Optimisation of the Flagging Criteria on the Sysmex SE-9500

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We used a total number of 702 blood samples to evaluate the automated analyser's differential of the SE-9500 with the results of the microscopic examination of peripheral blood smears. These results were than compared to results obtained after an adjustment of the Q-flag trigger levels. This significantly reduced the number of false positive samples by 37% and the microscopic workload by 14%. At the same time the specificity of flags was also improved.

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Key Words Automated Hematology Analyzer, SE-9500, Q-flag, Complete Blood Count (CBC)

INTRODUCTION

With the introduction of the Sysmex SE-9500 as the routine complete blood cell (CBC) CBC analyser in our laboratory and using the Q-flag default settings recommended by the manufacturer, we recognised a higher rate of specimens flagged "positive: Morph. Suspect" than with our previous analyser (Abbott Cell-Dyn 3500).
The SE-9500 allows the user to alter and optimise the Q-flag trigger levels which control the flag display, thus leading to elimination of unnecessary microscopic examination of morphologically normal blood films.
The present study was designed to reduce the number of false positive (FP) flags without degrading true positive (TP) detection nor increase false negative (FN) detection by the simple expedient of adjusting the Q-flag numerical limits on the analyser. This resulted in a 37 % reduction in flagging for white blood cells (WBC) differentials.

MATERIALS AND METHODS

Seven hundred and two (702) EDTA-anticoagulated blood specimens were analysed on the Sysmex SE-9500. In this analyser the morphology flagging triggers are expressed as Q-flag values (arbitrary unit scale 0 to 300, manufacturer default setting 100 for each parameter).
Microscopic differentiation of the May-Grünwald-Giemsa stained blood smears, based on examination of 200 WBC, was used as the reference. A microscope WBC differential count was considered abnormal if it met one or more of the following criteria:

Erythroblasts	≥ 1%
Blasts	≥ 1%
Promyelocytes	≥ 1%
Myelocytes	≥ 1%
Metamyelocytes	≥ 1%
Band neutrophils	≥ 6%
Atypical lymphocytes	≥ 1%

True Positive (TP) samples were flagged by the analyser and showed a microscopic abnormality. True Negative (TN) samples were not flagged by the analyser and showed no microscopic abnormality.
False Positive (FP) samples were flagged by the analyser but did not show microscopic abnormality. False Negative (FN) samples were not flagged by the analyser but did reveal a microscopic abnormality. The analyser, therefore, did not highlight the latter.

RESULTS

Modification of the morphology flagging numerical criteria

Four hundred and eighty one (481) morphologically flagged samples (Morph. Suspect) were studied retrospectively. All analytical data and the numerical default values for all Q-flags were entered into a spreadsheet. In addition a score 1 or 0, representing the presence or absence of each microscopic abnormality was included based on the criteria described above.
The Q-flag values for each flag were ranked and compared to the scores for microscopic abnormality. The highest Q-flag numerical level, which at the same time

did not create additional false negative flags, was selected as the new flagging trigger level. The modified values were then entered into the analyser according to the following table (***Table 1***).
The following numerical trigger levels for microscopic review were also verified by the same procedure:

White Blood Cell count	$< 2 \times 10^9$/L
Neutrophil count	$< 1.0 \times 10^9$/L
Platelet count	$< 25 \times 10^9$/L
Neutrophils	< 10% or > 95%
Lymphocytes	< 5% or > 70%
Monocytes	> 20%
Eosinophils	> 20%
Basophils	> 3%

All these triggers were left unchanged since all numerically abnormal samples were already flagged for morphological abnormalities.
Prior to adjustment of the Q-flag values, 294 samples out of the 481 were True Positive (TP) and 187 samples False Positive (FP), i.e. 61% and 39%, respectively.
Following adjustment of the Q-flag values, the number of microscopic WBC differentials was reduced by 69 (all with normal morphology). Therefore adjustment of the flagging threshold values resulted in a reduction in the number of false positive samples by 37%.

Verification of the modified flagging criteria

Two hundred and twenty one (221) specimens with potential morphological abnormalities (originating from hematology, nephrology, and pediatric wards) were analysed in a prospective study to determine the impact of modification of the threshold values on the reliability of the flagging and thus evaluate the occurrence of false negative (FN) results.

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100\ (\%)$$

$$\text{Spcificity} = \frac{TN}{TN + FP} \times 100\ (\%)$$

For each flag we calculated as followed;

Morphological flagging criteria

1. All morphological flags (***Table 2***)
2. Selected morphological flags (***Table 3***)

Numerical flagging criteria

We also calculated the results of the morphological flags for those samples that were triggered in association with numerical flagging as shown in ***Table 4***.
The flagging criterion PLT < 25×10^9/L was only used for non-haematological wards. There were no samples with this flag.
These data show that morphological flagging is less effective for leucopenic samples and needs to be backed up by additional flagging based on numerical criteria.
Overall performance (Morphological and Numerical Flags).

Table 1 *Modifide values*

Morphological Q-Flag values	Default	Modified
Blasts	100	100
Imm.Gran	100	180
Left Shift	100	250
Aty/Abn Ly	100	110
NRBC	100	120
NRBC/PLTCL	100	130
AbnLy/Aged	100	100

Table 2 *All morphological flags*

	TP	TN	FP	FN
All Morphological Flags	24%	68%	6%	2%

Sensitivity: 92%
Specificity: 92%

Table 3 *Selected morphological flags*

	TP	TN	FP	FN
Blasts	5%	88%	5%	2%
Immature Granulocytes	10%	82%	5%	3%
Erythroblasts	0%	93%	1%	6%

For specific morphological flags, sensitivity and specificity are not caluculated because of the low incidence of cases showing these flags.

Table 4 *Numerical flags*

	TP	TN	FP	FN
WBC < 2.0×10^9/L	19%	23%	12%	46%
Granulocytes < 1.0×10^9/L	60%	15%	25%	0%

Table 5 *Overall performance (Morphological and Numerical Flags)*

	TP	TN	FP	FN
Overall performance	27%	63%	9%	1%

Sensitivity: 96%
Specificity: 87%

The two false negative samples (1%) were caused by the low sensitivity of the erythroblasts flag.
The difference between “Overall performance” with 1 % false negatives and “All Morphological Flags” with 2 % false negatives shows the importance of the numerical criteria for microscopic review. The latter were unchanged throughout the study (***Table 5***).

CONCLUSIONS

Adjusting morphology flagging threshold values resulted in a reduction in the false positive flagging of WBC differentials by 37%, cutting the routine microscopy workload in our laboratory by 14%. This represents a half-time technician’s workload. In addition, this modification resulted in an improved specificity of the overall flagging (87% for this data set) and specific flags. The few false negative results were caused by the low sensitivity of the erythroblast flag.
This study demonstrates the value and versatility of the unique Q-flag system in meeting the case-mix requirements of the individual laboratory.