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Purpose and Criteria for Blood Smear Scan, Blood Smear Examination, and Blood Smear Review

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A microscopic examination of an appropriately prepared and well-stained blood smear by a knowledgeable laboratory professional is necessary and clinically useful in a number of circumstances and for a variety of reasons. In this article, an attempt is made to delineate the purpose and criteria for blood smear examination in a variety of circumstances that are encountered in everyday laboratory hematology practice. A blood smear scan serves to at least (a) verify the flagged automated hematology results and (b) determine if a manual differential leukocyte count needs to be performed. Blood smear examination/manual differential leukocyte count with complete blood count (CBC) provides the complete hematologic picture of the case, at least from the morphologic standpoint. Blood smear review with or without interpretation serves to ensure that no clinically significant finding is missed, besides providing diagnosis or diagnostic clue(s), particularly if and when interpreted by a physician.

Key Words: Blood smear scan, Blood smear examination, Blood smear review

INTRODUCTION

The most commonly performed test in a clinical hematology laboratory is a complete blood count, generally referred to as CBC, or infrequently as Hemogram. The second most commonly performed hematologic test is what is traditionally called differential leukocyte count or DIFF. Currently available automated hematology analyzers are capable of performing both of these tests fairly reliably, efficiently, and cost-effectively [1-5]. A microscopic examination of an appropriately prepared and well-stained blood smear by a knowledgeable laboratory professional is, however, necessary and clinically useful in a number of circumstances and for a variety of reasons [6-10]. The microscopic examination may be limited to a blood smear scan or may include a complete blood smear examination with manual differential leukocyte count and/or a blood smear review. In this article, we have attempted to define and delineate the purpose and criteria for each of these 3 types of smear examination, as practiced by professionals in hematology laboratories around the world.

THREE TYPES OF SMEAR EXAMINATION

1. Blood Smear Scan (BSS)
   Synonyms: platelet scan, platelet estimate, blood smear examination without a DIFF.
   A BSS is usually performed to verify the automated platelet count, particularly if it is flagged by the analyzer for verification or if it is significantly lower than the lowest limit of the reference range. Many laboratories opt to verify the automated platelet count when it is below 100×10⁹/L on a new patient or when a delta-check fails with a significant drop in the platelet count (≥50% drop) on follow-up blood counts. Verification of platelet count below 100×10⁹/L is important because pseudo-thrombocytopenia of this magnitude may unnecessarily trigger a hematology consult, additional laboratory work-up, postponement of surgery/special procedure, and/or a platelet transfusion. Additional reasons to perform a blood smear scan include (a) verification of the remaining CBC results that are flagged by the analyzer, (b) to determine if the automated DIFF result that is
flagged by the analyzer is reliable and thereby reportable or a manual DIFF needs to be performed and reported instead of the automated DIFF, and (c) to determine the suitability of the smear and its staining quality for the manual DIFF and to select the area for performing the manual DIFF, if needed. It is usually performed by a technical person in the laboratory.

For verification of the platelet count, the entire blood smear, including the feather edge, lateral edges, readable area and thick area, should be examined first under 10× dry objective (i.e. magnification of ×100) looking for clumps of platelets. Large clumps are easily discernible under this magnification but small clumps may not be clearly visible, thereby prompting examination under higher magnification, which may be 40× dry objective (i.e. ×400 magnification), 50× oil immersion objective (i.e. ×500 magnification) or 100× oil immersion objective (i.e. ×1,000 magnification). While examining under higher magnification, it is important to note if red cell fragments, organisms (bacteria and fungi), and/or giant platelets are present in significant number (more than occasional). The presence of significant number of red cell fragments is frequently associated with falsely high platelet count whereas the presence of significant number of giant platelets is often associated with falsely low platelet count [11, 12]. The presence of bacteria and/or fungi has been associated with falsely high platelet counts [13-16]. If clumps are present, the automated platelet count is often unreliable and consequently not reportable. In such cases, a platelet scan should be reported in qualitative terms as normal with clumps, increased with clumps or decreased with clumps.

To obtain a reliable platelet count on specimens revealing platelet clumps on blood smear, it is necessary either to (a) vortex the specimen for 1 to 2 min at the highest speed and rerun through the analyzer or (b) collect the specimen in a citrated tube (blue-top vacutainer tube) instead of EDTA-anticoagulated tube (lavender-top tube) and run through the analyzer. The former procedure is successful in disaggregating the platelet clumps and consequently generating a reliable platelet count, as judged by platelet estimate from the smear made from the vortexed specimen, in approximately 50% of cases [17]. In the other 50% of cases the disaggregation of platelet clumps is either incomplete or does not occur. The use of citrated blood specimen to count platelets is successful in over 90% of cases but understandably requires subjecting the patient to another venipuncture and multiplication of the platelet count by 1.1 for the citrated blood dilution factor, prior to reporting. In the absence of clumping or in cases with rare small platelet clumps, an estimated platelet count may be obtained by determining either (a) the average number of platelets per field under 100× oil immersion objective (i.e. ×1,000 magnification) and multiplying it by 15 (range of multiplication factors used by various laboratories varies between 10 and 20 for manually made wedge smears) or (b) the highest number of platelets in a field in the readable area of the smear under 100× oil immersion objective (i.e. ×1,000 magnification) and multiplying it by 10. The latter has worked fine for me personally with smears made and stained by automated smear makers/stainers (SP 100 and SP-1000) from Sysmex Inc. (Mundelein, IL, USA).

Based on our experience and a review of the literature on performance evaluation of currently available analyzers, we suggest that the criteria for a blood smear scan should include (a) CBC and/or DIFF results flagged by the automated analyzers for verification, (b) initial platelet count below 100×10⁹/L, whether flagged or not flagged by the analyzer, (c) follow-up platelet counts of over 30×10⁹/L with delta failure flag indicating ≥50% drop in the count, and (d) when the analyzer generates any one or more of these flags: platelet clumps, giant/large platelets, red cell fragments, and qualitative white cells-associated flags (also called morphologic, suspect, or interpretive flags), such as blasts, atyps, immature granulocytes, and left shift. The reason for the inclusion of criteria (c) is the clinical suspicion of heparin-induced thrombocytopenia in a majority of such cases. A delta failure flag associated with an increase in platelet count is not included in the criteria, however, because (i) its commonest cause is platelet transfusion with the expected rise in count, and (ii) it is a frequently encountered finding in daily laboratory hematology practice, at least in large medical centers.

If the smear scan reveals the presence of immature/abnormal/atypical white cells, a manual DIFF is generally reflex-ordered, performed, and resulted in place of the automated DIFF. Whether or not every smear with even one immature/abnormal/atypical cell needs a manual DIFF, irrespective of being the initial one or a follow-up one, is debatable. In our opinion, initial smears with a rare but clearly identifiable cell, such as a blast, a hairy cell, or a myeloma cell, or a suspect but not clearly identifiable cell, needs a manual DIFF. In the presence of other types of immature/abnormal/atypical cells, however, laboratories may choose to perform manual DIFF only if their number exceeds a predetermined threshold. While performing a blood smear scan, a notation should also be made of all significant morphologic abnormalities, if present, particularly if it is the initial smear examination of a patient at each admission or an infrequent outpatient visit.
2. Blood Smear Examination (BSE)

Synonyms: manual DIFF, DIFF.

BSE, traditionally referred to as DIFF or manual DIFF, is generally performed by a member of the technical staff of the clinical hematology laboratory. It typically includes a 100-cell DIFF and evaluation of morphology of blood cells (red cells, white cells and platelets), in addition to verification of the flagged automated CBC results and a platelet estimate. Clinical laboratories with the state-of-the-art hematology analyzers usually perform the blood smear examination only when (a) automated DIFF result is considered unreliable and thereby un-reportable, based on either the flags generated by the analyzer, morphologic, suspect or interpretive flags in particular, and/or by performing a blood smear scan, (b) a clinician specifically so requests, and/or (c) certain criteria developed by the clinical laboratory are met. The laboratory-developed criteria are generally based on the patient population served and the clinical significance of the abnormal CBC and/or automated DIFF results. Additional factors, such as the reliability of the automated flagging system and overall workload and the staffing-level of the laboratory, may also influence the criteria development process. Guidelines proposed by the International Consensus Group for Hematology Review are available at www.ISLH.org [10] for individual laboratories to consider while developing their own set of criteria. The laboratory-developed criteria may include numerical results and/or qualitative flags generated by the automated analyzers. Numerical results may include both the CBC and the DIFF results, whether flagged or not by the analyzer for verification. The list of criteria used in the Clinical Hematology Laboratory at Thomas Jefferson University Hospital in Philadelphia, Pennsylvania, USA is provided here as an example of a set of laboratory-developed criteria (Table 1). Any and all of these criteria may be modified to meet the needs of the patient population served at a given institution. At our institution, we have also modified this set of criteria a few times over the years based upon the changes in the patient population, the workload and the automated hematology system used for the CBC and the DIFF.

From the clinical standpoint, blood smear examination serves 3 important objectives. First, it serves as a quality control tool in verifying the results generated by the automated analyzers. Second, it allows for identification of abnormal/immature/atypical cells, if present. Third, it allows for recognition of clinically significant morphologic abnormalities, which the analyzers are incapable of either flagging or detecting and identifying. Currently available automated hematology analyzers do not generate any reportable information about the presence of many of the red cell abnormalities (elliptocytes/ovalocytes, target cells, sickle cells, acanthocytes, echinocytes, SC crystalloids, stomatocytes, tear

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**Table 1.** Criteria for blood smear scan and/or blood smear examination at Thomas Jefferson University Hospital

<table>
<thead>
<tr>
<th></th>
<th>Adults</th>
<th>Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Based on CBC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (×10^9/L) Initial*</td>
<td>&lt;2.0 or &gt;30.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Or Delta failure of -300% for WBC of 0.1 to 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+300% for WBC of &gt;10</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL)            Initial</td>
<td>&lt;8 (for ≥8 days old)</td>
<td>&lt;14 (for 0-7 days old)</td>
</tr>
<tr>
<td>(mmol/L)             Initial</td>
<td>&lt;5.0</td>
<td>&lt;8.7</td>
</tr>
<tr>
<td>RBC (×10^12/L) Initial</td>
<td>&gt;6.0 (for females over 7 days old)</td>
<td>&gt;6.5 (for males over 7 days old)</td>
</tr>
<tr>
<td>MCV (fL)             Initial</td>
<td>&lt;60 or &gt;110 (for &gt;7 days old)</td>
<td>&lt;85 or &gt;125 (for 0-7 days old)</td>
</tr>
<tr>
<td>PLT (×10^9/L)        Initial</td>
<td>&lt;100 or &gt;999 or &gt;30 with delta failure of -50% or greater</td>
<td></td>
</tr>
<tr>
<td>NRBC (per 100 WBC)   Initial</td>
<td>&gt;2 (for over 7 days old)</td>
<td>&gt;50 (for 0-7 days old)</td>
</tr>
<tr>
<td><strong>B. Based on automated DIFF results</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (×10^9/L) Initial</td>
<td>&gt;7.0 (for &gt;14 yr old)</td>
<td>&gt;10.0 (for 1-14 yr old)</td>
</tr>
<tr>
<td>Monocytes (×10^9/L)   Initial</td>
<td>&gt;3.0</td>
<td>&gt;14.0 (for &lt;1 yr old)</td>
</tr>
<tr>
<td>Eosinophils (×10^9/L) Initial</td>
<td>&gt;2.0</td>
<td></td>
</tr>
<tr>
<td>Basophils (×10^9/L)   Initial</td>
<td>&gt;0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Qualitative Flags</strong></td>
<td>WBC abnormal scattergram, immature granulocytes, Left shift, Atypical lymphocytes, Blasts, NRBC</td>
<td></td>
</tr>
</tbody>
</table>

*Initial: first smear on a new patient per admission or an infrequent outpatient visit.

Abbreviations: CBC, complete blood count; WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; PLT, platelet; NRBC, nucleated red blood cell; DIFF, differential leukocyte count.
drop cells, rouleaux, Howell Jolly bodies, Pappenheimer bodies, basophilic stippling, intraerythrocytic organisms, etc), white cell abnormalities (Auer rods, toxic granulation, toxic vacuolization, Dohle bodies, hypogranular/agranular granulocytes intraleukocytic organisms, etc), and platelet abnormalities (platelet satellitosis, abnormal granulation, and hypogranulation/agranulation bizarre platelets). The analyzers are fairly reliable, but not 100% sensitive and 100% specific, in generating (a) platelet clump flag in the presence of platelet clumping and (b) red cell agglutination flag in the presence of red cell agglutination. However, none of the analyzers currently generate any flag to indicate the presence of white cell clumps. In the face of these limitations of the automated analyzers, it would be ideal but perhaps neither practical nor cost-effective to include a blood smear scan or blood smear examination on either (a) every new patient, irrespective of CBC and DIFF results being normal or abnormal or (b) at least on those with any level of abnormality in any one or more parameters of CBC and/or DIFF.

A complete blood smear examination, like the blood smear scan, begins with a visual inspection for acceptable quality of the smear and the stain and for absence of macroscopic scratches and stain precipitate(s). An acceptable smear is then examined under 10× dry objective (×100 magnification), first to recheck the stain quality and then to look for (a) clumps of platelets, white cells and red cells, (b) extracellular organisms (microfilaria), (c) cryoprecipitate(s), (d) rouleaux formation, and (e) fibrin strands. All areas of the smear (feather edge, lateral edges, thin readable area next to the feather edge, and the thick area away from the feather edge) are examined at this magnification. The findings at ×100 magnification are confirmed by examining the smear at higher magnification, either under 40× dry objective (×400 magnification), 50× oil objective (×500 magnification), and/or under 100× oil objective (×1,000 magnification). All of these findings have clinical relevance. The clinical relevance of platelet clumping has been described above under the blood smear scan.

The presence of white cell clumps, an infrequently encountered finding, is generally associated with infectious conditions (clumping of neutrophils in particular) and/or lymphoproliferative disorders (clumping of lymphocytes in particular). It may cause a false decrease in the white blood cell (WBC) count and may cause difficulty in obtaining reliable DIFF results. To obtain reliable WBC result in such cases, one may attempt to either incubate the EDTA-anticoagulated blood specimen at 37°C for 10-15 min or vortex it at the highest speed for 1-2 min, prior to re-running through the analyzer. Alternatively, the blood specimen may be collected in citrated tube (blue-top tube), run through the analyzer, and the WBC result multiplied by 1.1 for the citrated blood dilution factor, prior to reporting. If all interventions fail, one may report the WBC result with a comment that the result may be unreliable due to clumps.

Red cell agglutination indicates the presence of cold-reacting red cell agglutinin(s) and it causes a false increase in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and a false decrease in the red blood cell (RBC) count and Hct. It may also cause a false increase in mean corpuscular volume (MCV). Prewarming of the EDTA-anticoagulated blood specimen at 37°C for 10-15 min is required to obtain reliable CBC results, the red cell parameters of the CBC in particular.

Cryoproteinemia has been associated with falsely increased WBC counts, falsely increased platelet counts, or both. Pre-incubation of cryoproteinemnic blood specimen at 37°C for 10-15 min often yields reliable CBC results, the WBC count in particular. Red cell rouleaux, if seen in the thin readable area of the smear, is indicative of elevated levels of fibrinogen and/or globulins. It is often associated with inflammatory or infectious conditions and/or malignant disorders, such as multiple myeloma and macroglobulinemia. It usually does not adversely affect the CBC and DIFF results. The presence of fibrin strands reflects questionable specimen integrity and is therefore a justification for withholding or cancelling the reported CBC results, the platelet count in particular. Damaged leukocytes, primarily granulocytes, denuded of their cell wall, with somewhat fuzzy-appearing red cells on the smear, are suggestive of hyperlipidemia. Severe hyperlipidemia causes a false increase in hemoglobin, MCH and MCHC. Reliable CBC results, the Hb, MCH and MCHC in particular, can be obtained from hyperlipidemic specimens by centrifuging the EDTA-anticoagulated blood specimen, replacing the plasma with an equal amount of isotonic diluent, and re-running through the analyzer.

After verifying the flagged automated CBC results and determining if the manual DIFF needs to be performed based upon the initial smear scan findings, typically a 100-cell DIFF is performed. Each white cell type is identified and classified into individual cell category until 100 white cells are counted. Total number of white cells counted may vary from the traditional 100-cell count if and when the WBC count is either lower than normal or greater than normal. One may count as low as 25 white cells or as high as 200 or 300 white cells depending upon the WBC count. Manual counters, laboratory information system (LIS)-based DIFF keyboards, and/or automated neural-network-based
systems (such as Cellavision) may be used to perform the DIFF. The results have traditionally been reported as a percentage of each cell-type but many, if not all, LIS-based DIFF keyboards can be set-up to also automatically calculate and report the absolute number of each cell-type, in addition to the results in percentage. The absolute numbers are generally considered more valuable because they reflect absolute and thereby the true increase or decrease in the number of each cell type. However, in the clinical arena, even a relative increase in percentage of bands in leukopenic patients has arguably received some credence towards sepsis work-up.

Completion of DIFF count is generally followed by evaluation of morphology of red cells, white cells, and platelets. Over the years, the lack of availability of a standardized approach to reporting the results of morphologic evaluation of blood cells have led laboratories to report the findings in a variety of ways with little, if any, attention being paid to maintaining consistency. Some laboratories choose to report individual specific abnormalities simply as present when seen in the smear, whereas others opt to grade individual abnormalities either as mild, moderate or marked or as 1+ through 4+. A grading system, whether in terms of mild, moderate and marked or 1+ to 4+, for reporting of morphologic findings is clinically useful at least in some cases. For example, a finding reported as 3+, 4+ or marked elliptocytes is essentially diagnostic of hereditary elliptocytosis. Similarly, a finding reported as 3+, 4+ or marked tear drop cells is highly suggestive of bone marrow fibrosis. In contrast, a finding reported as elliptocytes present or tear drop cells present is considered non-specific because a small number of elliptocytes may be seen in the blood smears of patients with anemia of various etiologies and an occasional tear drop cell is not an uncommon finding in patients with iron deficiency anemia or renal disease. Blood Cell Morphology Grading Guide, a recent publication from the American Society for Clinical Pathology Press (ASCP Press), provides a systematic approach and thereby some level of standardization to grading and reporting morphologic abnormalities of red cells, white cells and platelets [18]. Upon completion of the evaluation of blood cells morphology, the next step is to record other clinically significant findings, if any present, such as the presence of intracellular and/or extracellular organisms, non-heme malignant cells, etc. The blood smear examination is often completed with (i) qualitative platelet estimate reported as normal, increased or decreased and (ii) a determination by the blood smear examiner as to whether the smear needs a review and/or interpretation of findings by a hematopathologist and/or a pathologist.

3. Blood Smear Review (BSR)

Synonyms: blood smear interpretation, physician review of blood smear.

A BSR may be requested by the clinician or initiated by the laboratory staff. It may be performed with or without interpretation of findings. Clinical indications for a blood smear review request by a physician include (a) unexplained anemia, thrombocytopenia and/or leukopenia, (b) suspicion of microangiopathic hemolytic anemia (e.g., thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, etc.), hemoglobinopathy (e.g., SS, SC, CC, etc.), thalassemia, red cell membranopathy (e.g., hereditary elliptocytosis, hereditary spherocytosis, etc.), lymphoproliferative disorder, plasma cell dyscrasia, myeloproliferative disorder, myelodysplastic syndrome, parasitic infection, infectious mononucleosis, inherited leukocyte disorder (e.g., Pelger huet, May-Hegglin, etc.), or inherited platelet disorder (e.g., gray platelet syndrome). The laboratory staff usually initiates a blood smear review either as good laboratory practice and/or as required by regulatory or professional accreditation agencies in many countries around the globe. In many laboratories, at least the initial blood smears with potentially significant finding(s), as determined by the pre-set criteria, are subjected to a review by a qualified hematopathologist. Any person with expertise in blood cell morphology may serve as a qualified reviewer for confirmation of previously identified abnormal findings and for identifying those either unfamiliar to or missed by the initial blood smear examiner. However, a physician, that may be a hematopathologist, hematologist, or pathologist with training and experience in hematology, is most suitable for interpretation of blood smear findings in the light of other relevant clinical and laboratory information.

The list of criteria for smear review is usually developed by individual laboratories with input from pathologist(s), clinicians, and the hematology supervisory staff, and may be updated periodically as deemed appropriate. Although, clinical significance of the abnormal CBC and DIFF findings is the major determining factor in deciding which blood smears need review, several other factors may also influence such a decision. These factors may include patient population served, clinicians’ concerns pertaining to specific patient populations, training and experience of blood smear examiners(s) and reviewer(s), workload of the laboratory and the reviewer(s), initial vs. follow-up smears, QC/quality assurance (QA) consideration, and teaching/educational considerations. Published criteria [19-22] may be used by individual laboratories as a starting point in the process of developing their own set of criteria. The set of criteria utilized by the
Blood smear review by a qualified hematopathologist can serve several functions that are considered essential to good patient care. It can serve as a quality assurance tool for the CBC, DIFF (automated and/or manual), and reticulocyte count results, manual DIFF in particular, because there is no other commercially available QC material for daily use for this test. Interpretation of blood smear findings along with CBC and other available laboratory data in the clinical context may provide a definite diagnosis or suggest a strategy for additional work-up of the case in an efficient and cost-effective manner. Furthermore, blood smear review can serve as an excellent teaching resource for training of students, residents, fellows and newly hired staff, and for continuing education of the technical staff.

Blood smear review process encompasses every aspect of the blood smear scan and the blood smear examination described above, with one exception. The exception is that the re-
viewer may or may not choose to perform the actual 100-cell DIFF. The circumstances, which will necessitate a 100-cell DIFF by the reviewer include (a) if the review is being performed also for the purpose of QC for the DIFF or for assessing competency of the staff in performing manual DIFF and (b) if, in the judgement of the reviewer, the DIFF results reported by the blood smear examiner are either incomplete, inaccurate, or contain some unidentifiable cells reported as other cells with or without any comments. A blood smear review by a physician often generates a written report with interpretation of the findings in the clinical context. Blood smear reviewers often serve as consultants to clinicians and other laboratory professionals in explaining the abnormal findings and their clinical relevance, besides providing either a diagnosis whenever feasible or suggesting an appropriate strategy for an efficient and cost-effective additional work-up necessary for arriving at a diagnosis. At our institution, all initial blood smears meeting the pre-set criteria (Tables 2 and 3) are reviewed by a Hematopathologist and all follow-up smears meeting pre-set criteria (presence of any blast and/or organisms) are reviewed by an experienced technologist with good morphological skills.

SUMMARY

A blood smear scan serves to at least (a) verify the flagged automated hematology results and (b) determine if a manual DIFF needs to be performed. Blood smear examination/Manual DIFF with CBC provides the complete hematologic picture of the case, at least from the morphologic standpoint. Blood smear review with or without interpretation serves to ensure that no clinically significant finding is missed, besides providing diagnosis or diagnostic clue(s), particularly if and when interpreted by a physician.

REFERENCES