Elimination of Instrument-Driven Reflex Manual Differential Leukocyte Counts

Optimization of Manual Blood Smear Review Criteria in a High-Volume Automated Hematology Laboratory

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Abstract

Automated peripheral blood leukocyte differential counts (LDCs) are widely accepted in routine practice. However, many laboratories still reflexively perform manual LDCs based solely on abnormal automated results or instrument “flags,” before any manual triage step. We describe our transition to a procedure that uses manual methods to validate, rather than to replace, automated LDCs (an approach recommended early in the development of automated methods, but still not used in many clinical laboratories). Manual microscopic scans were performed in lieu of manual LDCs. Each scan that revealed cell types not quantifiable by the instrument triggered a manual LDC. However, if the manual scan simply confirmed the cell types seen on automated LDC, then the automated result was released, even if clinically significant quantitative abnormalities were present. This policy reduced manual LDCs by more than 70% and was validated by a manual retrospective audit.

Patient care and laboratory operations can be optimized by using manual microscopic examination as a validation procedure rather than as a reflexive substitute for automated methods. There is no clinical rationale for reflex performance of manual LDCs based solely on instrument warnings.

The accuracy and clinical usefulness of automated leukocyte differential counts (LDCs) have been validated in numerous studies. In most automated laboratories, manual differential counts are limited to cases in which instrument “flags” indicate the potential presence of cells not reliably identifiable by automated methods (such as blasts or other immature granulocytes) or of findings that may interfere with automated analysis (such as overlap in the distribution of different cell types or interference from matrix components such as cryoglobulin). Since the inception of automated differential counting methods, investigators have recommended the use of manual blood smear review as a validation of, rather than as a replacement for, automated methods. Although some clinical laboratories adhere to this principle, many laboratories continue to participate in the bias toward manual LDCs by building rules by which manual counts replace automated counts based solely on the presence of certain automated findings, before any manual scanning or triage. In essence, many laboratories use manual counts as reflexive substitutes for automated methods, rather than as validation steps.

Reflexively ordering manual LDCs based on instrument flags can be clinically problematic. For instance, an instrument flag may be issued for suspicion of cell types not actually in the blood sample, resulting in the manual LDC as a simple validation of the automated result, but with the release of the less accurate and less precise manual result. Alternatively, quantitative flags may trigger manual LDCs when the only finding at issue is confirmation of the correct qualitative identification of the quantitatively abnormal cell type. This arbitrary replacement of automated LDCs by manual LDCs can have clinical repercussions, particularly when clinical decisions are heavily dependent on accurate and precise absolute neutrophil counts or other LDC results.
We describe our transition to a system that eliminated the reflexive performance of manual LDCs based on instrument flags and replaced reflex manual LDCs with a manual microscopic triage system. Manual LDCs were reserved for those cases in which the microscopic triage resulted in the discovery of cell types or findings not quantifiable by the instrument. Our purpose was to design a system that directed the use of automated and manual LDCs toward greatest clinical benefit and optimized laboratory operations by fully using the potential of automated methods and permitting medical technologists to focus on tasks not achievable by these methods.

**Materials and Methods**

The University of Michigan (Ann Arbor) Medical Center hematology laboratory performs more than 340,000 CBC counts and more than 180,000 LDCs per year. Samples are run on 1 of 4 Beckman-Coulter GenS analyzers (Hialeah, FL). As with other systems, the GenS system provides a 5-part LDC (including neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and uses an array of flags that serve as signals of the potential need for manual review of automated results. “Suspect” flags are built into the instrument technology and generally are based on aberrations in the distribution of cell populations or the detection of potential interference with these distributions (eg, suspect blasts, suspect immature granulocytes). “Definitive” flags are user-defined and generally are based on quantitative thresholds defined by each individual laboratory (eg, lymphocytosis, macrocytosis).

**Previous Manual Review Policy**

Before implementing our revised manual review policy, our standard procedure included instrument criteria by which blood smears should be microscopically scanned before determining the appropriateness of a manual LDC and instrument criteria by which a manual LDC should be ordered reflexively, without initial microscopic scan. The process is illustrated in Figure 1, and specific criteria are outlined in Table 1.

**Table 1**

|---|
| **Scan Slide**
| MCV ≥115 µm³ (≥115 fL) | Manual LDC
| “Suspect” and definitive flags as follows: | Dots (::::) appear for automated differential parameters
| Anemia (hemoglobin <8.0 g/dL [80 g/L]) | R flag for automated differential percentages
| Anisocytosis >T+ (RDW >17) | Suspect and definitive flags as follows:
| Eosinophilia (>1,500/µL >1.5 x 10⁹/L) | Basophilia
| Hypochromia >1+ (MCH <21 pg [<21 pg]) | Blasts
| ImmNE2 and WBC count ≤15,000/µL (≤15.0 x 10⁹/L) | Dimorphic RBC population
| Leukocytosis (WBC count >50,000/µL >50.0 x 10⁹/L) | Giant or large platelets
| Microcytosis >1+ (MCV <77 µm³ [≤77 fL]) | ImmNE2 and WBC >15,000/µL (>15 x 10⁹/L)
| NRBCs | Lymphocytosis (>8,000/µL >8 x 10⁹/L)
| Thrombocytopenia (platelet count <50 x 10⁹/µL [≤50 × 10⁹/L]) | Micro/fragmented RBCs

**LDC**, leukocyte differential count; **ImmNE2**, suspect flag alerting to the possibility of immature granulocyte forms; **MCH**, mean cellular hemoglobin; **MCV**, mean cellular volume; **micro/fragmented RBCs**, suspect flag alerting to the possible presence of microcytic or fragmented RBCs; **NRBC**, suspect flag alerting to the possibility of nucleated RBCs; **RDW**, RBC distribution width.

| **Manual LDC**
|---|
| Dots (::::) appear for automated differential parameters
| R flag for automated differential percentages
| Suspect and definitive flags as follows:
| Basophilia
| Blasts
| Dimorphic RBC population
| Giant or large platelets
| ImmNE2 and WBC >15,000/µL (>15 × 10⁹/L)
| Lymphocytosis (>8,000/µL >8 × 10⁹/L)
| Micro/fragmented RBCs
| Microcytosis (>2,000/µL >2.0 × 10⁹/L)
| Pancytopenia
| RBC agglutination
| Review slide
| Variant lymphocytes
| WBC count ≥500/µL (≥0.5 × 10⁹/L) and ≤1,000/µL (≤1.0 × 10⁹/L) (buffy coat) differential count

* Conditions requiring manual microscopic scan of peripheral blood smear before release of the automated LDC.

* Conditions requiring manual LDC based solely on instrument flags.

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In our laboratory, medical technologists perform manual microscopic scans using a 50× oil-immersion lens. The exact extent of the scan is left to the professional discretion of the individual technologist based on the leukocyte count and distribution on the slide being scanned.14 Technologists are strongly encouraged to evaluate at least as many cells as they would evaluate for a manual LDC. The purpose of the scan is to evaluate for discordance between instrument-reported values and manual microscopic findings; the scan is not limited to an evaluation of the “flagged” result.

**Design of Revised Manual Review System**

The revised system is illustrated in **Figure 2**. Under the revised system, criteria for the instrument-driven reflexive performance of manual differential counts were eliminated, except when the instrument was unable to issue any differential result owing to marked interference (so-called dot-out results) **Table 2**. The specific purpose of the microscopic scan was to identify blood smear findings that the automated system is incapable of formally quantifying or reporting (eg, immature granulocytes, blasts, lymphoma cells). If the criteria outlined in **Table 3** were met based on the microscopic scan, then the automated differential count was canceled and replaced by a 100-cell manual differential count. If the criteria outlined in Table 3 were not met, then the automated LDC was validated and released. For patients with multiple orders over time, criteria were applied independently to each sample for which an LDC was ordered, regardless of previous findings.

Since there are no evidence-based guidelines regarding the level at which immature granulocytes (metamyelocytes and myelocytes) become clinically significant,11,19 the arbitrary limit of greater than 2 immature granulocytes discovered on scanning was chosen for the performance of a manual differential count. If 1 or 2 immature granulocytes (other than promyelocytes or blasts) were seen on scan, the technologist performing the scan was instructed to release the automated LDC with the following comments, as applicable: “Rare metamyelocyte seen on scan,” or “Rare myelocyte seen on scan.” The same threshold rule also was applied to the presence of nucleated RBCs. The finding of even a single promyelocyte or a single blast on scanning triggered the ordering of a manual LDC.

**Retrospective Audit of Slides Scanned and Released Under New Policy**

Once the policy was implemented, the performance of the new system was analyzed by a series of audits involving a total of 204 cases for which manual scans were performed but for which the automated LDC was released. The purpose of the auditing process was to determine whether, and to what extent, clinically useful information was being withheld by the new manual review procedure. Each of the 204 cases was subjected to a 100-cell manual LDC (the standard manual LDC in our laboratory). The result of this manual LDC was subjected to the criteria outlined for the performance of a manual LDC under the new system of manual scanning. If the audited manual LDC result included findings that would have triggered a manual LDC under our revised scanning rules, this was noted as a discordant result. Quantitative comparisons between automated and manual LDCs were made using the Student t test and Pearson correlation coefficients.

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### Table 2


<table>
<thead>
<tr>
<th>Scan Slide*</th>
<th>Manual LDC†</th>
</tr>
</thead>
<tbody>
<tr>
<td>All criteria for scan slide and manual LDC noted in Table 1</td>
<td>Dots (::::) appear for automated LDC parameters</td>
</tr>
<tr>
<td>Unusual scatter plots from instrument LDC‡</td>
<td></td>
</tr>
</tbody>
</table>

LDC, leukocyte differential count.

* Conditions requiring manual microscopic scan of peripheral blood smear before release of the automated LDC.

† Conditions requiring manual LDC based solely on instrument flags.

‡ “Unusual scatter plots” were at the technologist’s discretion, with general guidelines provided in the laboratory procedure manual.
LDC, leukocyte differential count.

“Suspect” cells are observed, including the following:

- Any blasts or promyelocytes
- Immature granulocytes, including the following:
  - >2 metamyelocytes (if ≤2, include comment with automated LDC result)
  - >2 myelocytes (if ≤2, include comment as above)
  - >2 metamyelocytes/myelocytes combined (if ≤2, include comment as above)
- Variant (“atypical”) lymphocytes
- >2 nucleated RBCs (if ≤2, include comment as above)

When scanning slides, a manual LDC is indicated when:

Automated LDC percentages seem inaccurate (technologist discretion)

Table 3

<table>
<thead>
<tr>
<th>Reason for Scan (Instrument Flag)</th>
<th>Manual LDC Under Old Rules</th>
<th>Oversight Noted on Audit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspect blasts† (n = 34)</td>
<td>34 (100)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>ImmNE2† (n = 27)</td>
<td>14 (52)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>Monocytosis† (n = 7)</td>
<td>7 (100)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Lymphocytosis† (n = 9)</td>
<td>9 (100)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Variant lymphocytes† (n = 8)</td>
<td>8 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>WBC interference‡ (n = 13)</td>
<td>6 (46)</td>
<td>3 (23)</td>
</tr>
<tr>
<td>Miscellaneous† (n = 10)</td>
<td>1 (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>RBC or platelet flags only</td>
<td>28 (29)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Total (n = 204)</td>
<td>107 (52.%)</td>
<td>15/204 (7.4)</td>
</tr>
</tbody>
</table>

LDC, leukocyte differential count.

Retrospective Recount of Previous Manual LDCs

For comparison, we also performed repeated manual LDCs on 100 cases for which manual LDCs had been done based on the criteria outlined in the new policy.

Results

A summary of the retrospective audit findings is given in Table 4.

Discordant Findings

The audit of 204 cases that had been previously scanned and released without manual LDC revealed 15 cases (7.4%) that should have had manual LDCs ordered under the new system. Of these 15 cases, 10 would have qualified for reflexive manual differential counts under the old system. For 12 of these 15 cases, the discordance was due to the presence of 3% or more metamyelocytes and/or myelocytes, including 11 cases with 5% or fewer metamyelocytes and/or myelocytes and 1 with 8% metamyelocytes. The remaining 3 discordant results were for the presence of blasts (1 case), promyelocytes (1 case), and greater than 2 nucleated RBCs (1 case). The sample in which blasts were overlooked was obtained from a patient on the second day of induction chemotherapy for acute myeloid leukemia. A manual LDC had been performed on this patient approximately 14 hours earlier and had reported 2% blasts. There were no clinical repercussions related to this particular oversight.

Of the 204 audited cases, 107 (52.5%) would have qualified for reflexive manual LDC by the old rules, as outlined in Table 4. Overall, the revised policy resulted in a decrease in manual LDCs of more than 70%, from an average of approximately 125 per day (or about 24% of LDC orders) to an average of approximately 35 per day (or about 6% of LDC orders).

The recount of 100 previously performed manual LDCs resulted in discordant results in 13 cases (13.0%), using oversight definitions analogous to those applied to the audit of scanned and released cases. The interobserver discordant results for these manual LDCs included 11 cases in which blasts and/or promyelocytes were seen in one but not the other manual LDC and 2 cases in which more than 2 metamyelocytes and/or myelocytes were seen in one manual LDC when the other manual LDC revealed no immature granulocytes.

Quantitative Discordance

One of our criteria for performance of a manual LDC under the new policy was the individual technologist’s perception of a quantitative mismatch between the automated LDC and what was observed on the blood smear. This is, by definition, a subjective evaluation. Nevertheless, we analyzed potential quantitative discordance between manual and automated neutrophil counts and lymphocyte counts in the audited sample of scanned slides released as automated LDCs. Overall, quantitative concordance between automated and manual results in this sample was excellent ($R^2 = 0.87$ for neutrophil percentage [$P < .0001$]; and $R^2 = 0.90$ for lymphocyte percentage [$P < .0001$]).

Within the group of 204 audited cases, special note was made of cases flagged for interference in the WBC channel (“*” flags; n = 15) and cases flagged for interference among LDC population distributions (R flags; n = 8). The difference between automated and manual counts (calculated as a percentage of the automated LDC result) was not significantly higher for either neutrophils or lymphocytes in the group of WBC “*” flagged cases. The difference between
automated and manual neutrophil counts was significantly higher in cases with LDC R flags ($P = .017$), but this was attributable solely to 1 outlier that showed a difference of more than 300% between automated (10.2% [0.10]) and manual (43% [0.43]) neutrophil values. The difference between automated and manual lymphocyte values was significantly higher in cases with LDC R flags (44% [0.44] vs 28% [0.28]; $P = .004$).

**Impact on Laboratory Operations**

It is important to note that the revised policy was implemented not for the sake of expediency but to assure performance of the tests that would be of greatest benefit to patient care. Nevertheless, we noted an impact of the new policy on laboratory operations. The average turnaround time for LDC orders showed a modest decrease (ranging from 3 to 10 minutes in a limited retrospective sampling). However, there was strong subjective evidence of increased technologist productivity with the new policy. Specifically, the manual review bench, which required 2 full-time technologists, was redesigned to be run by 1 full-time technologist and 1 technologist rotating between that bench and the stat oncology review bench, which required 2 full-time technologists, was eliminated not for the sake of expediency but to assure performance and not any abnormal cells, there is no need to invalidate the automated result. The approach we adopted attempts to optimally apply automated and manual LDCs to maximize the clinical value added to patient care by hematology laboratory test results.

The revised policy for manual review of LDCs resulted in savings of technologist time. This may seem counterintuitive, since the manual scan should, in theory, be at least as thorough as the manual differential count. However, the performance of formal manual LDCs requires cancellation and reordering of tests in the laboratory information system, physical documentation of 100 cells on a keypad, entry of these data into the laboratory information system, and validation of the manual result. The time savings under the revised policy presumably are attributable to the elimination of these administrative steps. Subjectively, the technologists reported a strong preference for performing scans over manual LDCs.

In the present study, the presence of metamyelocytes and/or myelocytes in peripheral blood smears was problematic. Since there are no guidelines regarding the clinical importance of low numbers of immature granulocytes in LDCs, we arbitrarily determined that 1 or 2 metamyelocytes and/or myelocytes on a manual scan did not warrant cancellation of the automated LDC, but instead warranted a separate comment indicating the presence of these cells. Subsequently, 80% of the discordant results found in a retrospective audit were due to the presence of these immature granulocytes, generally in quantities that fell near the borderline of our decision criteria. There is no evidence that these low numbers of immature granulocytes add independent clinical value over the accurate determination of an absolute neutrophil count (a function better performed by automation); furthermore, our policy mandates a qualitative comment alerting clinicians to the presence of these cells. In addition, a recount of previously performed manual LDCs
resulted in a discordance rate substantially higher than that noted on the manual audit of slides that had been scanned and released without manual LDC. Therefore, we do not consider the audited findings sufficient to invalidate the approach outlined herein. It will be important for future studies to focus on setting thresholds for the clinical importance of immature granulocyte forms, particularly with the advent of automated hematology analyzers that have the capability to accurately measure these cells.

Our audit also revealed 1 case in which blasts were present, but manual review resulted in release of the automated result. This was an unfortunate error in cell identification by the operator and, therefore, would not have been solved by issuing a manual LDC. Of note, the case in question was marked by the instrument with a definitive “mono- cytosis” flag. In our experience and the experience of others, such flags can indicate the presence of abnormal cells, even in the absence of instrument-generated suspect flags.11

Quantitative discordant results in our audited sample were more challenging to evaluate, since it is difficult to assess the relevance of numeric differences between 100-cell manual LDCs and 8,000-cell automated LDCs. Nevertheless, outliers were noted and were weighted toward (although not consistently associated with) interference flags for LDC percentages (R flags). Therefore, although quantitative correlations generally were excellent, technologists in our laboratory are now advised to be particularly diligent in examining for potential quantitative LDC discordant results when these R flags are present.

An important finding of our study was that almost half of the cases selected for manual review by the instrument were flagged for RBC or platelet abnormalities only, without evidence of interference with the total WBC count or LDC. Four such cases were found in our audit to harbor immature granulocyte forms; all 4 were associated with RBC interference flags (the NRBC [nucleated RBC] flag, or the micro/fragmented reds [microcytic/fragmented RBCs] flag), but were not associated with purely quantitative flags such as anisocytosis, microcytosis, or macrocytosis. This study focused on the prudent application of automated and manual methods for LDCs and not on RBC morphologic features. Nevertheless, our findings highlight the impact of RBC flags on the manual review process. The specifics of when and how to manually evaluate RBC morphologic features in response to instrument RBC flags is somewhat controversial, and the approach varies among laboratories.25-27 We observed, however, that quantitative RBC flags that were not related to interference with cell population distributions did not affect the LDC result. Therefore, careful consideration should be given to the need for routine manual scrutiny of leuocyte populations in cases flagged solely for RBC index abnormalities.

Clinical hematology laboratories can optimize patient care by using manual methods for validation, rather than replacement, of automated methods. The manual LDC should be ordered only following the determination of its appropriateness by a triage method such as manual microscopic scan of a peripheral blood smear. There is no clinical rationale for reflex performance of manual LDCs based solely on quantitative or qualitative abnormalities reported by automated methods.

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