Validation and Optimization of Criteria for Manual Smear Review Following Automated Blood Cell Analysis in a Large University Hospital

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• ***Context.***—**Each laboratory should have criteria for manual smear review that limit workload without affecting patient care. The International Consensus Group for Hematology Review established guidelines for action after automated blood cell analysis in 2005.**

Objective.—**To compare the consensus group criteria with our laboratory criteria and optimize them for better efficiency.**

Design.—**A total of 2114 first-time samples were collected consecutively from daily workload and were used to compare 2 criteria as well as establish the optimized criteria. Another set of 891 samples was used to validate the optimized criteria. All samples were run on either Sysmex XE-5000 or Coulter LH750 hematology analyzers and were investigated by manual smear review. The efficiency of each set of criteria was compared and optimized to obtain better efficiency, an acceptable review rate, and a low false-negative rate.**

Results.—**From 2114 samples, 368 (17.40%) had positive smear results. Compared with that of our laboratory criteria, the efficiency of the consensus group criteria was higher (83.63% versus 78.86%, $P < .001$), the review rate was higher (29.33% versus 22.37%, $P < .001$), and the false-negative rate was lower (2.22% versus 8.09%, $P < .001$). After optimizing the rules, we obtained an efficiency of 87.13%, a review rate of 24.22%, and a false-negative rate of 2.98%. We validated the optimized criteria with another set of samples, and the efficiency, review rate, and false-negative rate were 87.32%, 25.25%, and 1.12%, respectively.**

Conclusions.—**Each laboratory should verify the criteria for smear review, based on the International Consensus Group for Hematology Review, and optimize them to maximize efficiency.**

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The complete blood count (CBC) with leukocyte differential counts is one of the most frequently requested hematologic tests in medical laboratories.[1,2] Despite the great precision, high accuracy, and expandability of automated hematologic analyzers, manual slide review (MSR) is still necessary to identify some morphologic abnormalities that may be relatively unremarkable in automated methods.[3] Each laboratory has its own criteria about when to perform manual smear review following automated blood count analysis. The laboratory productivity of CBC is inversely related to the number of manual differential count review rates; in addition, the rate of MSR is variable in each institution.[4]

The commission on laboratory accreditation of the College of American Pathologists (CAP) requires each laboratory to have criteria for blood smear review and keep evidence of such reviews.[1] Siriraj Hospital is a tertiary university hospital located in Bangkok, Thailand. The hospital has a capacity of about 2200 beds and more than 1 million outpatient visits per year. The clinical pathology laboratory has 2 types of hematology analyzers: 2 Sysmex XE-5000 analyzers (Sysmex, Kobe, Japan), and 1 Coulter LH750 analyzer (Beckman Coulter Inc, Brea, California), which can produce 900 to 1300 CBC samples per day. The clinical pathology laboratory received specimens from inpatients and outpatients from all departments except the hematology clinic in Siriraj Hospital. The average analytic turnaround time is about 30 minutes and the MSR rate was approximately 22%.

Our laboratory used the list of criteria based on that of Gulati et al[5] and modified several parameters in accordance with the consensus between clinical pathologists and hematologists in the hospital. The sensitivity and specificity of these criteria had not been validated. In 2005, the International Consensus Group for Hematology Review established guidelines composed of 41 rules for action after automated analysis of a blood sample that had false-negative and false-positive rates of 2.90% and 18.60%, respectively.[6] This study compared the efficiency of the series of criteria for first-time samples established by the International Consensus Group (consensus group criteria)

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and our laboratory criteria, and we optimized these criteria to improve efficiency.

MATERIALS AND METHODS

Study Samples

The study was performed in the clinical pathology laboratory of Siriraj Hospital, Mahidol University, in Thailand and was approved by the institutional review board. The samples were collected from daily workload, including outpatient and inpatient populations, from May 2010 to June 2010. All samples were first-time samples and collected consecutively.

The Automated Analyzers

Automated CBC and white blood cell (WBC) differential counts were performed with Coulter LH750 and Sysmex XE-5000 hematology analyzers. Reticulocyte counts were performed in several samples upon clinician request with Sysmex XE-5000 analyzers.

Manual Slide Review

Blood films were prepared by using a Sysmex SP-1000i automated hematology slide preparation unit. If blood volume was low, the blood films were smeared manually and then stained with the SP-1000i unit. Each blood film was examined independently by 2 experienced technicians who were not aware of the automated results. The technicians performed either manual scans (a cursory examination for a specific purpose such as to verify platelet [PLT] count or red blood cell [RBC] morphology) or manual leukocyte differential counts of peripheral smears according to the presence of WBC abnormalities or nucleated red blood cells (NRBCs). The positive smear result was defined as per the International Consensus Group criteria, which included RBC morphology at 2+ or greater, malaria, giant PLTs at moderate or greater, PLT clumps at greater than rare/occasional, Döhle bodies/toxic granulation/vacuoles at moderate or greater, blasts at 1 or greater, metamyelocytes at greater than 2, myelocytes/promyelocytes at 1 or greater, atypical lymphocytes at greater than 5, NRBCs at 1 or greater, or plasma cell at 1 or greater.[6] All positive smear results and discrepant results between 2 technicians were reviewed by 2 laboratory physicians.

Statistical Analysis

Statistical analysis was performed with SPSS version 13.0 (SPSS Inc, Chicago, Illinois) and Excel software (Microsoft, Redmond, Washington). If a rule was triggered and the smear result was positive, the sample was graded as a "true positive." If a rule was triggered and the smear did not have any positive findings, the sample was graded as a "false positive." If a rule was not triggered and the smear result was negative, the sample was graded as a "true negative." If a rule was not triggered but the smear contained a positive finding, the sample was graded as a "false negative." χ^2 and Fisher exact tests were used to compare the efficiency, false-positive rate, false-negative rate, and review rate between different sets of criteria. A P value of $\leq .05$ was considered statistically significant.

Efficiency is the ability of a test to correctly classify the true outcome, that is, the true-positive and true-negative results. Efficiency can be calculated as follows[7]:

$$\text{Efficiency} = (\text{True Positives} + \text{True Negatives})/\text{All Cases}.$$

Study Design

Optimization Set.—First, 2114 samples were used to compare the efficiency, false-negative rate, and review rate between the consensus group criteria and our laboratory criteria. We adjusted and selected the threshold of each parameter to achieve the highest efficiency. We accepted a false-negative rate of less than 5%, as recommended by the consensus group, and accepted a manual review rate of less than 30%, according to our capacity for smear review and the average review rate of the CAP survey.[4]

Validation Set.—We applied the optimized group of criteria to a separate set of 891 samples. The efficiency and smear review rates of validation set were calculated. The false-positive and false-negative cases were enumerated and clarified.

Additional Positive-Smear Cases.—We collected additional positive-smear samples, including 12 cases of confirmed thalassemia, 12 cases with atypical lymphocytes of more than 5%, and 12 cases with blasts of more than 1%. We tested the optimized criteria to determine whether they could detect the case.

RESULTS

Analysis of Smear Review Findings

A total of 2114 samples were collected from 825 males and 1289 females (mean age, 44 years; age range, 0–97 years). Only 200 samples came from pediatric patients (younger than 12 years). Samples were collected from 1840 outpatients (87.04%) and 274 inpatients (12.96%), and 1752 samples (82.88%) were analyzed by Sysmex XE-5000 analyzers.

From 2114 samples used in optimizing the smear review criteria, 368 (17.40%) had positive smear results according to the definition of the International Consensus Group. Among the positive samples, 230 (62.50%) had RBC abnormalities, 15 (4.08%) had WBC abnormalities, 67 (18.21%) had PLT abnormalities, 34 (9.24%) had both RBC and PLT abnormalities, 17 (4.62%) had RBC and WBC abnormalities, 3 (0.82%) had both WBC and PLT abnormalities, and 2 (0.54%) had RBC, WBC, and PLT abnormalities. The 3 most common findings of abnormal RBC morphology were microcytic RBC (215 occurrences), anisocytosis (85 occurrences), and hypochromic RBC (70 occurrences). For abnormal WBCs, the 3 most common findings were atypical lymphocytes (18 occurrences), blasts (9 occurrences), and myelocytes (6 occurrences). Platelet clumps (84 occurrences) were found more often than giant PLTs (34 occurrences). An automated CBC and WBC differential count analysis was performed on all 2114 samples; an automated reticulocyte count was performed on 58 samples upon clinician request.

Comparison of the Performance Between the Consensus Group Criteria and Laboratory Criteria

The criteria from the consensus group and our laboratory were different in several parameters (Table 1). We used a total of 2114 samples to compare the efficiency, false-negative rate, and review rate between both criteria. The efficiency was 83.63% in the consensus group criteria and 78.86% in the laboratory criteria ($P < .001$). The false-negative rate was 2.22% with the consensus group criteria and 8.09% with the laboratory criteria ($P < .001$). The review rate was 29.33% with the consensus group criteria and 22.37% with the laboratory criteria ($P < .001$).

Optimized Criteria

We used both criteria as guidelines to adjust the threshold of each parameter to establish optimized criteria with better efficiency. From a total of 23 rules, 5 rules were related to CBC parameters, including hemoglobin, mean corpuscular volume (MCV), red cell distribution width (RDW), WBCs, and PLT count; 6 rules were related to the differential parameters for WBCs, including no or incomplete differential and absolute counts for 5 WBC types; 1 rule related to reticulocytes; and 11 rules related to suspect flags such as

Table 1. Criteria for Blood Smear Review of the International Consensus Group for Hematology Review and of Our Laboratory

Parameters	Consensus Group Criteria	Our Laboratory Criteria
CBC		
HGB, g/dL	<7 or >2 above upper reference range for age and sex	<10 or >18
MCV, fL	<75 or >105	>105
RDW, %	>22	NA
WBC, /μL	<4000 or >30 000	<1500 or >20 000
PLT, × 10^3/μL	<100 or >1000	<100 or >600
Differential		
No differential	No differential	No differential
No. of neutrophils, /μL	<1000 or >20 000	NA
No. of lymphocytes, /μL	>5000 (adult) or >7000 (<12 years old)	>4000
Lymphocyte, %	NA	>70
No. of monocytes, /μL	>1500 (adult) or >3000 (<12 years old)	>2000
Monocyte, %	NA	>20
No. of eosinophils, /μL	>2000	>1000
No. of basophils, /μL	>500	NA
Basophil, %	NA	>4
Reticulocyte		
Absolute reticulocyte, × 10^3/μL	>0.100	NA
Suspect flags		
Nucleated red blood cell	Flag	Flag
Blast	Flag	Flag
Atypical lymphocyte	Flag	Flag
RBC fragment	Flag	NA
Dimorphic RBC	Flag	NA
Lyse resistant	Flag	NA
Immature granulocyte	Flag	NA
Left shift	Flag	NA
PLT clump	Flag	NA
Platelet (except PLT clump)	Flag	NA
Suspect flag (except ImmG/band in adult)	Flag	NA
Suspect flag (child)	Flag	NA

Abbreviations: CBC, complete blood count; HGB, hemoglobin; ImmG, immature granulocyte; MCV, mean corpuscular volume; NA, not available; PLT, platelet; RDW, red cell distribution width; WBC, white blood cell; RBC, red blood cell.

NRBC, blast, RBC fragment, and PLT clumps (Table 2). We did not include neonatal specimens in the criteria because most neonatal samples (75 of 77 samples) were triggered by the optimized criteria. One sample not triggered was the negative-smear sample; another was the positive smear because of the presence of polychromasia, PLT clumps, and giant PLTs, with a normal PLT count (PLTs, 330 × 10^3/μL). Currently, our laboratory does not use an automated method to enumerate NRBCs, so our optimized criteria did not contain absolute NRBC counts.

Validation of the Optimized Criteria

After we obtained all of the rules for the optimized criteria, we validated them with the previous set of samples (n = 2114). Compared to the consensus group criteria and the laboratory criteria, the efficiency was improved ($P = .001$ when compared to the consensus group criteria and $P < .001$ when compared to the laboratory criteria). The false-negative rate in the optimized criteria slightly increased when compared to the consensus group criteria ($P = .15$), but decreased when compared to the laboratory criteria ($P < .001$). The review rate declined in the optimized criteria when compared to the consensus group criteria ($P < .001$) but increased slightly when compared to the laboratory criteria ($P = .15$, Table 3).

In the optimized criteria, we used a stricter threshold for MCV, low WBC counts, absolute neutrophil counts, and reticulocyte counts, leading to a reduction in peripheral smear reviews. Altering the threshold increased the false-negative rate only in the low MCV rule; in contrast, the

Table 2. Optimized Criteria for Smear Review

Rule No.	Parameters	Optimized Criteria
	CBC	
1	HGB, g/dL	<7 or >19
2	MCV, fL	<70 or >110
3	RDW, %	>22
4	WBC, /μL	<1500 or >30 000
5	PLT, × 10^3/μL	<100 or >600
	Differential	
6	No differential	No differential
7	No. of neutrophils, /μL	<500 or >25 000
8	No. of lymphocytes, /μL	>7000
9	No. of monocytes, /μL	>3000
10	No. of eosinophils, /μL	>2000
11	No. of basophils, /μL	>500
	Reticulocyte	
12	Absolute reticulocyte, × 10^3/μL	>0.250
	Suspect flags	
13	Nucleated red blood cell	Flag
14	Blast	Flag
15	Atypical lymphocyte	Flag
16	RBC fragment	Flag
17	Dimorphic RBC	Flag
18	Lyse resistant	Flag
19	Immature granulocyte	Flag
20	Left shift	Flag
21	PLT clump	Flag
22	Platelet (except PLT clump)	Flag
23	Suspect flags	Flag

Abbreviations: CBC, complete blood count; HGB, hemoglobin; MCV, mean corpuscular volume; PLT, platelet; RDW, red cell distribution width; WBC, white blood cell; RBC, red blood cell.

Table 3. Truth Table Summary of the Optimized Criteria, the Consensus Group Criteria, and Our Laboratory Criteria (n = 2114)

	Optimized Criteria, % (No.)	Consensus Group Criteria, % (No.)	*P* Value[a]	Our Laboratory Criteria, % (No.)	*P* Value[b]	*P* Value[c]
True positive	14.43 (305)	15.18 (321)	.52	9.32 (197)	<.001	<.001
False positive	9.89 (209)	14.14 (299)	<.001	13.06 (276)	.001	.32
True negative	72.71 (1537)	68.45 (1447)	.003	69.54 (1470)	.02	.46
False negative	2.98 (63)	2.22 (47)	.15	8.09 (171)	<.001	<.001
Efficiency	87.13	83.63	.001	78.86	<.001	<.001
Review rate	24.22	29.33	<.001	22.37	.15	<.001

[a] *P* value (optimized criteria versus consensus group criteria).
[b] *P* value (optimized criteria versus laboratory criteria).
[c] *P* value (laboratory versus consensus group criteria).

threshold from the optimized criteria significantly reduced the false-positive rate in the low MCV, low WBC counts, and high neutrophil counts rules. We used a lower cutoff value for high PLT counts, which caused 16 more cases to be reviewed. However, the false-positive and false-negative rates for the high PLT rule in the optimized criteria and the consensus group criteria were not significantly different (Table 4).

When we separated samples into inpatient and outpatient sources, the false-negative rates from both inpatients (3.65%) and outpatients (2.88%) were not different when compared with total samples (2.98%, $P = .68$ and .93, respectively). However, the false-positive rate was very high in the inpatient group (21.90%) as compared to total samples (9.89%, $P < .001$), while that rate in the outpatient group was 8.10% ($P = .06$) when compared with the total samples. The smear review rate in inpatient samples, outpatient samples, and total samples was 51.09%, 20.33%, and 24.31%, respectively.

We repeated validation of the optimized criteria by using another separate set of samples (n = 891). In the validation set, the false-negative rate was only 1.12%, which was less than the optimization set ($P = .004$); the review rate and efficiency were 25.25% and 87.32%, respectively, which were similar to the optimization sample set ($P = .62$, $P = .94$, respectively).

False-Negative and False-Positive Analysis

We conducted false-negative analysis of 3 sets of criteria, and we found that PLT morphology was the most frequent false-negative finding in the consensus group–suggested criteria and the optimized criteria. In laboratory criteria, the most frequent false-negative finding was RBC morphology because this set of criteria did not include the low MCV and RDW rule. The most common RBC morphology (grade 2+ or greater) missed was microcytic RBC (107 occurrences), followed by anisocytosis (21 occurrences), hypochromic RBC (21 occurrences), and target cell (21 occurrences). False-negative results that had abnormal PLT morphology from the consensus group criteria, laboratory criteria, and optimized criteria had PLT counts of $107 \times 10^3/\mu L$ to $725 \times 10^3/\mu L$, $102 \times 10^3/\mu L$ to $598 \times 10^3/\mu L$, and $102 \times 10^3/\mu L$ to $598 \times 10^3/\mu L$, respectively. False-negative cases by PLT morphology that had PLT counts less than $150 \times 10^3/\mu L$ only occurred in 4 of 42 cases (9.52%) in the consensus group criteria, 10 of 55 cases (18.18%) in the laboratory criteria, and 7 of 48 cases (14.58%) in the optimized criteria. No cases of blast were missed by all 3 criteria (Table 5).

After false-positive analysis, the data indicated that the MCV criteria (117 occurrences) caused the most false-positive smear reviews in the consensus group criteria. One hundred and twelve cases were triggered by MCV less than 75 fL, whereas 5 cases were triggered by MCV greater than 105 fL. Lymphocyte count greater than 4000/μL or >70% and hemoglobin levels lower than 10 g/dL caused the most false-positive smear reviews in the laboratory criteria. Immature granulocyte, blast, and NRBC flags caused the most false-positive results with the optimized criteria (Table 6).

Table 4. False-Positive and False-Negative Rate in Samples Between Cutoff Values of the Consensus Group Criteria and the Optimized Criteria

Parameters	Cutoff[a]	No. of Cases Between 2 Cutoff Values	False-Positive (%)	*P* Value	False-Negative (%)	*P* Value
Low MCV, fL	<75	138	73.91	<.001	0.00	.003
	<70		49.28		6.52	
High MCV, fL	>105	10	40.00	.30	0.00	NA
	>110		20.00		0.00	
Low WBC, /μL	<4000	46	63.04	<.001	0.00	.13
	<1500		17.39		8.70	
High PLT, $\times 10^3/\mu L$	>600	16	12.50	>.99	12.50	.48
	>1000		18.75		0.00	
Low No. of neutrophils, /μL	<1000	6	100.00	.18	0.00	NA
	<500		50.00		0.00	
High No. of neutrophils, /μL	>20 000	16	62.50	.01	0.00	NA
	>25 000		12.50		0.00	
No. of reticulocytes, $\times 10^3/\mu L$	>0.1	39	66.67	.64	0.00	NA
	>0.25		58.97		0.00	

Abbreviations: MCV, mean corpuscular volume; NA, not applicable; PLT, platelet; WBC, white blood cell.
[a] Cutoff value between the consensus group criteria (upper) and the optimized criteria (lower).

Table 5. False-Negative Analysis			
	Consensus Group Criteria, No. (%)	Laboratory Criteria, No. (%)	Optimized Criteria, No. (%)
Metamyelocyte, myelocyte, promyelocyte	1 (1.92)	1 (0.54)	1 (1.41)
Blast	0 (0.00)	0 (0.00)	0 (0.00)
Atypical lymphocyte	1 (1.92)	4 (2.17)	2 (2.82)
NRBCs	1 (1.92)	0 (0.00)	1 (1.41)
RBC morphology	7 (13.46)	121 (65.76)	17 (23.94)
Platelet morphology	42 (80.77)	55 (29.89)	48 (67.61)
WBC morphology	0 (0.00)	3 (1.63)	1 (1.41)
Total No. of false-negative occurrences	**52** (100.00)	**184** (100.00)	**70** (100.00)
Total false-negative cases	47	171	63

Abbreviations: NRBCs, nucleated red blood cells; RBC, red blood cell; WBC, white blood cell.

Additional Positive-Smear Cases

In all 12 thalassemic cases, 12 samples with more than 5% atypical lymphocytes (range, 6%–35.20%; mean ± SD, 14.39 ± 8.28%) and 12 samples with more than 1% blasts (range, 5%–73.20%; mean ± SD, 22.74 ± 21.49%) were triggered by our optimized criteria. The 3 most common criteria were triggered in each group of samples as follows: in thalassemic cases, 12 of 12 (100%) were triggered by RDW criteria, 11 of 12 (91.67%) were triggered by the NRBC flag, and 8 of 12 (66.67%) were triggered by PLT counts greater than $600 \times 10^3/\mu L$. In samples with atypical lymphocytes, 11 of 12 (91.67%) were triggered by PLT counts less than $100 \times 10^3/\mu L$, 10 of 12 (83.33%) were triggered by atypical lymphocyte flags, and 9 of 12 (75%) were triggered by blast flags (abnormal lymphocytes/lymphoblast flags). In samples with blasts, 11 of 12 (91.67%) were triggered by blast flags, 11 of 12 (91.67%) were triggered by PLT counts less than $100 \times 10^3/\mu L$, and 8 of 12 (66.67%) were triggered by atypical lymphocyte flags.

COMMENT

The International Consensus Group for Hematology Review suggests that each laboratory adopting the criteria for action, following automated blood cell analysis, validate its operation before implementation.[6] No guidelines can be used universally and also be economically feasible.[8] Our laboratory had been using the set of criteria established by our expert opinion consensus, but it had not been validated.

In this study, first we compared the consensus group criteria with our current laboratory criteria. The consensus group criteria had higher efficiency and a lower false-negative rate; however, the review rate of 29.33% was significantly higher than that in the laboratory criteria. After we optimized the criteria, the efficiency was improved, and the review rate of 24.31% was lower than the review rate from the consensus group criteria but higher than the rate from the laboratory criteria.

The manual smear review is labor-intensive, time-consuming, and may not be necessary. In the CAP Q-Probes Program study with 263 participating hospitals and laboratories, the rates of MSR varied among participants, with a median of 26.70%. The manual scan rate increased with a greater number of hospital beds, but the manual leukocyte differential count rate decreased. That study illustrated that reducing the review rate was directly related to the efficiency of generating CBC results.[4] Our optimized criteria increased the rate of MSR insignificantly, but the significantly improved efficiency made the criteria satisfactory.

Our optimized criteria were the same as those of the consensus group criteria for low hemoglobin values, RDW, high WBC counts, low PLT, eosinophil counts, basophil counts, no or incomplete differential counts, and the presence of suspect flags. However, after we selected the cutoff values that achieved the highest efficiency, our optimized criteria were different from those of the consensus group in several parameters.

The stricter threshold we used in the optimized criteria reduced the peripheral smear review rate without increasing the false-negative rate in high MCV, low WBC count, absolute neutrophil count, and reticulocyte criteria. In the low MCV rule, the false-negative rate increased after we adjusted the threshold; however, in patients with microcytosis, the blood smear review may not be useful to discriminate between iron-deficiency anemia, thalassemia minor, and anemia of chronic disease.[9] We did not specify criteria for different age and sex, so we selected a single cutoff value for high hemoglobin values, high lymphocyte counts, and high monocyte counts. For high PLT counts, we used a lower cutoff value, which slightly increased the smear review rate.

Our samples were first-time samples, so most of them were collected from outpatient sources. The false-negative rate was not significantly different between inpatient,

Table 6. Top 6 Rules That Cause a False-Positive		
	False-Positive Rule	Rate, %
	Consensus group criteria	
1	MCV <75 fL, >105 fL	24.43
2	PLT flag (except PLT clump)	8.56
3	Immature granulocyte flag	8.56
4	Neonate	7.72
5	Blast flag	6.89
6	WBC <4000/μL or >30 000/μL	6.68
	Laboratory criteria	
1	Lymphocyte >4000/μL or >70%	27.03
2	HGB <10 g/dL	23.88
3	Eosinophil >1000/μL	9.71
4	NRBC flag	8.66
5	Blast flag	8.66
6	WBC >20 000/μL	5.77
	Optimized criteria	
1	Immature granulocyte flag	16.46
2	Blast flag	13.92
3	NRBC flag	13.08
4	Platelet $<100 \times 10^3/\mu L$ or $>600 \times 10^3/\mu L$	10.13
5	Left-shift flag	9.28
6	PLT clump flag	5.91

Abbreviations: HGB, hemoglobin; MCV, mean corpuscular volume; NRBC, nucleated red blood cell; PLT, platelet; WBC, white blood cell.

outpatient, and total samples. Therefore, optimized criteria can be used in both inpatient and outpatient samples. The disadvantage was that the high false-positive rate in inpatient samples would lead to a high smear review rate.

The false-negative rate in the optimized criteria (2.98%) was insignificantly higher than that from the consensus group criteria (2.22%) but was significantly decreased when compared with the laboratory criteria (8.09%). According to the International Consensus Group, the false-negative rate should be less than 5% to ensure patient safety.[6]

When validating the optimized criteria with another set of samples, the efficiency and the review rate in the validation set were nearly similar to the optimization set. The false-negative rate was slightly lower in the validation set. As a result, our optimized criteria were reproducible.

The most common cause of false-negatives when we used the laboratory criteria was RBC morphology. We did not include the low MCV and high RDW in our laboratory criteria because there are many patients with hemoglobinopathies in Thailand,[10] which may cause a very high review rate. Clinicians can use information from hemoglobin, MCV, and RDW from automated results to further investigate for final diagnosis. However, when we included low MCV and high RDW in the optimized criteria, as well as reduced the threshold of hemoglobin, the review rate did not increase significantly, while the false-negative rate decreased significantly. The reporting of RBC morphology was still useful for clinicians,[11] so we decided to include these 2 parameters in our optimized criteria.

For false-negatives caused by abnormal PLT morphology, only a few cases had PLT counts less than $150 \times 10^3/\mu L$, which was defined as thrombocytopenia.[12] However, patients with PLT counts between $100 \times 10^3/\mu L$ and $150 \times 10^3/\mu L$ have only a 6.90% chance of developing persistent thrombocytopenia of PLT counts less than $100 \times 10^3/\mu L$ in the subsequent 10 years. Moreover, normal PLT values in healthy individuals in non-Western populations may be between $100 \times 10^3/\mu L$ and $150 \times 10^3/\mu L$, and a cutoff value of $100 \times 10^3/\mu L$ would decrease concerns about mild physiologic thrombocytopenia during pregnancy.[13] For these reasons, physicians may examine patients with thrombocytopenia when PLT counts are less than $100 \times 10^3/\mu L$. Hence, the presence of abnormal PLT morphology in these samples, which had PLT counts of $100 \times 10^3/\mu L$ to $150 \times 10^3/\mu L$, would be acceptable.

Most of the neonatal samples were triggered by the optimized criteria. One positive case with the presence of polychromasia, PLT clumps, and giant PLTs was missed by the criteria. However, polychromasia, macrocytic normochromic cells, and a few NRBCs can be found in normal newborn infant blood films.[14] The presence of PLT clumps and giant PLTs in this case, which had a normal PLT count, was also not clinically significant.

No cases of blasts would have been missed by the consensus group criteria, laboratory criteria, or optimized criteria. However, there were only cases with blasts in 2114 cases, so we selected 12 more cases with the presence of blasts to check our criteria. Still, no cases with blasts were missed by the optimized criteria, although the blast flag was only detected in 11 of 12 cases. One additional case was triggered by low WBC counts, a low neutrophil count, and low PLT count criteria.

Since lymphocyte findings were difficult to classify, either within the reference range or as atypical lymphocytes, and were varied among individual observers,[15] we thought that occasionally missing cases with increased atypical lymphocytes was acceptable. However, only 3 in 18 cases of increased atypical lymphocytes were missed. In addition, in 12 additional atypical lymphocyte cases, all cases were triggered by our optimized criteria. Ten of 12 cases were triggered by atypical lymphocyte flag and 11 of 12 cases were triggered by PLT counts lower than $100 \times 10^3/\mu L$ because most of these cases had Dengue viral infections, which is prevalent in Thailand.[16]

The causes of false-positive cases in our current laboratory criteria mostly come from the lymphocyte count criteria and hemoglobin levels lower than 10 g/dL. After we adjusted the threshold of both criteria, the false-positive rate improved in our optimized criteria. Also, the most common cause of false-positives in the consensus group criteria was MCV criteria, especially low MCV (<75 fL). Thailand has many people with the thalassemia trait who may have slightly low hemoglobin levels and low MCV but whose blood smear would be slightly abnormal (less than grade 2+) and give rise to false-positive results.

There are a number of limitations to the current study: (1) There were small samples collected from patients younger than 12 years, so we did not define the age-specific criteria for absolute lymphocyte and monocyte counts. On the other hand, non–age-specific criteria will be easy to use in routine clinical practice, (2) These data were generated from a single geographic area with a high prevalence of thalassemia and Dengue fever. Because of these regional factors, the precise cutoffs and rules that proved most efficient in our laboratory may not be the optimal rules to apply in laboratories working in other regions of the globe, (3) Samples were collected consecutively in a period of 2 months, so some uncommon positive findings were not observed during the study, such as malaria, RBC autoagglutination, and rouleaux formation. As a result, the efficacy of the optimized criteria in these positive findings could not be assured.

In summary, we compared 2 sets of criteria, the International Consensus Group for Hematology Review criteria and our laboratory criteria. The consensus group criteria had higher efficiency with a higher review rate. From these 2 criteria, we adjusted each parameter to improve overall efficiency and generate optimized criteria. All laboratories should have their own criteria for smear review. Criteria can be based on the consensus group criteria but should be verified before adoption or optimized to be suitable for different requirements.

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