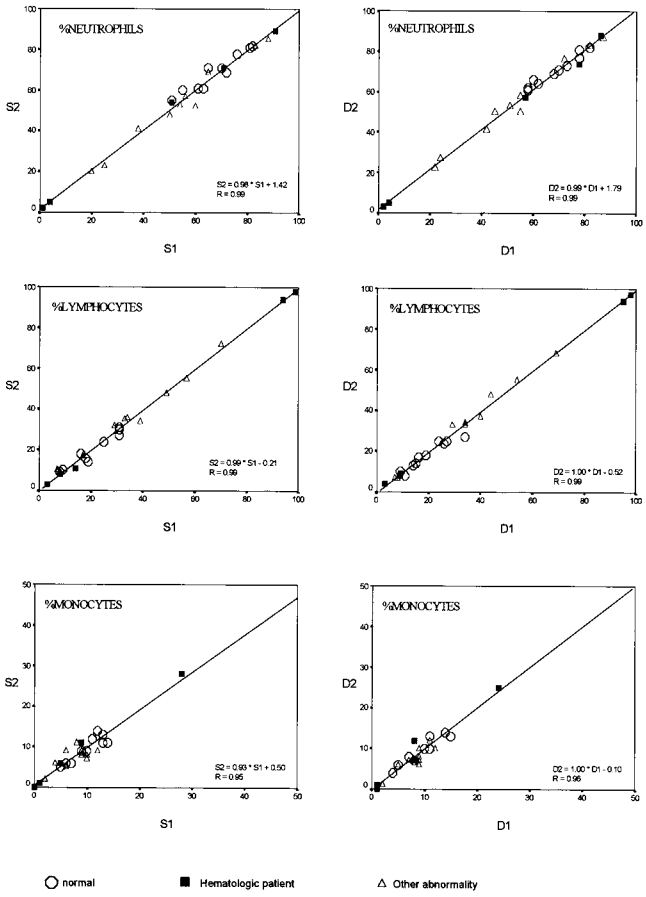

Fig. 3 *Quality control differentials (% neutrophils, lymphocytes and monocytes) analyzed on duplicate smears (D1 vs D2, right panel) compared to two singlely prepared smears (S1 vs S2, left panel). Correlation coefficients are given in the figures.*

at a procedure where two slides are generated by a separate double aspiration procedure. Evidently, this will need some software adaptation.

CONCLUSION

The SP-100 performed sub-optimally in our hands, as well as in most other laboratories in The Netherlands with the default settings following installation. The most important shortcomings dealt with abnormalities in white blood cell morphology. These settings are, however, user selectable and a systematic study has demonstrated that the quality of the blood smears can be very much improved. However, in our hands, certain categories such as CLL always resulted in less satisfactory lymphocyte morphology in automatically prepared blood films. The following adaptations appeared to have been the most important for major improvements of the SP-100:

1. Prolongation of the pre-fix time period to 60 seconds.
2. Adaptations of the angle and speed of the spreader glass adjusted for each hematocrit.
3. Change in the five Hct cohorts used for smear levels aiming at smaller cohorts

Factors that were *not* important consisted of:

1. Variations in the temperature of the May-Grünwald stain.
2. Variations in the fixation by replacing May-Grünwald stain by Methanol.
3. Variations in the slides used.
4. Prolongation of the storage time of blood samples up to 3 hours.

It is clear that the flexibility and user selectability of the instrument settings can assist in optimizing the desired smear appearances for the individual laboratory.

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