Optimization of Criteria for Verification of Automated Platelet Counts Generated by the Sysmex XE-2100 Hematology Analyzer

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Abstract

Background: Verification of automated platelet counts by blood smear examination is necessary but time consuming. This study was undertaken to optimize the criteria for performing platelet scans.

Methods: Platelet scans were performed on 796 blood smears made from specimens that were either flagged for (a) platelet abnormal distribution or PAD (group I), (b) platelet clumps or CLP (group II), (c) PAD + CLP (group III), or (d) had a platelet count below 100,000/μL (group IV). Based on the findings of the platelet scans, a percent positive yield was determined for each group of specimens.

Results: Percent positive yields of 8.9, 30.0, 35.3, and 1.9 were obtained from platelet scan findings for groups I, II, III, and IV, respectively.

Conclusions: Optimal criteria for performing platelet scans excluded the PAD flag and the follow-up platelet counts below 100,000/μL but included specimens flagged for CLP and the initial platelet counts below 100,000/μL.

Automated hematology analyzers used by clinical laboratories around the world to perform complete blood counts (CBCs) and differential leukocyte counts (Diffs) generate reliable results on essentially all blood specimens containing normal cellular elements and no interfering substance(s).[1-8] In contrast, the results generated by these analyzers on blood specimens containing abnormal cellular elements and/or potentially interfering substances may or may not be reliable and are consequently flagged for verification by alternate means.[9-12] Blood smear examination represents the most commonly employed method for verification of automated platelet counts, automated white cell differential counts, or both. Manual blood smear examination is a labor-intensive and time-consuming procedure that impacts overall laboratory efficiency. In order to maintain a reasonable degree of efficiency, many, if not all, laboratories try to minimize the number of blood smear examinations performed daily. The clinical laboratory at Thomas Jefferson University Hospital processes approximately 700 blood specimens per day for CBCs and/or platelet counts. Approximately one-half of these specimens are also processed for Diffs and nucleated red cell counts (NRBCs), and a reticulocyte count is performed on a small fraction of these. Blood smears are made, stained, and examined microscopically on approximately 170 of the 700 (24%) specimens. Platelet scans performed to verify only the platelet counts account for approximately 70 of the 170 (41%) blood smear examinations. The criteria for reflex order of a platelet scan in our laboratory routinely included (a) 1 or more of the analyzer-generated flags (platelet abnormal distribution or PAD, platelet clumps or CLP, and giant/large platelets) and/or (b) an automated platelet count $<100 \times 10^3/\mu L$, irrespective of whether it is an initial or a follow-up count.

Since the platelet scans, though comparatively less time consuming than the manual Diffs, contributed significantly to the total workload and adversely impacted the turnaround time of platelet count results, we decided to examine the outcome in terms of percent positive yield of individual criteria cited above for performing platelet scans and to find ways, if possible, to improve efficiency by reducing the number of daily platelet scans without an adverse effect on patient care.

Material and Methods

The clinical laboratory at our institution uses 2 XE-2100 analyzers (Sysmex America Inc, Mundelein, IL) for performing CBCs and Diffs and 2 SP100s (Sysmex America Inc, Mundelein, IL) for automatically preparing and staining smears. Both analyzers were calibrated and quality controlled according to manufacturer's recommendations.[13] The XE-2100 employs impedance technology as the primary method to generate platelet counts, but it also has an optical methodology built-in for use at the laboratory's discretion. The platelet-associated flags routinely generated by the XE-2100 are PAD, CLP, and giant/large platelets. Among these, only the first 2 are encountered frequently in daily work while the latter is encountered rarely and hence was not included in this study. The threshold for the CLP flag was adjusted by the manufacturer's representative to an optimal setting prior to the start of the study.

A total of 796 smears made from 796 K2-EDTA anticoagulated blood specimens collected in either lavender-top tubes (737 of 796) from BD Vacutainer Systems, NJ or lavender-top Microtainers (59 of 796) also from BD Vacutainer Systems, NJ, from 651 patients as part of their routine clinical care over a period of several months were reviewed by the primary author. The purpose of the smear examination was to determine if the automated platelet count was acceptable to be reported and to note the presence, if any, of the substances or factors (eg, platelet clumps, giant/large platelets, fibrin strands, schistocytes, cytoplasmic fragments, etc.[14]), which could be considered potential causes of results deemed unacceptable. The automated platelet count was accepted and reported if the platelet count estimated from the smear was within 10% of the automated count for counts $>/=40 \times 10^3/\mu L$ and within 20% for counts $<40 \times 10^3/\mu L$ and if none of the interfering substances/factors were present in significant number (1+ or greater) in the smear.

The data were analyzed retrospectively and divided into 4 groups based on the type of platelet-associated flag generated by the analyzer and the platelet count. Specimens flagged for PAD comprised group I, and those flagged for CLP made up group II. Group III consisted of specimens flagged for both, the PAD and the CLP. Specimens with platelet counts $<100 \times 10^3/\mu L$, whether

flagged or not, comprised group IV. The distribution of specimens and the platelet count range for each group are shown in **Table 1**.

The data from each of the 4 groups were analyzed to determine (a) percent positive yield, (b) fraction of specimens with non-platelet flags, which would have also required smear examination, (c) specific details of specimens, which did not generate any non-platelet flags but revealed a positive smear finding, and (d) fraction of specimens collected in Microtainers, which revealed positive smear findings compared to that of specimens collected in lavender-top tubes. To determine percent positive yield, a smear was considered positive if it revealed any 1 or more of the following findings: 1 or more platelet clumps, giant/large platelets (1+ or greater), red cell fragments (1+ or greater), any fibrin strands, and cytoplasmic fragments (1+ or greater). The grading of morphologic findings was based on published guidelines.[15]

Specimens generating the so-called non-platelet flags, ie, either 1 or more white cell-associated flags (eg, left shift, immature granulocyte, blast, atypical lymphocyte, etc) and/or 1 or more red cell-associated flags (eg, red cell fragments and NRBC >/=2.0 per 100 WBC), in addition to the platelet- associated flag(s) and/or platelet count $<100 \times 10^3/\mu L$, were tallied to calculate the percentage of specimens with additional flags, which would have also required smear examination. The specifics of the specimens that did not generate any flag other than the platelet-associated flag(s) were also tallied to determine their impact on the operational efficiency. A comparison of the fraction of specimens collected in Microtainers revealing positive smear findings with that of the specimens collected in lavender-top tubes was performed to confirm or refute the general belief among laboratory professionals that platelet clumping is relatively more commonly seen in smears made from heel-stick or finger-stick specimens collected in Microtainers.

Table 1_Distribution of Specimens and the Platelet Count Ranges Among the Defined Groups

Group	# of Specimens	From # of Patients	Platelet Count Range ($x10^3/\mu L$)
I	101	101	1-861
II	130	108	11-908
III	102	75	10-658
IV	463	367	1-99

Group IV had the largest number of specimens because a large majority of the platelet scans (approximately 80%) were reflex ordered due to platelet counts below $100 \times 10^3/\mu L$.

Table 2_Blood Smear Findings of Groups I, II, and III

	Group I	Group II	Group III
	"PAD"	"CLP"	"PAD"+"CLP"
# of specimens	101	130	102
# of patients	101	108	75
# of specimens with non-platelet flags	41	124	82
# of specimens with positive smear findings:	9 (8.9%)	39 (30%)	36 (35.3%)
Platelet clumps	1	31	16
Giant/large platelets (>/= 1+)	1	2	10
Schistocytes (>/= 1+)	0	0	2
Fibrin strands	7	4	8
Cytoplasmic fragments (>/= 1+)	0	2	0

Abbreviations: PAD, platelet abnormal distribution; CLP, platelet clumps.

Results

Group I: Nine of 101 blood smears from specimens generating only the PAD flag among platelet-associated flags, revealed a positive finding on microscopic examination, resulting in a positive yield of 8.9% **(Table 2)**. Among the 9 smears with positive findings, 1 revealed CLP, 1 revealed giant platelets, and the remaining 7 revealed fibrin strands. Five of the 9 specimens with positive findings (55.6%) also generated non-platelet flags, including 3 with WBC-associated flags, 1 with a red cell fragment flag, and 1 with an NRBC of 2.2 per 100 WBC. These 5 specimens would, therefore, have received a smear review regardless of the PAD flag. Of the 4 specimens that did not generate any additional flags, 1 revealed CLP, 1 revealed giant platelets, and the remaining 2 revealed occasional fibrin strands on smear examination **(Table 4)**. The automated platelet counts for the 2 specimens, which revealed occasional fibrin strands, were unexpectedly within the normal range and considered acceptable upon smear review and comparison with the previous and/or follow-up platelet count(s). In contrast, the automated platelet counts for the remaining 2 specimens with positive smear findings were considered unacceptable due to discrepancies with the platelet estimate obtained from smear examination. The specimen revealing giant platelets had an automated count of $5 \times 10^3/\mu L$ and an estimated count of $50 \times 10^3/\mu L$, while the specimen revealing platelet clumps had an automated count of $333 \times 10^3/\mu L$ and an estimated count of $430 \times 10^3/\mu L$ with occasional clumps noted. Only 1 of the 101 blood specimens in this group and none of the specimens with positive smear findings was a Microtainer specimen (ie, a heel-stick collected in a Microtainer).

Group II: Thirty-nine of 130 blood smears from specimens generating only the CLP flag among platelet-associated flags, revealed a positive finding on microscopic examination, resulting in a positive yield of 30% **(Table 2)**. Among the 39 smears with positive findings, 31 revealed CLP, 2 revealed giant platelets, 4 revealed fibrin strands, and the remaining 2 revealed cytoplasmic fragments. Thirty-five of the 39 specimens with positive findings (89.7%) also generated non-platelet flags, including 32 with WBC-associated flags and 3 with NRBC >/= 2.0 per 100 WBC. These 35 specimens would, therefore, have received a smear review regardless of the CLP flag. All 4 specimens that did not generate any additional flags revealed CLP on smear examination **(Table 4).** The automated platelet counts from 3 of these 4 specimens were within the normal range, but only 1 with a platelet count of $239 \times 10^3/\mu L$ was considered acceptable. The remaining 3 specimens with automated counts of 28, 182, and $202 \times 10^3/\mu L$ respectively were unacceptable, as judged by smear examination. The corresponding approximate platelet counts estimated from the smears were 350–450, 250–300, and 230–300, respectively. Twenty-eight of the 130 blood specimens in this group and 16 of the 39 specimens with positive smear findings were Microtainer specimens (ie, heel-sticks collected in Microtainers).

Group III: Thirty-six of 102 blood smears from specimens generating both platelet-associated flags, ie, the PAD and the CLP, revealed a positive finding on microscopic examination, resulting in a positive yield of 35.3% **(Table 2).** Among the 36 smears with positive findings, 16 revealed CLP, 10 revealed giant platelets, 8 revealed fibrin strands, and the remaining 2 revealed red cell fragments. Twenty-nine of the 36 specimens with positive findings (80.6%) generated additional non-platelet flags (1 or more WBC-associated flags). These 29 specimens would, therefore, have received a smear review regardless of the platelet-associated flags. Of the 7 specimens that did not generate any additional flags, 1 revealed CLP, 1 revealed giant platelets, 1 revealed red cell fragments, and the remaining 4 revealed fibrin strands on smear examination **(Table 4)**. The automated platelet counts from all 4 specimens revealing fibrin strands and the 1 revealing CLP along with fibrin strands were also unexpectedly within the normal range and considered acceptable upon smear review and comparison with the previous and/or follow-up platelet count(s). The automated platelet count of 658 × 10^3/μL from the specimen, which revealed red cell fragments, was also considered acceptable upon smear review and comparison with the previous and/or follow-up count(s). The remaining specimen, with the finding of giant platelets upon smear review, had an automated platelet count of 29 × 10^3/μL and an estimated count of 80 × 10^3/μL, and was, therefore, considered unacceptable. Twenty-three of the 102 blood specimens in this group and 8 of the 36 specimens with positive smear findings were Microtainer specimens (ie, heel-sticks collected in Microtainers).

Table 3_Blood Smear Findings of Group IV

	Group IV (PLT <100x10^3/μL)
# of specimens	463
# of patients	367
# of specimens with:	
"PAD" flag	176
"CLP" flag	3
"PAD" + "CLP" flags	1
# of specimens with non-platelet flags	300
# of specimens with positive smear findings:	9 (1.9%)
Platelet clumps	2
Giant/large platelets (>/= 1+)	2
Schistocytes (>/= 1+)	5
Fibrin strands	0
Cytoplasmic fragments (>/= 1+)	0

Abbreviations: PAD, platelet abnormal distribution; CLP, platelet clumps.

Group IV: Nine of 463 blood smears from specimens with automated platelet counts <100 × 10^3/μL revealed a positive

Table 4_Specifics of Specimens With Positive Smear Findings But No Additional Flags

			Automated Platelet			Platelet Scan Reflex-Ordered By	
	Specimen #	Smear Finding	Count (x10^3/μL)	Acceptability*	Platelet Estimate From Smear (x10^3/μL)	Current Policy	Revised Policy
Group I	1	Giant PLT	5	Unacceptable	50	yes	yes
(PAD)	2	Fibrin	252	Acceptable		yes	no
	3	Fibrin	282	Acceptable		yes	no
	4	Clumps	333	Unacceptable	430-clp	yes	no
Group II	1	Clumps	28	Unacceptable	Norm/Inc-clp (~350-450)	yes	yes
(CLP)	2	Clumps	182	Unacceptable	Normal-clp (~250-300)	yes	yes
	3	Clumps	202	Unacceptable	Normal-clp (~230-300)	yes	yes
	4	Clumps	239	Acceptable		yes	yes
Group III	1	Giant PLT	29	Unacceptable	80	yes	yes
(CLP+PAD)	2	Fibrin	244	Acceptable		yes	yes
	3	Fibrin	263	Acceptable		yes	yes
	4	Clumps and fibrin	282	Acceptable	Normal-clp (~200-300)	yes	yes
	5	Fibrin	363	Acceptable		yes	yes
	6	Fibrin	379	Acceptable		yes	yes
	7	RBC frag	658	Acceptable		yes	yes
Group IV	1	Giant PLT	37	Unacceptable	90	yes	yes**
PLT<140	2	Clumps	44	Unacceptable	Normal-clp (~300-400)	yes	yes**
	3	RBC frag	89	Acceptable		yes	yes**

PAD, platelet abnormal distribution; CLP, platelet clumps.
**as judged by blood smear review.*
Norm, normal; Inc, increased.
***only if either initial result, delta check failure, or previous result unreliable.*
PLT, platelets; RBC frag, red cell fragments; CLP, with occasional clumps.
~ approximately

finding on microscopic examination, resulting in a positive yield of 1.9% (**Table 3**). Among the 9 smears with positive findings, 2 revealed CLP, 2 revealed giant platelets, and the remaining 5 revealed red cell fragments. All of these 9 specimens generated only the PAD flag among the platelet-associated flags. Six of the 9 specimens (66.7%) also generated the non-platelet flag(s), including 4 with WBC-associated flags and 2 with NRBC >/= 2.0. These 6 specimens would, therefore, have received a smear review regardless of the presence of a platelet count <100 × 10^3/μL. Of the 3 specimens that did not generate any additional flags, 1 revealed CLP, 1 revealed giant platelets, and 1 revealed red cell fragments on smear examination (**Table 4**). The automated platelet counts from these 3 specimens were all <100 × 10^3/μL, but, as judged by smear examination, only the count of 89 × 10^3/μL from the specimen, which revealed red cell fragments, was considered acceptable. The specimen revealing giant platelets had an automated count of 37 × 10^3/μL and an estimated count of 90 × 10^3/μL, while the specimen revealing CLP had an automated count of 44 × 10^3/μL and an estimated count of 300-400 × 10^3/μL. Seven of the 463 blood specimens in this group and 1 of the 9 specimens with positive smear findings were Microtainer specimens (ie, heel-sticks collected in a Microtainer).

Comparison of Relative Frequency of Positive Smear Findings Between the Microtainer and non-Microtainer (ie, Lavender-top Tubes) Specimens: As indicated in **Table 5**, the smears from 25 of the total 59 Microtainer specimens (42.4%) revealed positive findings in comparison with 60 of the total 737 non-Microtainer specimens (8.1%). Although a large majority of the smears with positive findings came from specimens in groups II and III regardless of the type of specimen (capillary vs venous) or specimen-container type (Microtainer vs non-Microtainer), the highest percentage of smears with positive findings (57.1%) came from Microtainer specimens in group II.

Discussion

Among the 3 groups of specimens identified by 1 or more PLT-associated flags, the highest percent positive yield of 35.3 was obtained from blood smear examinations of specimens generating both the PAD and CLP flags. Examination of smears from specimens generating only the CLP flag resulted in a slightly lower percent positive yield of 30.0. In contrast, the percent positive yield was only 8.9 from blood smear examination of specimens generating only the PAD flag. The lowest percent positive yield of 1.9 was derived from blood smear examination of specimens with a PLT count <100 × 10^3/μL, irrespective of the flagging status. These findings associated with the Sysmex XE-2100 analyzer, for the CLP flag in particular, are in agreement with the only available reports of efficiency of flagging systems for older models of Coulter analyzer (STKS and GenS) published by our group almost a decade ago.[16-17] To the best of our knowledge, there are no other similar studies published in the recent literature assessing the efficiency of individual flags generated by various analyzers currently in use in laboratories around the world.

The data presented here lends support to the concept of verification of the automated platelet counts by examination of blood smears from specimens flagged for either CLP alone or in combination with PAD but not for specimens flagged for only PAD or those generating an automated platelet count <100 × 10^3/μL. Consequently we have revised our practice/policy for reflex ordering of platelet scans to exclude specimens flagged for PAD alone. Our decision to do so was based on 2 findings: (a) a percent positive yield of less than 10 and (b) the reliability of the automated platelet count results for 99 out of 101 specimens flagged in this way by the analyzer. Among the 2 specimens with unacceptable results were, 1 with an automated platelet count of 5 × 10^3/μL, which would have generated a reflex order for platelet scan according to both the current and the revised policies and another with an automated platelet count of 333 × 10^3/μL, which would not have generated a reflex order of a platelet scan with either the current or the revised policy. The latter, however, is unlikely to have an undesirable effect in patient management because it is within the normal range. Based on the findings of (a) the lowest percent positive yield of 1.9 and (b) the reliability of over 99% of results (459 out of 463), achieved for the group of specimens with platelet count <100 × 10^3/μL, one could also understandably exclude platelet count <100 × 10^3/μL as a criteria for a reflex order of a platelet scan. However, considering (a) the likelihood physicians making quick patient management decisions based on

Table 5_Relative Frequency of Smears With Positive Findings: Lavender-Top Tube Specimens vs Microtainer Specimen

	Lavender-Top Tube			Microtainer		
Group of Specimens	Total #	Smears With Pos* Findings #	Smears With Pos* Findings (%)	Total #	Smears With Pos* Findings #	Smears With Pos* Findings (%)
Group I (PAD)	100	1	(1)	1	0	(0)
Group II (CLP)	102	23	(22.5)	28	16	(57.1)
Group III (CLP+PAD)	79	28	(35.4)	23	8	(34.8)
Group IV PLT<100x10^3/μL	456	8	(1.8)	7	1	(14.3)
All Together	737	60	(8.1)	59	25	(42.4)

*Abbreviations: PAD, platelet abnormal distribution; CLP, platelet clumps. *Pos = positive.*

unverified low platelet counts (eg, ordering an additional work-up including a hematology consult, postponing a surgery, or administering platelet transfusions), which in our experience is higher than desirable and (b) less than ideal sensitivity of the automated flagging system for CLP,[16-17] we decided to keep the platelet count $<100 \times 10^3/\mu L$ as a criterion, but limit it to only the initial result. According to our revised policy, platelet scans will not be performed on follow-up platelet counts that are $<100 \times 10^3/\mu L$ unless accompanied by either a delta check failure or flagged for CLP by the analyzer, and/or platelet clumping noted on previous smear examination. The delta failure limit for our policy is defined as a 50% reduction in the platelet count from the most recent previous result. In our opinion, a delta failure based on an increase in the platelet count has limited value because in clinical practice pseudothrombocytosis is encountered infrequently when compared to pseudothrombocytopenia.

Additionally, our current policy of blood smear examination from all specimens collected in Microtainers will remain unchanged as it is supported by the data showing a higher rate of positive findings among specimens collected by heel-stick in Microtainers when compared to those collected by venipuncture in lavender-top tubes (42.4% vs. 8.1%). Furthermore, it will not impact the operational efficiency of the laboratory because many, if not all, of the automated Diff results from specimens collected in Microtainers, which essentially are limited to heel-sticks from newborn babies and finger-sticks from hematology/oncology patients, are generally flagged for verification by the smear examination or the manual Diff. We did not investigate the outcome of platelet scans performed on specimens flagged by the analyzer for giant/large platelets because of its relatively very low frequency of occurrence. Consequently, the revised policy continues to include giant/large platelets flag as a criteria for reflex order of a platelet scan.

The implementation of the first part of our revised policy, ie, exclusion of the PAD flag as a criterion for reflex ordering of a platelet scan, has resulted in a 20% reduction in the number of platelet scans performed daily in our laboratory. Based on the current ratio of initial to non-initial platelet counts and the latter with or without delta failures, an additional 40% reduction in the number of platelet scans performed everyday in our laboratory is expected with the implementation of reflex ordering of platelet scans with only an initial platelet count $<100 \times 10^3/\mu L$. This second part of the revised policy is anticipated to coincide with an upgrade of our Sysmex automated hematology system over the next few months.

The use of optical methodology for platelet counting has been favored over the impedance method by some [18-20] but the superiority of 1 method over the other in terms of reliability of the results and the fraction of specimens requiring verification by smear examination, is debated by others.[3,8] In our experience with selective use of optical method of XE-2100 on specimens with the smear findings of significant number of schistocytes (1+ or greater), we have been able to obtain acceptable platelet counts on some, but not all, specimens. In contrast, we have rarely, if ever, obtained acceptable automated platelet counts by either impedance or optical method available on the XE-2100 on specimens revealing a significant number of giant platelets (1+ or greater). These observations vouch for the need of smear examination for verification of automated platelet counts on appropriately selected specimens irrespective of the methodology used for obtaining the counts.

In conclusion, optimization of criteria for verification of automated platelet counts generated by the Sysmex XE-2100 hematology analyzer was achieved by 2 policy modifications. The first modification consisted of eliminating the PAD flag from the list of criteria for generating a reflex order for a platelet scan. The second and more significant modification comprised of performing a platelet scan only from specimens that are either (a) flagged for CLP with or without a PAD flag, (b) reveal a platelet count of less than $100 \times 10^3/\mu L$ at the initial request for such a test, and/or (c) fail a platelet count delta check (defined as a 50% reduction in the platelet count from the most recent previous result). The current policy of performing platelet scans from specimens flagged for giant/large platelets and all specimens collected in Microtainers remains unchanged. This revised policy offers a means to improve efficiency without adversely affecting the quality of patient care. LM

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