Original Article
Adjustments made to the Q-flags affect the performance of the flags generated by the IMI channel

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Abstract: Introduction: IMI-DC (immature information-direct current) is a useful index for efficient morphologic classification of cells. This study assessed the usefulness of IMI-DC thresholds to retain less than 5% false negative rate on automated cell counters. Methods: In total, 526 blood samples supplemented with EDTA-K2 (1.8 mg/mL) were analyzed on Sysmex XE2100. Then, 5 WBC-specific flags including blasts, immature granulocytes, left shift were verified by manual slide review. Laboman EasyAccess 4.2.44 was used for data analysis and measuring efficiency. False-negatives were counted by standard microscopy following the laboratory's standard operating procedure. Optimized IMI-DC thresholds were assessed by receiver operating characteristic (ROC) curve through maximized YI. The efficiency of the optimized IMI-DC thresholds was verified by 200 additional samples. Results: The 526 samples examined by the Laboman software showed 63.40% positive predictive value (PPV), 89.81% negative predictive value (NPV), 82.13% efficiency, 10.65% false-positives, 7.22% false-negatives and 29.09% review rate. Maximized Youden index (YI) altered the false negative rate to 3.5%, PPV to 64.15%, NPV to 95.24%, efficiency to 87.00%, and false-positive to 9.5%. Finally, review rate was reduced from 29.09% to 26.5%. Discussion: IMI-DC is associated with false negative rate in automated cell counters. A low false negative rate helps improve the accuracy of automated cell counters.

Keywords: Q flag, smear slide review, thresholds, IMI channel, automated cell counters

Introduction

Automated laboratory hematology analyzers are very efficient at performing leukocyte differential count and blood morphologic evaluation, with greater precision and accuracy compared with manual screening of blood smears [1, 2]. When this novel technology was introduced by Wallace Coulter in 1953, the manual screening of blood smears was replaced [3]. Performing manual blood morphology analysis and using automated cell counters yield identical results. However, the latter procedure ensures efficiency and productivity in a medical laboratory.

Furthermore, the International Society for Laboratory Hematology (ISLH) through the International Consensus Group for Hematology Reviews, founded by the hematologist Berend Houwen, published a set of 41 applicable guidelines as criteria for reviewing automated complete blood count and leukocyte differential results obtained with automated hematology analyzers [4]. These guidelines have been applied in different laboratories with various choices according to specific laboratory conditions [5-7], aiming to reduce cost and save time without sacrificing data quality.

In addition, quality control data to identify tests that utilize internal quality control processes to confirm performance is important in evaluating the accuracy of an instrument [8].

IMI-DC is an IMI channel detection control parameter that directs current information of the IMI channel. Direct current methods detect the size of the blood cells by changes in direct-current resistance, and the density of the blood cell by changes in radio-frequency resistance. Both RF and DC monitor immature cell accuracy and precision of IMI detector. The affected flags by IMI-DC are: Left Shift, Blasts, and Immature

Table 1. Abnormal cell criteria for positive smear based on the International Society of Laboratory Hematology recommendations

Abnormal cell types
Blasts ≥1%
Myelocytes/promyelocytes ≥1%
Metamyelocytes >2%

Gran. In our laboratory, review criteria were established for the Sysmex XE2100 (Sysmex, Kobe, Japan) instrument. Based on these parameters, blood smear examination was performed to assess the validity of reported flags. In order to ensure the accuracy of validated results for reported flags, an external control daily was assessed and numeric QC data was monitored. In the process of online quality control monitoring, we found that IMI-DC was below target value. This indicated that sensitivity of immature cell assessment is reduced, with the "Left Shift, Blasts, Immature Gran" not reported. A microscopic slide review may not be performed. As a result, accuracy of verifying "Left Shift, Blasts, Immature Gran" presence and identifying/excluding other pathologic cells, e.g. blasts, in peripheral blood, will be altered.

To our knowledge, no studies have investigated the usefulness of IMI-DC quality control data. The aim of this study was thus to evaluate analytical and diagnostic performance of IMI-DC measurements as well as the usefulness of IG flag reports by Sysmex XE-2100, assessing the effect of manual slide review. IMI-DC thresholds of Sysmex XE-2100 were altered. The accuracy of IMI-DC and usability of "Left Shift, Blasts, Immature Gran" flag reports were studied by comparing with manual review.

Materials and methods

Sysmex XE2100 automated cell counters

Routine hematology samples from inpatient and outpatient populations were analyzed randomly on Sysmex XE2100 at the First Affiliated Hospital of Chongqing Medical University. During a period of three days, 526 blood samples supplemented with ethylenediaminetetraacetic acid (EDTA)-K2 (1.8 mg/mL) were selected and reanalyzed on Sysmex XE2100. All specimens were kept anonymous. Only samples initially reported with one or more WBC-specific suspect flags by microscopic slide review were included. The 3 WBC-specific flags affected by

the channel IMI were: (1) blasts, (2) immature granulocytes, (3) left shift. These flags were generated by patterns in scatter grams, and are typical for certain abnormalities [9].

Manual blood smear and differential WBC counts

Manual blood smear and differential WBC counts using standard microscopy following the laboratory's standard operating procedure, based on the Clinical and Laboratory Standards Institute guidelines [4]. In this process, atypical lymph was excluded. Manual slide review was performed by 2 observers, with consistency certified by comparison [10]. At least 200 nucleated cells were counted by at least 2 observers independently, in a blinded manner. The criteria defining positive smear are summarized in **Table 1**, and based on recommendations by the International Consensus Group for Hematology Review [4, 9]. Laboman EasyAccess 4.2.44 (Sysmex, Kobe, Japan) was then used for data analysis. Positive and negative microscopic review results were considered true positive (TP) and false positive (FP), respectively. Positive microscopic review results with negative automated count results were considered false-negatives (FN). A negative microscopic review result was considered true negative (TN).

IMI-DC threshold reduction

Improving the IMI-DC threshold was used to adjust IMI-DC data that mainly reflect sensitivity of "Left Shift, Blasts, Immature Gran" detection. However, decreasing IMI-DC threshold was carried out by optimization. Optimal settings for flagging thresholds were assessed using the YI. The threshold was selected for an assay with YI maximized.

YI = Sensitivity + (specificity - 1)

Optimization began by reducing the cut-off value of "Left Shift, Blasts, Immature Gran" flags from factory default settings declining by 10 units and calculating the YI for identifying the specific abnormality denoted by the flag [11]. The threshold yielding the highest YI was selected for each flag.

Validation of optimized criteria

Another randomly selected 200 samples (selection criteria were the same as described above) were used to validate the optimized flag

Table 2. False negative rate, false positive rate, PPV, NPV and efficiency of former and optimized criteria in the validation set

Criteria	False negative, %, NO.	False positive, %, NO.	PPV, %	NPV, %	Efficiency, %
Former	7.22, 38/526	10.65, 56/526	63.40	89.81	82.13
Optimized criteria	3.50, 7 (total number = 200)	9.50, 19 (total number = 200)	64.15	95.24	87.00
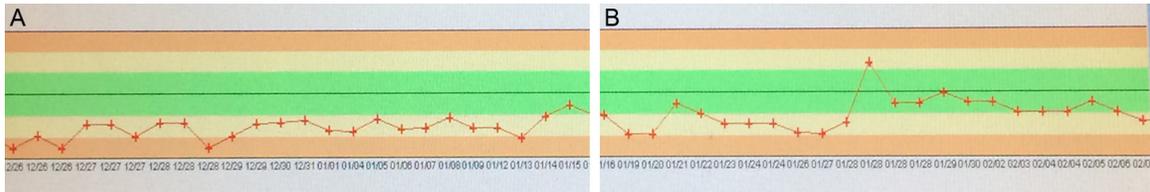
Figure 1. IMI-DC of automated cell counter assessed by quality control online. A. Quality control data before IMI-DC threshold improvement. B. Numeric quality control data of IMI-DC threshold improved by optimization. Horizontal axis, date; vertical axis, IMI-DC data.

threshold derived from the optimization as well as a new IMI-DC setting. Samples were assessed as described above.

Statistical analysis

Optimal settings for flag thresholds were described using the Youden index (YI). The thresholds were selected for an assay with YI maximized. YI was derived by ROC using the SPSS software 17.0.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), microscopic review rate, and efficiency were evaluated by the Laboman software as follows [12]:

Sensitivity (%) = TP/(TP + FN) × 100;

Specificity (%) = TN/(TN + FP) × 100;

PPV (%) = TP/(TP + FP) × 100;

NPV (%) = TN/(TN + FN) × 100;

Review rate (%) = (TP + FP)/(TP + FP+ FN + TN) × 100;

Efficiency (%) = (TP + TN)/(TP + FP + FN + TN) × 100

Results

Performance of 526 samples on Sysmex XE2100

A total of 526 samples were assessed by Sysmex XE2100 by experienced technologists. The results obtained with the Laboman soft-

ware are shown in **Table 2**. PPV, NPV, and efficiency were 63.40%, 89.81%, and 82.13%, respectively. A false-positive rate of 10.65% was obtained; false-negative rate was 7.22%, for a review rate of 29.09%. To reduce the false-negative rate, we analyzed 526 additional samples. There are 13 false negative samples were Myelocytes/Promyelocytes/Metamyelocytes among the 526 samples.
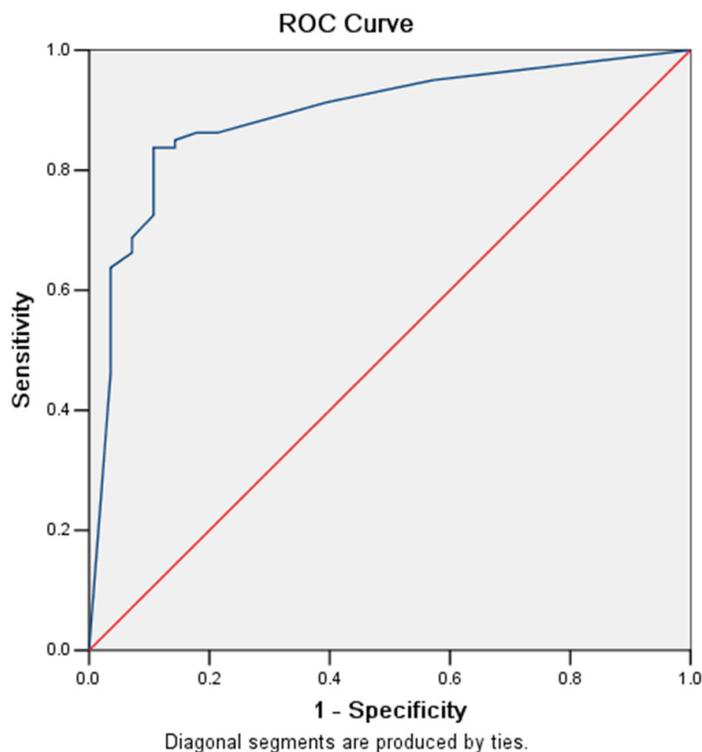
Optimized thresholds

Online quality control was a procedure using control material. The target value was setting according to short term quality control method. When online quality control data were assessed, IMI-DC data were found to be below the target values (**Figure 1A** and **1B**). The threshold for IMI-DC was optimized for the detection of specific abnormalities. Quality data were reflected by the machine reducing the sensitivity of estimating "Left Shift, Blasts, Immature Gran"; this resulted in a high false-negative value. Therefore, the threshold for "Left Shift, Blasts, Immature Gran" was optimized from 100 to 90; a receiver operating characteristic (ROC) curve was used to evaluate the efficiency of optimizing the flag threshold for "Left Shift, Blasts, Immature Gran". As shown in **Figure 2**, the AUC of optimizing the threshold from 100 to 90 was 0.730, with 83.8% sensitivity and 80.8% specificity.

Validation of the optimized setting

An independent set of 200 samples were next used to validate the optimized settings. App-

ROC Curve

Figure 2. ROC analysis was used to optimize the flag threshold for immature granulocytes.

lication of the optimized thresholds instead of factory-default settings investigated the number of false-negatives from 38 (total number = 526) to 7 (total number = 200), indicating a false negative rate reduction from 7.22% to 3.5%; overall PPV, NPV and efficiency were 64.15%, 95.24%, and 87.00%, respectively. A false-positive rate of 9.5% was obtained (**Table 2**).

Effects of IG flagging threshold adjustment on review rate

The review rate was 29.09% for all complete blood cell counts with differential values when the factory-default threshold was used. When the optimized threshold was applied, the review rate dropped to 26.5%, indicating that the optimized threshold did not increase the burden of manual slide review.

Clinical effect of false-negatives

Among the abnormalities observed in manual differential WBC counts of the validation set (n = 200), 2 (1.0%), 2 (1.0%), 2 (1.0%), and 1 (0.5%) samples had promyelocytes, metamy-

elocytes, neutrophil particle toxicity, and erythroblasts. No cases of blasts were missed.

Discussion

In this study, we adjusted the IMI-DC, which is an IMI channel detection control parameter by ROC curve and found the best cutoff value for the Q-flag in question. A total of 526 samples were selected to evaluate the efficiency of Q-flags. A ROC curve was used to optimize threshold and reduce the false negative rate affected by IMI channel. In addition, 200 additional samples were selected to verify the optimized threshold. By maximizing the YI, we were able to adjust Q flag thresholds and reduce the false negative rate form 7.22% to 3.5%. The overall PPV was increased to 64.15%; NPV and efficiency were 95.24% and 87.00%, respectively. Additionally, the false-positive rate was decreased to 9.5% [**Table 2**]. The importance of IMI-DC for testing samples on automated cell counters is emphasized to allow laboratories to safely increase the efficiency of morphologic classification of cells.

In general, the usefulness of instrument-generated flags is evaluated by their sensitivity and specificity [13-17]. Overall, optimized thresholds lead to increased PPV, NPV, and efficiency rate compared with factory-default settings because of low false negative rates.

This study demonstrates the value of IMI-DC in decreasing the false negative rate. However, Heidi Eilertsen et al. [18] reported that the "IG Present" flag gives almost no information regarding blast detection in addition to the "Blasts" flag. In this study, because of the number of missed immature myeloid cells, adjusting to the Q-flags affected the performance of the flags, with no cases of blasts being missed and "IG Present" being detected. The high false negative rate was reduced and the efficiency of automated hematology analyzer was improved. Maximized YI, which could optimize the relationship between true-positives and false-posi-

tives, was used to improve PPV. An additional factor is the XE-IG master software, which could report a parameter directly called immature granulocytes (IG) from the analyzer. CAP Laboratory Improvement Programs [19] reported that adopting certain laboratory practices may be possible to reduce the rates of manual reviews of peripheral blood smears and increase the efficiency of generating CBC results.

In conclusion, the IMI-DC threshold is important in reducing the false-negative rate and improving the overall PPV and NPV. In another, this study finds the best cut-off value for the Q-flags which provide a more adequate assessment in order to indicate the blood smear review.

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Disclosure of conflict of interest

None.

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