Educational Objectives

Upon completion of this module, the participant will be able to:

1. State the preferred anticoagulant for most hematologic testing.

2. Discuss the most common principles utilized in table top hematology analyzers.
   a. Cell counts
   b. Hemoglobin
   c. Hematocrit

3. Briefly discuss sources of error in CBC results.
   a. Cell counts
   b. Hemoglobin
   c. Hematocrit

4. Correlate hemoglobin and hematocrit results.

5. Discuss the use and reliability of hemacytometer cell counts, centrifuged hematocrits, and alternative hemoglobin procedures.

6. Discuss the centrifuged hematocrit, including sources of error, preventive maintenance, and quality control.

7. Discuss the differences between automated and centrifuged hematocrits.

8. Discuss the derivation and use of red cell indices.

I. Introduction

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Hematology testing has long been part of the Physician office laboratory. Historically, the centrifuged hematocrit has been one of the most popular laboratory tests. The value of this test
has not diminished; however the availability of cost-effective tabletop instrumentation utilized in testing hematocrit has become more popular than the manual method.

Simple equipment that requires minimal operator intervention has made a complete blood count (CBC) readily available. The throughput of these instruments is fast enough to give cell counts, indices, hemoglobin, hematocrit, and even a differential in the same time it takes to perform a manual hematocrit. Most of the instruments currently marketed to physician office laboratories are specifically designed to be user friendly and can be purchased with ready to use charts and procedures for the required preventive maintenance and quality assurance.

This unit will discuss the hematology testing most often done in a physician office laboratory. The intent is to discuss general information on instrumentation and commonly performed tests. Specific manufacturer's information will not be included. This can be obtained from the individual manufacturer.

II. Samples

Sample procurement is addressed in the phlebotomy module, but proper sample collection and handling cannot be over emphasized. Most hematology testing is done on blood anticoagulated with di- or tri- potassium EDTA (ethylene diamine tetraacetic acid).

Bullet tubes, with EDTA, are available for collecting blood from skin punctures. The bullet tube must fill quickly and the blood mixed with the EDTA before coagulation begins. The quality of the skin puncture sample is very technique dependent.

Samples collected in EDTA must be well mixed at the time of draw and before sampling depends on the types of centrifuges used. However, the time ranges from two to five minutes on centrifuge or an aliquot mixer or, if they are mixed manually, a minimum of sixty inversions. Avoid over mixing because prolonged mixing on aliquot mixers causes hemolysis.

As a safety precaution, always open evacuated tubes under a protective shield.

Residual vacuum causes aerosols.

http://www.bd.com/vacutainer/pdfs/plus_plastic_tubes_wallchart_tubeguide_VS5229.pdf
http://labmed.ascpjournals.org/content/38/12/723.full.pdf
These are examples of freestanding protective shields. They are available through laboratory supply and safety product distributors.

III. Complete Blood Count (CBC)

Over the years, the components of the CBC have expanded, as the instrumentation has become more sophisticated. The CBC test menu on basic instruments usually includes cell counts (red cells, white cells, and platelets), hemoglobin, hematocrit, and red cell indices. More complex instrumentation adds automated differentials, platelet indices, and white cell differentials. Performing complete blood counts using multiple manual or semi-automated methods is no longer cost or time effective. The manual and semi-automated methods are useful for measuring single parameters and as back up procedures.

A. Table Top Analyzers
These analyzers vary in the extent of the automation involved. The simplest analyzers require the operator to mix the sample and present an open sample tube to the instrument. Sampling units that pierce the cap of the tube save time, are readily available, and are safer. The more sophisticated systems read bar coded labels, mix, and sample through the cap of the tube. Skin puncture samples may need to be diluted and/or presented to a different sampling probe. The ease of testing skin puncture samples and 2 or 3 ml tubes may be an important consideration in
choosing an instrument. The top of the line hematology systems can make peripheral smears, store thousands of results, and store and manipulate quality control data. Most of the instruments can be interfaced with a laboratory information system.

The following URLs will connect to some of the manufacturers of table top hematology analyzers.

   Beckman Coulter

   Siemens

   Dade Sysmex

1. Cell Counts

On any given instrument, all of the cell counts are based on the same principle. The Coulter Principle of electrical impedance or a modification is the most common principle used in the smaller hematology instruments. This principle is based on the ability of the saline diluent to conduct electricity while the suspended cells are nonconductive. As the cells pass through an aperture they break the current between the external and internal electrode and are enumerated and sized. Red cells and white cells are counted in separate baths or channels, with the red cells lysed when white cells are counted. Platelets are usually counted with the red cells and the cells are differentiated by size.

Red cell histograms derived from these counts have a small tail to the right of the curve. This represents coincidence, which is multiple cells passing through the aperture at the same time. The correction for coincidence takes place in the cell count calculation and is not a concern.

The other basic counting principle is based on light scatter. Here, a single cell passes through a beam of light from a laser or tungsten-halogen light source. The cell is counted as it breaks the beam of light and the light is scattered. The scattered light is measured and translated into cell size. The counts are reported in units per volume of whole blood.
Some hematology instrumentation prints the red cell and platelet histograms as part of the report. These histograms give a visual interpretation of the cell population and correlate with the indices.

Sources of error in cell counts include:

1. **Cold agglutinins** - low red cell counts and high MCVs can be caused by a decreased number of large red cells or red cell agglutinates.

   If agglutinated red cells are present, the automated hematocrits and MCHCs are also incorrect. Cold agglutinins cause agglutination of the red cells as the blood cools. Cold agglutinins can be present in a number of disease states, including infectious mononucleosis and mycoplasma pneumonia infections. If red cell agglutinates are seen on the peripheral smear, warm the sample in a 37 degrees C heating block and mix and test the sample while it is warm. Strong cold agglutinins may not disperse and need to be redrawn in a pre-warmed tube and kept at body temperature.

2. **Fragmented or very microcytic red cells**

   these may cause red cell counts to be decreased and may flag the platelet count as the red cells become closer in size to the platelets and cause an abnormal platelet histogram. The population is visible at the left side of the red cell histogram and the right end of the platelet histogram.

3. **Platelet clumps**

   and platelet satellitosis

   - these cause falsely decreased platelet counts. Platelet clumps can be seen on the right side of the platelet histogram. Decreased platelet counts are confirmed by reviewing the peripheral smear. Always scan the edge of the smear when checking low platelet counts.
4. Giant platelets

- these are platelets that approach or exceed the size of the red cells. They cause the right hand tail of the histogram to remain elevated and may be seen at the left of the red cell histogram.

5. Nucleated red blood cells

- these interfere with the WBC on some instruments by being counted as white cells/lymphocytes

Red cell histograms - histograms are derived by plotting the size of each red cell on x axis and the relative number on the y axis. They are used to determine the average size, distribution of size, and to detect subpopulations.

Red Cell Histogram

Red cell histograms are derived by plotting the size of each red cell on x axis and the relative number on the y axis. They are used to determine the average size, distribution of size, and to detect subpopulations. This histogram represents a normal red cell distribution. The small tail to the right of the curve represents coincidence, multiple cells passing through the aperture at the same time.

Agglutinated Red Blood Cells
The arrow points to a red cell agglutinate.

**Microcytic and Fragmented Red Cells**

**Red Cell and Platelet Histograms**
The black curves on the red cell and platelet histograms indicate expected or normal cell distributions. The red curves demonstrate the effect of very microcytic red cells on the histograms. Since microcytic red cells only affect the right end of the platelet curve, the black and red lines are superimposed for most of the curve.

**Platelet clumps**

There is a large clump of platelets in the center of the field.

**Platelet Satellitosis**
The neutrophils in the center of the field are surrounded by platelets. This is an uncommon phenomenon seen in EDTA samples. The platelets surround the neutrophils and "stick". This results in a false decrease in the platelet count.

**Giant Platelets**
Platelets that are 6 microns in diameter or larger, are considered giant platelets. Multiple giant platelets are visible in this field.

**Red Cell and Platelet Histograms**
The black curves on the red cell and platelet histograms indicate "expected" or normal cell distributions. The red curves demonstrate the effect of giant platelets on the red cell and platelet histograms.

**Nucleated Red Blood Cells**

The two nucleated cells are immature red cells that have been released from the bone marrow ahead of schedule.

**2. Hemoglobin**

Hemoglobin, on most automated systems, is measured as cyanmethemoglobin. This is a long-standing method that reliably measures all the hemoglobin variants except sulfhemoglobin. However, some manufacturers indicate that their instruments now include sulfhemoglobin in the total hemoglobin measurement.

Red cells are lysed and potassium ferricyanide oxidizes hemoglobin to methemoglobin, which combines with potassium cyanide forming cyanmethemoglobin. The brown color is measured spectrophotometrically and the corresponding hemoglobin reported. The end point of the
reaction is stable and the reaction is linear to 20 g/dL or higher. Reagents for cyanmethemoglobin are light sensitive and poisonous.

However, the Sysmex systems can use a sulfhemoglobin methodology to measure total hemoglobin. The reagent sodium lauryl sulfate disrupts the red cell membrane and binds to the globin chains, causing methemoglobin to be formed. This binds to the sulfate group producing a conjugate with an absorption peak at 535 nm. No hazardous waste is produced.

Common sources of error in measuring hemoglobin include anything that will cause turbidity and interfere with a spectrophotometric method. Examples are a very high WBC or platelet count, lipemia and hemoglobins that are resistant to lysis, such as hemoglobins S and C. The hemoglobin will be falsely increased with turbidity. Manufacturer's guidelines address interferences.

A simple method for obtaining a hemoglobin value from a lipemic sample is to use plasma replacement.

**Plasma replacement** - centrifuge a whole blood specimen and remove the plasma with an automatic pipettor. Record the volume removed and, with the same pipettor and a clean tip, replace the exact volume removed with isotonic saline. Mix the sample well and retest.

### 3. Hematocrit

A hematocrit is the volume of the red cells as compared to the volume of the whole blood sample. Hematocrits on the automated systems are calculated. The volume of each red cell is measured as it is counted and a mean cell volume is derived. The calculations are not precisely the same. But, they can be summarized as mean corpuscular red cell volume (MCV) multiplied by the red cell count (RBC). Hematocrits are reported in L/L or the traditional %.

When electrolyte displacement is used, the resulting hematocrit is sometimes called the CCV, or conductance cell volume.

Hematocrits calculated by automated instruments depend on correct red cell counts and red cell volumes to arrive at an accurate hematocrit. Hence, anything affecting the red cell count or volume measurement will affect the hematocrit. This method is not as sensitive to the ratio of blood to EDTA as the centrifuged hematocrit. Since the red cells are resuspended in isotonic
saline, they regain their normal shape and size. The practical side of this is that a reliable automated hematocrit can frequently be obtained from a short sample.

It is very useful to have a hematocrit centrifuge for back up. It can be used when the main instrument is down and to trouble shoot or check hemoglobins that do not correlate with the other results.

**Short sample** - an evacuated tube that is not full or does not have enough blood to satisfy the manufacturer's recommended anticoagulant to blood ratio.

### 4. Correlating Hemoglobin and Hematocrit Values

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The hemoglobin times three roughly equals the hematocrit in most patients.

**Example:**

14.8 x 3 = 44 (patient's hematocrit result is 45 L/L)
11.0 x 3 = 33 (patient's hematocrit result is 32 L/L)

The exception to this rule is in patients with hypo chromic red cells. These patients will have hematocrits that are more than three times the hemoglobin.

**Hypochromic red cells**- red cells that have an increased zone of pallor on Wright's stain. These cells contain decreased hemoglobin for their size and have a decreased MCHC.

**Hypochromic Red Cells**
Hypochromic red cells have an increased zone of pallor on Wright's stain. These cells contain decreased hemoglobin for their size and have a decreased

5. Comparing Manual and Automated Hematocrits

Understanding the differences in hematocrit methodology helps explain why some patient's hematocrits vary more than others do when comparing an automated hematocrit with a centrifuged hematocrit. Theoretically, measuring each red cell and adding those volumes to get a total red cell volume is the most accurate method. However, we use the centrifuged hematocrit as our "gold standard" and may even calibrate the automated hematocrit to match the packed cell volume (PCV). Centrifuged hematocrits have a built in bias due to the trapped plasma. When red cells have very abnormal shapes, this trapped plasma may be increased enough to cause a significant change in the centrifuged hematocrit. Some studies have shown that the two methods show better correlation if K$_2$EDTA or K$_3$EDTA is used as the anticoagulant.

Understanding how the automated hematocrit is derived is very important when trouble shooting or using back up methods such as a centrifuged hematocrit. If the hematocrits appear incorrect on the automated equipment, it means that the MCV and/or red cell count are also suspect. If a centrifuged hematocrit is substituted for an automated value, the MCV must be manually calculated.

1. Cell Counts
**Thrombo-Tic**

Thrombo-tic is a system that has made manual cell count much easier, reliable, and safe by use of a capillary holder. It is precise due to cell specific dilution. The Thrombo-tic reagent device allows an individual to enumerate platelets in whole blood. The first step in counting platelet is adding 1% oxalate reagent which lyses the RBC before counting. The process makes the platelet disaggregate and rounded, which in turn makes easier identification. When a discrepancy exists between the instrument platelet count and platelet estimate, techs use the phase platelet method to review Wright's stained peripheral smear in order to resolve the issue. An increase in white cells, the presence of immature cells, or reactive lymphocytes may answer the immediate questions. However, platelet counts performed by phase microscopy must correlate with platelet estimate. The duplicate value must agree within 10% otherwise the test must be rerun using a clean hemocytometer with well mixed thrombo-tic reagents. If the result is still not within the 10%, a new sample should be obtained and rerun using a new thrombo-tic mixture.

A properly collected sample is crucial in this process. A free flowing capillary or thoroughly mixed sample in K₂ or K₃ EDTA anticoagulated blood needs to be obtained. This sample can be used up to 4 hours when properly stored in a refrigerator with temperature that ranges from 2 – 8 °C. The sample should never be frozen. Diluting a sample with thrombo-tic reagent allows a tech to use the sample to count platelet up to 12 hours. Thrombo-tic reagent vial contains 990 µl of 1% oxalate buffer.

Quality control in this process is crucial. The diluent must be sterile. The sterility is checked monthly by charging the chamber with diluent. When charged, a count of zero must be obtained and recorded on the fluids and special maintenance sheet. If there is a discrepancy where the value is ≥ 1 then a reagent vial needs to be used. However, if the result is still the same, then use a new box of thrombo-Tic reagent vial. Make sure you check expiration date and discard any unacceptable reagent vials and document on the maintenance sheet. It is also necessary to do counts on proficiency samples and to ensure that everyone who is performing counts is getting equivalent results. i.e., competency testing.

Hemocytometers are necessary for body fluid counts. Laboratories that examine synovial and other body fluids may perform cell counts as well as a crystal analysis on a polarized microscope.

2. **Hematocrits**
When considering back up equipment, the hematocrit centrifuge is probably the most useful. It requires little expenditure after the initial cost and has simple preventive maintenance and quality control (PM and QC) requirements.

A centrifuged hematocrit is also called a packed cell volume (PCV). As in the automated hematocrit, a hematocrit is the volume of the red cells as compared to the volume of the whole blood sample and is reported in L/L or as a %. The packed cell volume is determined by centrifuging the specimen in capillary tubes and measuring the height of the red cell column.

The sample is venous blood drawn in EDTA or capillary blood collected in heparinized (red banded) microhematocrit tubes. A short EDTA sample will have an increased anticoagulant to red cell ratio, which causes the red cells to shrink and the hematocrit to be falsely decreased. EDTA must not exceed 2 mg/ml of whole blood. For EDTA samples use blue banded, plain capillary tubes. Traditional tubes are glass and unplugged. Mylar coated tubes, plastic tubes, and pre-plugged tubes are available. Plastic pre-plugged tubes are a good choice. They decrease the risk of breaking a tube and puncturing the finger of the person performing the test and avoid some sources of error.

To perform a microhematocrit:

1. Fill two red or blue banded capillary tubes, from the end without the colored band, 1/2 to 2/3 full. Tilt each tube so that the blood is near the colored band. Hold the tube horizontally and wipe all of the excess blood off of the tubes before it dries. Be careful not to wipe across the end of the tube. Absorbent material will pull out more plasma than cells.

2. For unplugged tubes, hold each tube horizontally and seal the end with the colored band by inserting it into the clay.

This is the fire-polished end. Add the sealant until it is just above or below the colored band. Filling and sealing this way keeps the clay from becoming contaminated with blood, helps prevent leakage, is safer for the person testing, and keeps the gasket in the centrifuge from being cut by the capillary tubes.
3. Insert the capillary tubes into the centrifuge with **duplicate samples across from each other**. Place the sealed end toward the outside, making sure that the tubes are seated in the groves and firmly against the gasket.

4. If there is an internal cover, make sure it is screwed down. The tubes will break if the cover is not on correctly. Centrifuge the specimens for five minutes at 11,000 to 12,000 rpms.

5. Open the centrifuge **after it has come to a complete stop**. Read the results immediately after the centrifuge stops. If this is not possible, place the tubes upright until they are read. The red cells will slide if the tubes are left in a horizontal position and the hematocrit will be falsely increased.

6. When reading hematocrits, make sure the clay red cell interface is aligned with the 0% line and the bottom of the plasma meniscus is at the 100% line. The reading that corresponds to the top of the red cell column is the hematocrit.

7. Duplicate readings should match within 1 L/L and must be within 2 L/L. Readings that match can be averaged and reported in 0.5 L/L. Centrifuged hematocrits are always reported in whole numbers or halves.

**Centrifuge Hematocrit Errors**

<table>
<thead>
<tr>
<th>Effect of Error on the Hematocrit</th>
<th>Error</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased</td>
<td>Sample drawn above the IV line</td>
<td>Redraw sample from another site</td>
</tr>
<tr>
<td>Decreased</td>
<td>Red cell leakage</td>
<td>Check sealant. Hard sealant causes leaks.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Check gasket for cuts.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Do not fill and seal from the same end of the capillary tube.</td>
</tr>
<tr>
<td>Decreased</td>
<td>Short sample, red cells shrink</td>
<td>Perform an automated hematocrit or redraw the sample.</td>
</tr>
<tr>
<td>Decreased/Increased</td>
<td>Mixing errors</td>
<td>Mix sample well before each use.</td>
</tr>
<tr>
<td>Increased/Decreased</td>
<td>Reading errors</td>
<td>Check reading device by reading the hematocrits on a card reader or with a ruler. Check employee competence.</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------</td>
<td>-----------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Increased</td>
<td>Time too short, timer not working Inadequate rpms</td>
<td>Centrifuge longer, check timer. Check centrifuge with tachometer.</td>
</tr>
<tr>
<td>Increased</td>
<td>Sample allowed to stay in the centrifuge after it has stopped</td>
<td>Remove samples immediately and store them upright.</td>
</tr>
<tr>
<td>Increased</td>
<td>Buffy coat included when reading the red cell column</td>
<td>Carefully read the top of the red cell column below the layer of white cells and platelets.</td>
</tr>
<tr>
<td>Increased</td>
<td>Abnormal red cell morphology that results in increased trapped plasma</td>
<td>Perform an automated hematocrit.</td>
</tr>
</tbody>
</table>

**Drawing of a centrifuged Hematocrit**
Hematocrit Tubes
Sealing a Hematocrit Tube

Filling and sealing with the clay perpendicular to the table keeps the clay from becoming contaminated with blood, helps prevent leakage and is safer.

Correctly Aligned Hematocrit Tube
The 100% line must bisect the bottom of the meniscus and the zero line must align with the red cell/clay interface. When both the 0% and 100% lines are positioned correctly, the hematocrit in percent is the line that aligns with the top of the red cells. The buffy coat, which is comprised of white cells and platelets, is not included.

### a. Hematocrit centrifuge quality assurance

Quality assurance includes documented quality control and preventive maintenance plus the proficiency and competency testing. Quality control Good quality control includes running duplicate samples on all patients and performing at least two levels of commercial controls with known values. If clay is used to plug the tubes, it must be soft, pliable, and inserted into the end that has not been coated with blood. Duplicate readings must be within 2% and are frequently within 1%.

Everyone performing hematocrits must demonstrate competency. An easy way to do this is to have everyone run the same sample or a split sample and compare the results. This should include samples with normal and abnormal hematocrits and be done on a quarterly basis. Quality assurance could also include documenting that multiple people read the same spun hematocrits and obtained the same results. Proficiency testing samples are available and may be combined with the proficiency testing done on a hematology multi parameter instrument.
**Preventive maintenance**
These are generic recommendations. Always check manufacturer's guidelines for specific instructions.

**Daily:**
Clean the inside of the centrifuge and the gasket.

**Monthly:**
Check the reading device. Misuse and zeroing of the reading devices can inject large errors. Always use a second, simple reading device to check the fancier devices. Use a ruler or a flat plastic card.

![Image](image_url)

These cards are available from laboratory vendors and are inexpensive.

**Quarterly:**
1. Check the brushes if they are present - this may need to be done more often.
2. Check the gasket for cuts and breaks. Cut gaskets allow tubes to leak and need to be replaced.
3. Check the timer with a stopwatch.
4. Check the speed of the centrifuge with a tachometer. These can easily be shared among multiple laboratories.
5. Perform a maximum cell pack to verify the time required for complete packing.
Centrifuge hematocrits (low, normal, high) for 2 minutes and then repeat the procedure adding 30 seconds each time you centrifuge until the results are the same for two consecutive centrifugations. The required time is 30 seconds longer than the second time the hematocrit results match.

Example: The required time is 5 minutes.

**Two Consecutive Centrifugation Results**

<table>
<thead>
<tr>
<th>Time</th>
<th>2 min.</th>
<th>2.5 min.</th>
<th>3 min.</th>
<th>3.5 min.</th>
<th>4 min.</th>
<th>4.5 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>26%</td>
<td>23%</td>
<td>22%</td>
<td>22%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>52%</td>
<td>49%</td>
<td>47%</td>
<td>46%</td>
<td>45%</td>
<td>45%</td>
</tr>
</tbody>
</table>
An easy way to check the maximum pack on a weekly basis is to read a sample and then centrifuge it for one minute. The results should be the same. If they are not, perform preventive maintenance on the centrifuge and redo the maximum pack procedure.

Micro-Hematocrit Capillary Tube reader

3. Hemoglobins

Equipment that only performs hemoglobins is available. One of them is the HemoCue®. This small hand-held equipment measures the hemoglobin in 10 microliters of blood. It measures azide methemoglobin by mixing reagents and blood in a small disposable cuvet and measuring at two wavelengths. The higher wavelength compensates for turbidity so lipemic samples are not a problem.

Stand-alone hemoglobinometers are also available. These are usually based on the same spectrophotometric principle as CBC analyzers and use cyanmethemoglobin as the end point.

With the inexpensive small multi parameter analyzers available, hemoglobin analyzers are no longer popular. The use of the HemoCue® for back up and lipemic specimens is an exception.
C. Red Cell and Platelet Indices

Red cell indices include the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC) and red cell distribution width (RDW).

**MCV** is **Mean Corpuscular Volume** in femtoliter. On automated instruments, it is computed using the measurements of each red cell. With manual methods, it is calculated using the hematocrit and the red cell count. Hint: do not worry about the decimal points. This applies to the MCV, MCH and MCHC. Just divide the raw numbers and place the decimal where it makes sense. It is a physiologic impossibility to have an MCV of 9.0 or 900. It must be 90.

\[
\text{Manual MCV =} \frac{\text{spun hematocrit in L/L}}{\text{red cells in millions/L}}
\]

Automated Hematocrit = RBC x MCV

MCV correlates to the cell diameter on the peripheral smear. Macrocytes have a high MCV and microcytes have a low MCV.

It is possible to have abnormal sized cells and a normal MCV because the MCV is an average. Agglutinated red cells also cause an increased MCV. These samples need to be warmed in a 370 degrees C heating block and rerun warm.

MVC generic reference range: 82 - 99 femtoliters

**MCH** is **Mean Corpuscular Hemoglobin** weight in picograms. This is the average weight of the hemoglobin in picograms in a red cell. It is a calculated value.

\[
\text{MCH =} \frac{\text{hemoglobin in gm/L}}{\text{red cell count in millions/L}}
\]

MCH generic reference range: 27 - 32 picograms
**MCHC** is Mean Corpuscular Hemoglobin Content. This indicates the average weight of hemoglobin as compared to the cell size. It is traditionally a calculated value. However, some instruments may measure the density of the cells as they are counted and use this value to compare to the calculated value. Bayer calls this measured value a CHCM (Cellular Hemoglobin Concentration Mean) on the Bayer/Technicon hematology instruments.

\[
\text{MCHC} = \frac{\text{Hemoglobin in g/mL}}{\text{Red cell count in millions/L}}
\]

or

\[
\text{MCHC} = \frac{\text{MCH in picograms}}{\text{MCV in femtoliter}}
\]

MCHC correlates with the degree of hemoglobinization of the red cells on the peripheral smear. It is reported in gm/dL, picograms/100 femtoliter or in %. A decreased MCHC corresponds to cells with increased zones of central pallor on a Wright's stained peripheral smear. These cells are called hypochromic red cells.

An increased MCHC is rarely a true value. MCHCs above the reference range are suspect. A large number of spherocytes, the most common physiologic reason, is not common. A more likely reason is an error in the hemoglobin or the hematocrit. Solutions include: checking the smear for spherocytes, retesting the sample, performing a spun microhematocrit, performing an alternate hemoglobin method, and checking the quality control and other patient results to identify shifts or trends in the hemoglobin or hematocrit determinations.

MCHC generic reference range: 32-36 g/dL or pg/fL

**RDW** is the Red cell Distribution Width. This value indicates the degree of red cell size variation or how much difference exists between the largest and smallest red cells. This value is derived from the MCV histogram. An increased RDW corresponds with an increase in anisocytosis on the peripheral smear.
The RDW is only available if it is included in the instrument menu. Although different manufacturers use slightly different methods of obtaining data the RDW is generally thought of as the coefficient of variation of red cell volume distribution.

\[
\text{RDW} = \frac{\text{standard deviation} \times 100}{\text{mean MCV}}
\]

RDW generic reference range: 9.0 - 14.5

The RDW, coupled with the MCV, gives more relevant information than an individual index. The following is an attempt to clarify the relationship of the MCV and RDW.

1. Red cells that are all microcytic or macrocytic will have a RDW in the reference range and a decreased or an increased MCV.
2. Red cells that vary in size and have an average size within the reference range will have an increased RDW and a normal MCV.
3. Red cells that vary in size and have an average size below or above the reference range will have an abnormal MCV and RDW.

There are varying opinions on the clinical value of red cell indices. They are used to morphologically classify anemias and to select additional tests to determine the cause of an anemia. Indices also change in response to treatment of some anemias.

**Platelet Indices:** These include the platelet distribution width and average platelet volume. They have limited use, but do correlate to flags on the platelet histogram that indicate giant platelet or platelet clumps.

Anisocytosis - variance in red cell size as determined by viewing the diameter of the cells on a stained peripheral blood smear.

Anemia - A reduction in the oxygen carrying capacity of the blood. Almost always manifested by a decreased hemoglobin and frequently accompanied by a decreased hematocrit and red cell count.

**Macrocytic Red Cells**
Microcytic Red Cells

Macrocytic and Microcytic Red Cells
These red cells vary in size and have an average cell size that falls within the reference range.

**Hypochromic Red Cells**

Hypochromic red cells have an increased zone of pallor on Wright's stain. These cells contain decreased hemoglobin for their size and have a decreased MCHC.
Anisocytosis: Variation in Red Cell Size

These cells exhibit a large variation in size. The RDW (Red Cell Distribution width) is a numerical indication of this morphologic abnormality.