Q. How often should we document hematology competencies to ensure consistency of morphologic observations for manual differentials and fluids? Should we do this every six months or annually? It would be helpful if this were defined in the hematology and urinalysis checklists. How do laboratories typically address checklist items HEM.34400, HEM.35566, and URN.30800?

A. It has been proposed for the next edition of the checklists that the phrase "at least annually" be added to those checklist questions that require a documented system to ensure consistency of morphologic observations among all personnel performing blood cell microscopy (HEM.34400), body fluid cell differentials (HEM.35566), morphologic classification of sperm and other cells, and urine sediment microscopy (URN.30800). For now, however, no defined frequency is specified.

Including these elements as part of your competency program can aid efforts to quantify and identify cells and sediment consistently. The program should describe thoroughly how to achieve consistency using competency assessments. The key is for the lab to follow a documented system. In other words, it should generate employee records to show it is striving for consistency among staff members.

Proficiency testing materials, such as photomicrographs, from PT providers or the laboratory’s own alternative performance assessment program are often used to meet the intent of these questions. They also may be used as part of the ongoing competency and continuing education programs in the laboratory.

Laboratorians too should document and share with staff instances when they encounter rare or unusual cells and rare urine sediment elements from patient samples. The laboratory can foster consistency, as well, by including definitions of semi-quantitative measures, such as 1+, 2+, and 3+, in its policies and procedures. Having these defined measures posted and readily available to employees helps lab staff make consistent morphologic observations.
Q. What is the protocol for Kleihauer-Betke stain testing? We are seeing an increase in testing on positive moms, premature labor, repeat KB stain within 24 hours, and trauma cases. I am concerned because the test is so dependent on technique and interpretation.

A. Currently, fetal-maternal hemorrhage can be documented by such methods as flow cytometry, gel agglutination, and Kleihauer-Betke (acid elution). The most commonly used method is Kleihauer-Betke, or KB. While this procedure is used to determine the number of vials of Rh immune globulin to be administered to an Rh-negative mother, we are finding our obstetricians-gynecologists are using it more and more for assessing placental injury in cases of trauma and premature labor, as was stated in the question (along with fetal monitoring).

We have performed the KB test using the Sure-Tech Kit for more than 15 years. We have found this method to be reliable and to offer a good turnaround time. We have also performed this procedure on a stat basis. Our proficiency tests, which can be purchased from the CAP, have been good. As indicated in the question, the test must be performed as indicated on the instruction sheet. I reviewed our 2007 CAP Survey; the expected results are in the first set of parentheses and our results are in the second parentheses: 2007 HBF-1 (0.5 percent) (0.3 percent), HBF-2 (2.0 percent) (1.6 percent), HBF-3 (1.0 percent) (0.8 percent), HBF-4 (0 percent) (0 percent). Our 2008 results were HBF-1 (2.0 percent) (1.9 percent) and HBF-2 (0 percent) (0 percent). These results are in good agreement with the Surveys.

Though immunophenotyping has a greater accuracy, the KB test can give reliable results at a lower cost and with a faster turnaround time. I recently called five national laboratories that perform flow on leukemia/lymphoma samples. None of the labs performs determination on HbF. The sixth laboratory I called does do flow on HbF with a 48-hour turnaround time at a cost of $467. In addition, I checked with our larger regional medical center, which does flow on leukemia/lymphoma samples; it does not perform flow on HbF and, instead, uses the KB test.

If performed correctly, the KB test can give reliable results with a good turnaround time and at reasonable cost. A possible source of error is in the interpretation. Nucleated cells such as granulocytes and lymphocytes must not be counted. (I wrote a reference manual for Sure-Tech that addresses this issue.) Additionally, our blood bank specialist uses cord blood along with male blood at various dilutions for quality control. Because interpretation is critical, only a few of our medical technologists are trained in this procedure. This way, the technologists are more familiar and more comfortable with the procedure.

Reference


Robert H. Davis, MD, PhD
Department of Pathology
Q. Are there accepted policies regarding the performance of repeated manual differential white blood cell counts for patients with persistent low total leukocyte counts?

A. Practice varies widely on this topic. The CAP Q-Probes group recently published the results of its study on the rate of manual peripheral blood smear review (including scans and differential counts) at 263 institutions in 45 states and four foreign countries.¹ A total of 95,141 complete blood count determinations were studied. The study found that the percentage of CBCs with a manual review, scan, or differential performed ranged from a 10th percentile of 9.9 percent to a 90th percentile of 50 percent, with a median of 26.7 percent. In other words, 10 percent of laboratories did a manual review, scan, or differential on less than 9.9 percent of their CBCs; another 10 percent did more than 50 percent; and the median group did reviews on approximately 25 percent of their CBCs. The most frequent prompts resulting in reviews were hematology analyzer flags, including WBC flag (36.7 percent), immature cell flag (25.5 percent), red blood cell flag (13 percent), and platelet flag (5.9 percent). Physician request accounted for only 3.7 percent of reviews. When the prompts were set at higher levels, the number of reviews decreased, as would be expected. In addition, the number of reviews was lower if the laboratory had a policy of limiting reviews of repeat specimens within certain time frames or if the reviews were done only for abnormal RBC parameters (and not other hematologic abnormalities). Only 41 laboratories reported having a time interval limit, and of those 41, the most common interval limit was 24 hours. When all reviews were considered, they were thought to have contributed new information in about one-third of cases.

The International Society for Laboratory Hematology has suggested criteria for action following automated CBC and WBC differential analysis.² A group of 20 hematology laboratorians from six countries and 17 laboratories reached consensus on rules that should be used in various circumstances, which it then tested on 1,000 samples, with 200 repeated to test the delta rules. The group developed criteria to determine if the use of these rules revealed a positive smear finding. Analysis of this method showed a true positive rate of 11.² percent, true negative rate of 67.3 percent, false-positive rate of 18.6 percent, and false-negative rate of 2.9 percent. The group indicated that it hopes individual laboratories will consider applying these rules in their own institutions, and it has provided a protocol to facilitate this validation.

As for the question of repeated low total leukocyte counts, the group recommends a slide review with the first low result (<4.0) and then a repeat slide review if the delta check failed and within three days. In addition, it recommends a manual differential and slide review in circumstances in which the analyzer is unable to give a differential or the differential is incomplete. Therefore, the group would recommend a slide review (or manual differential) with the first low total leukocyte count, but would only repeat it in the first three days if the delta check failed.

References

Q. Is a differential cell count needed in CSF samples with normal cell counts? A.
Cerebrospinal fluid, or CSF, is submitted to the laboratory most often to evaluate for infection, hemorrhage, or malignancy. A cell count and differential count are routinely requested as part of this evaluation. It is logical to perform a differential cell count on a CSF sample with an increased number of cells. However, the issue has been raised of whether a differential count is needed in CSF samples with normal cell counts. Literature addressing this question is scant, but the following are guidelines on how members of the CAP Hematology/Clinical Microscopy Resource Committee handle the differential cell count on CSF samples.

A Wright-Giemsa-stained cytocentrifuged slide is prepared for all CSF samples, regardless of the total cell count. In cases with a normal CSF cell count, the slide is used differently depending on the patient population. Cytocentrifuged slides from adults are always scanned for organisms or abnormal cells, particularly in patients with a history of malignancy. When a malignant cell is identified, it is always reported whether or not a differential cell count is performed. CSF-cytocentrifuged slides with organisms or malignant cells are always reviewed by a pathologist. A differential cell count is typically performed on CSF samples from children even if the cell count is normal. In cases where very few cells are present on the smear (as would be expected if the cell count is normal), the number of cells counted is also reported.

Beverly Nelson, MD
Northwestern University
Feinberg School of Medicine
Chicago, IL
Q. How should one deal with a hemolyzed or icteric specimen for a CBC?

A. Specimens with increased bilirubin (icteric specimens) do not generally interfere with the performance of a CBC. Elevated bilirubin levels may be associated with hemolysis, which does affect the CBC.

Hemolysis, if significant, is always a problem primarily because of the plasma hemoglobin and, depending on the mechanism of the hemolysis, secondarily because of effects on cellular elements. Whenever there is significant hemolysis there will be a discrepancy between the hemoglobin (measured after lysis of red cells so it includes both cellular and plasma hemoglobin) and red cells/hematocrit (based only on intact cells). If the hemolysis is a result of improper specimen collection, it is a cause for specimen rejection and re-collection. If the hemolysis truly represents the condition of the patient (intravascular red cell destruction), then the discrepancy is expected and the laboratory should note it and indicate its cause. In centers that perform plasma hemoglobin determinations, it would be possible to correct the hemoglobin value by measuring and subtracting the plasma hemoglobin and substituting that hemoglobin value in the CBC, which should match the red cells/hematocrit and allow for calculating correct indices. The problem of the plasma hemoglobin could also be avoided with plasma substitution techniques. In practice, these corrections are rarely done.

Certain causes of hemolysis can cause problems with other CBC parameters. Strong cold agglutinins, in addition to causing hemolysis, cause cell clumping that leads to undercounting of red cells and false increases in the MCV and MCH. The clumping can be avoided, to some extent, by keeping the specimen warm (body temperature) until analysis. An anemia that causes red cell fragmentation, especially one that results in a number of small fragments, can lead to a spuriously low red count and elevated platelet count due to miscounting the red cell fragments as platelets. In such a case, review of the blood smear is essential to recognize the problem and perform a platelet estimate from the smear, which will more accurately reflect the platelet number. In centers that have patients with problems that invariably lead to intravascular hemolysis (for example, patients on ECMO or with severe burns), a system should be in place to recognize the hemolysis, review clinical diagnosis and previous results and a blood smear, and based on these findings, provide the most appropriate and accurate results possible.

Reference

Robert Novak, MD
Department of Pathology
Children’s Hospital
Medical Center of Akron
Akron, Ohio
Chair, CAP Hematology and Clinical Microscopy Resource Committee