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FROM THE CHAIR

By definition, Hematology is the study of blood, the blood forming organs, and blood diseases. In practice, the most common Hematology test is the CBC or Complete Blood Count. This is because the cell counts and other parameters included in the CBC are affected differently by different blood diseases. By looking at these effects in conjunction with the patient’s clinical symptoms, the clinician is able to identify or rule out various disease states. Once a diagnosis is determined, the CBC is also essential for monitoring the progression of the disease.

The articles in this issue of Insights concentrate on the automated testing of the CBC and the possible problems associated with that testing. In “Basic Hematology: The CBC,” we touch on the test itself and the most common methods used to determine CBC results. Problems that you could see while running this test are the focus of “Instrument Flags.” The remaining articles spotlight a specific COLA criterion, HE 4, and offer tips on how to maintain compliance with it.

Understanding the theory behind the testing helps you provide higher quality patient results since you will be able to recognize potential complications that could develop during testing, troubleshoot the causes, and perform corrective actions if needed. This, in turn, leads to our ultimate goal of higher quality patient care.

W. James Stackhouse, MD, MACP
Chair, COLA Board of Directors
Basic Hematology: The CBC

The CBC or Complete Blood Count consists of several different parameters. Cell counts for Red Blood Cells (RBCs), White Blood Cells (WBCs), and platelets (plts) and values for Hemoglobin (Hgb) and Hematocrit (Hct) are the minimum parameters reported for a CBC. Most also report Mean Platelet Volume (MPV) and the red cell indices:

- Mean Cell (or Corpuscular) Volume – MCV
- Mean Cell (or Corpuscular) Hemoglobin – MCH
- Mean Cell (or Corpuscular) Hemoglobin Concentration – MCHC
- RBC Distribution Width – RDW

Many automated hematology instruments are also capable of providing a three- or five-cell differential, which gives the clinician more details about the patient’s WBC population. A three-cell differential will categorize the white cells as granulocytes, lymphocytes, or mononuclear cells. The five-cell differential further classifies the granulocytes as neutrophils, basophils, or eosinophils. The differential can be reported as percentages and/or absolute counts, depending on the hematology instrument.

Electrical Impedance (The Coulter Principle)

Electrical Impedance, also known as The Coulter Principle, is a common methodology used to count and size RBCs, WBCs, and platelets. Within the analyzer, cells are suspended in a liquid capable of conducting electricity. This suspension passes through an aperture of known size that has an electrical current passing through it. (The aperture is large enough to allow only single cells to pass through it.) When a cell passes through the opening, the electrical current is interrupted (or impeded) creating a measurable “pulse.” The amplitude of that pulse correlates with the volume of the cell that produced it.

The number of pulses indicates the number of cells that pass through the aperture, which translates into the cell counts. Through analysis of the amplitudes of the pulses, the instrument classifies the cells according to size. This is one of the steps required to perform the white cell differential.

To perform the different cell counts, the patient sample is divided into separate portions. One portion is used to perform the WBC count and differential. In this portion, RBCs are lysed by exposing the cell suspension to a lysing agent for a specified period of time. Since the lysing agent also reveals the WBC nuclei, some analyzers will also expose the cells to a stain. The analyzer can then utilize the fact that cell nuclei stain at various intensities to differentiate between the white cell lines.

Abbreviations Used Throughout This Issue of Insights

- CBC Complete Blood Count
- RBC Red Blood Cell
- WBC White Blood Cell
- plts Platelets
- Hgb Hemoglobin
- Hct Hematocrit
- MCV Mean Cell (or Corpuscular) Volume
- MCH Mean Cell (or Corpuscular) Hemoglobin
- MCHC Mean Cell (or Corpuscular) Hemoglobin Concentration
- RDW RBC Distribution Width
- nRBC Nucleated Red Blood Cell
- MPV Mean Platelet Volume
- diff White cell differential

>> CONTINUED ON PAGE 4
Utilizing a separate, non-lysed portion of the patient sample, the instrument also uses electrical impedance to determine red cell and platelet counts. Other parameters (MPV, MCV, RDW, and hematocrit) are determined through calculations and further analysis of the data obtained from this portion of the patient sample.

**Light Scatter and Absorption Spectrophotometry**

Cells not only impede electrical current, they scatter light in all directions. Through the use of various light sources and properly placed sensors, light scatter and absorption can be analyzed. If the instrument utilizes a stain, the light scatter and absorption of the cells are compared to values obtained when no cells are present.

This data is further analyzed to determine the physical and chemical characteristics of the cells being analyzed, which allows them to be categorized to produce the white cell differential.

Since hemoglobin is directly proportional to the absorbency of the sample, it can be determined by comparing the light absorption of the sample to the light absorption of a blank. This value is then used (with other parameters) to determine MCH and MCHC.

There are several factors that can interfere with the instrument's specimen analysis, including patient condition, specimen integrity, and mechanical issues within the instrument itself; however, there are mechanisms to alert testing personnel when such interference occurs. These are discussed in the next article in this issue of *Insights*.

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**CONTINUED FROM PAGE 3**

**BASIC HEMATOLOGY: THE CBC**

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**REFERENCES**


COULTER® AC-TM 5diff Autoloader Hematology Analyzer, Instructions for Use, version PN 624026AA, May 2010, Beckman Coulter, Inc., 250 S. Kraemer Blvd, Brea, CA 92821

**RESOURCES**

Refer to “Abbreviations Used Throughout This Issue of Insights” on page 3.
**Instrument Flags**

Instruments flag results to alert the user to possible interferences or problems. It is extremely important to know what these flags mean and how to react to them.

Regardless of the analyte, automated instruments will flag results that exceed the analyzer’s ranges; some instruments do not report these results at all. The result could be above or below the limits of the reportable or linearity ranges, or it could be beyond the instrument’s capacity altogether. Additional limits (e.g., reference ranges and alert values) can be entered by the user. This allows you to personalize the instrument to flag results that exceed limits you establish for your patient population.

Instruments will also flag control results when the controls do not perform as expected. Therefore, it is important to accurately enter the correct expected ranges for the current lots of Quality Control materials.

Due to the nature of Hematology, Hematology analyzers also flag results for many other reasons, and a single problem may affect several different parameters. For example, if the instrument cannot report a value for the Red Blood Cell (RBC) count, any parameter that requires the RBC count in its determination (Hct, MCV, MCH, MCHC, RDW) will not be reported either.

Different manufacturers will flag issues in different ways; therefore, it is imperative that you familiarize yourself with your instrument's flags and what they mean. This information can be found in the instrument’s operator’s manual, which also offers suggestions of action steps to take when flagged results are obtained. The actual protocol followed in your laboratory, however, should be determined by your Laboratory Director and Technical Consultant/Technical Supervisor.

**Instrument Conditions**

Analyzers are equipped with internal control mechanisms to monitor their operations and measurements. If any of these control monitors fail, patient results will be flagged or will not be reported. For example, the Cell-Dyn® Emerald™ must be operated in an environment with an ambient temperature of 63°-91°F (17°-33°C). If instrument sensors indicate that the temperature is too low or too high, results are invalidated, flagged with *, and “INS_T” appears in the flags section of the report.

The Coulter® AC•T™ manual provides another example of a flag resulting from an internal control failure. The instrument performs the WBC, RBC, and plt counts twice. If the results obtained in the two counts differ by more than a predefined limit, these counts are flagged with a “V” flag. This flag affects other parameters in addition to the cell counts. Examples include:

- the WBC count is flagged with a “V” flag: the diff results are also flagged
- the RBC count is flagged with a “V” flag: the red cell indices (MCV, MCH, MCHC, and RDW) are not reported

Additional built-in control mechanisms exist for these and other automated hematology analyzers. Results will be flagged in ways that vary depending on the instrument and the internal control mechanism. Therefore, the operator’s manual should be available at all times for testing personnel to review when flagged results are obtained.

**Specimen Integrity**

Specimen integrity issues can also affect hematology analysis and cause the instrument to flag results. The most common problems are seen when the specimen is clotted. This is so common that checking the specimen for clots is the first step recommended by many manufacturers when troubleshooting flagged results.

Cells are bound in the clot and are not available as free cells to be counted, causing falsely decreased cell counts. If the clot binds too many WBCs, additional flags may be seen on the differential results. Platelet aggregates that form during the clotting process can be mistaken for other cell lines, which leads to inaccurate and flagged cell count and differential results.

In addition to the inaccurate, flagged results, clots may damage or clog the tubing within the instrument causing problems with subsequent specimens. To help prevent specimen clotting, ensure that tubes are gently inverted (end-to-end) at least 10 times immediately after specimen collection, and again prior to running the test. Properly mixed tubes help ensure accurate, reliable test results.
Patient Condition

Results can also be flagged due to pathogenic reasons. Various disease states, illnesses, and conditions affect cell lines differently. Anemic patients may have significant circulating nRBCs as the bone marrow attempts to compensate for the low RBC count, but have normal WBC populations. Immature white cells (up to and including blasts) may be released into circulation as the disease progresses in leukemic patients with or without affecting RBCs. Other disease states may cause platelet clumping, rouleaux formation, fragmented cells, inclusion bodies, or any number of other circumstances that will cause an analyzer to misidentify or flag test results.

Table 1 provides examples of how various factors related to specimen integrity and patient condition can affect some of the CBC parameters. Most, but not all of these conditions, will lead to flagged test results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Factors Causing Increased Results</th>
<th>Factors Causing Decreased Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>In-vivo hemolysis, hyperlipidemia, hyperbilirubinemia, WBC &gt;50,000/mm^3</td>
<td>Clotting</td>
</tr>
<tr>
<td>MCV</td>
<td>Cold agglutinins, hyperglycemia, WBC &gt;50,000/mm^3</td>
<td>Cryoglobulins</td>
</tr>
<tr>
<td>MCHC</td>
<td>Hyperlipidemia, cold agglutinins</td>
<td>WBC &gt;50,000/mm^3</td>
</tr>
<tr>
<td>RDW</td>
<td>Recent blood transfusion</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>Nucleated red cells, platelet clumps, unlysed red cells, cryoglobulins</td>
<td>Clotting</td>
</tr>
<tr>
<td>pltS</td>
<td>WBC fragmentation, severe microcytosis, cryoglobulins</td>
<td>Satellitism, clumping</td>
</tr>
</tbody>
</table>

Conclusion

Depending on the patient population, some testing personnel may be more comfortable dealing with flagged results than others. For example, specimens tested in a Hematology-Oncology practice have a higher possibility of having flagged results than specimens tested in a well-patient practice; therefore, testing personnel in the first lab would be more familiar with flags than those in the second lab.

Regardless of their comfort level, it is imperative that your testing personnel understand the significance of the flags reported by the instrument(s) in use in your laboratory. Flagged results must be addressed through clearly defined procedural steps established by your Laboratory Director and/or Technical Consultant/Technical Supervisor. Your protocol should be customized to your laboratory based on your patient population, test menu, and testing personnel. However, a step that should be included in your procedure is the review of a stained peripheral blood smear by competent personnel, if differential results are flagged. If necessary, send the specimen to a reference laboratory for review.

Understanding what can cause flagged test results and knowing what to do when these flags appear will lead to higher quality test performance and better patient care.

REFERENCES

1 Refer to “Abbreviations Used Throughout This Issue of Insights” on page 3.

Spotlight on COLA Criterion: HE 4

HE 4: If you perform automated differential counts, have criteria been established for when a manual cell count must be performed to verify the automated count?

You may also answer yes to this question if you have established criteria for when to send a differential to a reference laboratory. The differential does not have to be performed in-house.

The focus of this criterion is to ensure that testing personnel are aware of when a patient specimen requires further review. The “review” is usually the performance of a manual differential; however, other actions may be necessary. The review may be performed in-house or by reference laboratory personnel.

The review may be required because the patient has an illness or condition that causes abnormal results. An elevated or reduced WBC count, an increase or decrease in number of a single white cell line (lymphocytes, eosinophils, etc.), or abnormal RBC or platelet counts may be triggers for further specimen review.

Reviews may also be required due to possible instrument errors. Instruments that perform automated differentials usually have a mechanism (result flags, histograms, not reporting results, etc.) for alerting the user when an error may have occurred. The error may be due to failure of instrument internal controls, mechanical problems, or electrical interference. Unusual specimen circumstances, such as platelet clumping, red blood cell agglutination, nRBCs etc., may also trigger a review. Testing personnel should be familiar with the instrument’s alert mechanism and should know what to do if they receive such an alert.

The operator’s manual for the analyzer will have a description of the alert mechanism as well as suggestions for follow-up actions to take. See Figure 1 for an example. Your Laboratory Director (LD) and/or Technical Consultant/Technical Supervisor (TC/TS) should review these actions to see if they are appropriate for your laboratory. If so, the operator’s manual will satisfy the intent of this criterion as long as there is documentation of the initial and annual reviews, which is consistent with the review requirement for all laboratory procedures.

If the actions provided by the instrument manufacturer are not appropriate for your laboratory, your LD and/or TC/TS must establish written criteria detailing what actions need to be taken in your laboratory. Patient to patient flexibility is acceptable, particularly for hematology/oncology practices.

Figure 1 - Example: You see Gran 73% R3 as part of your patient’s differential results. Since the result is flagged, you check the operator’s manual and find this:

<table>
<thead>
<tr>
<th>Probable Cause(s)</th>
<th>Corrective Action(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Shift in WBC distribution due to EDTA anticoagulant equilibration</td>
<td>• Check specimen for clots or agglutination</td>
</tr>
<tr>
<td>• Granulocytosis</td>
<td>• Ensure 20 minutes have passed since collection and rerun specimen. Redraw specimen if necessary</td>
</tr>
<tr>
<td>• Neutropenia</td>
<td>• If flag persists, manually review stained smear to confirm differential</td>
</tr>
<tr>
<td>• Eosinophilia</td>
<td></td>
</tr>
<tr>
<td>• Agranular neutrophils</td>
<td></td>
</tr>
<tr>
<td>• Bands</td>
<td></td>
</tr>
</tbody>
</table>

If your LD and/or TC/TS have approved this protocol, perform the stated corrective actions. When manually reviewing the stained smear, confirm the existence of any of the probable causes.

The written procedure can be very detailed, listing actions to be followed when specific instrument flags are seen. Alternatively, it can be as simple as “Specimens will be sent to ABC reference laboratory for further review at the request of the ordering clinician.”

>> CONTINUED ON PAGE 8
Regardless of how simple or complex the procedure is, it should reflect what is actually done in your laboratory and it should include:

- What triggers the review
  Clinician request, LD/supervisor request, elevated cell count, result flags, etc.

- What actions should be taken
  Perform manual differential or slide review, check specimen for clots, redraw or rerun the specimen, send to reference lab, etc.

- Who performs the review
  Testing personnel, supervisor, reference lab staff, etc.

- Additional criteria as needed for your patient population

When manual differentials are required, the blood smears must be reviewed by personnel who have been trained, and are deemed competent, to perform manual differentials. Therefore, manual differentials may be referred to a reference laboratory, they do not have to be done in-house.

The instrument may alert you to problems with CBC components other than the differential, therefore, your laboratory procedure should address these components as well. The action steps to take depend on the reason for the alert, and should be determined by your LD and/or TC/TS. As with the differential, if necessary, the specimen may be sent to a reference laboratory.

*This information has been condensed and formatted as a Compliance Tip for you to print and keep.*
Want to learn more about Hematology?

Join us for either or both of these upcoming opportunities!

OPPORTUNITY #1
WHEN:  Friday, April 20, 2012, 10:30am-12:00pm PST
WHERE: The Symposium for Clinical Laboratories, Tropicana Hotel, Las Vegas
WHAT:  Session C24, Clinical Hematology Review

This talk will provide a refresher on the fundamentals of clinical hematology. The most recent analytical methods will be presented as well as a discussion of the most common pre-analytical errors associated with hematology testing. Common clinical conditions (iron deficiency anemia, polycythemia, sickle cell disease, etc.) will be reviewed using a case study approach.

OPPORTUNITY #2
WHEN:  Wednesday, April 25, 2012, 2:00pm EST
WHERE: Anywhere you have a computer and a phone
WHAT:  COLA Live Webinar: The Key of Sample Integrity in Hematology Testing

This talk will provide insight into why proper specimen collection is vitally important in hematology testing. Basic hematology principles and common errors seen in hematology testing will also be discussed.

Go to www.COLA.org for more information on these and other exciting COLA events and educational products.

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Yes, it’s that easy.
1: Open.  2: Dispense.  3: Run.
Focus: COLA criterion HE 4

If you perform automated differential counts, have criteria been established for when a manual cell count must be performed to verify the automated count?

You may also answer yes to this question if you have established criteria for when to send a differential to a reference laboratory. The differential does not have to be performed in-house.

The focus of this criterion is to ensure that testing personnel are aware of when a patient specimen requires further review. The “review” is usually the performance of a manual differential; however, other actions may be necessary. The review may be performed in-house or by reference laboratory personnel.

You should have a written procedure that states

- What triggers the review
  - Clinician request, LD/supervisor request, elevated cell count, result flags, etc.
- What actions should be taken
  - Perform manual differential or slide review, check specimen for clots, redraw or rerun the specimen, send to a reference lab, etc.
- Who performs the review
  - Testing personnel, supervisor, reference lab staff, etc.
- Additional criteria as needed for your patient population

The operator’s manual for your analyzer will have suggestions for follow-up actions to take when result flags are seen. See the example below. Your Laboratory Director (LD) and/or Technical Consultant / Technical Supervisor (TC/TS) should review these actions to see if they are appropriate for your laboratory. If so, the operator’s manual will satisfy the intent of this criterion as long as there is documentation of the initial and annual reviews, which is consistent with the review requirement for all laboratory procedures.

If the action steps provided by the instrument manufacturer are not appropriate for your laboratory, your LD and/or TC/TS must establish written criteria detailing what actions need to be taken in your lab. Patient to patient flexibility is acceptable, particularly for hematology / oncology practices.

Example: You see GRAN 73% R3 as part of your patient’s differential results.

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<td>* Bands</td>
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If your LD and/or TC/TS have approved this protocol, perform the stated corrective actions.

When manually reviewing the stained smear, confirm the existence of any of the probable causes.

You must establish criteria for when manual differentials have to be performed; however, they do not need to be performed in-house. They can be sent to a reference lab.