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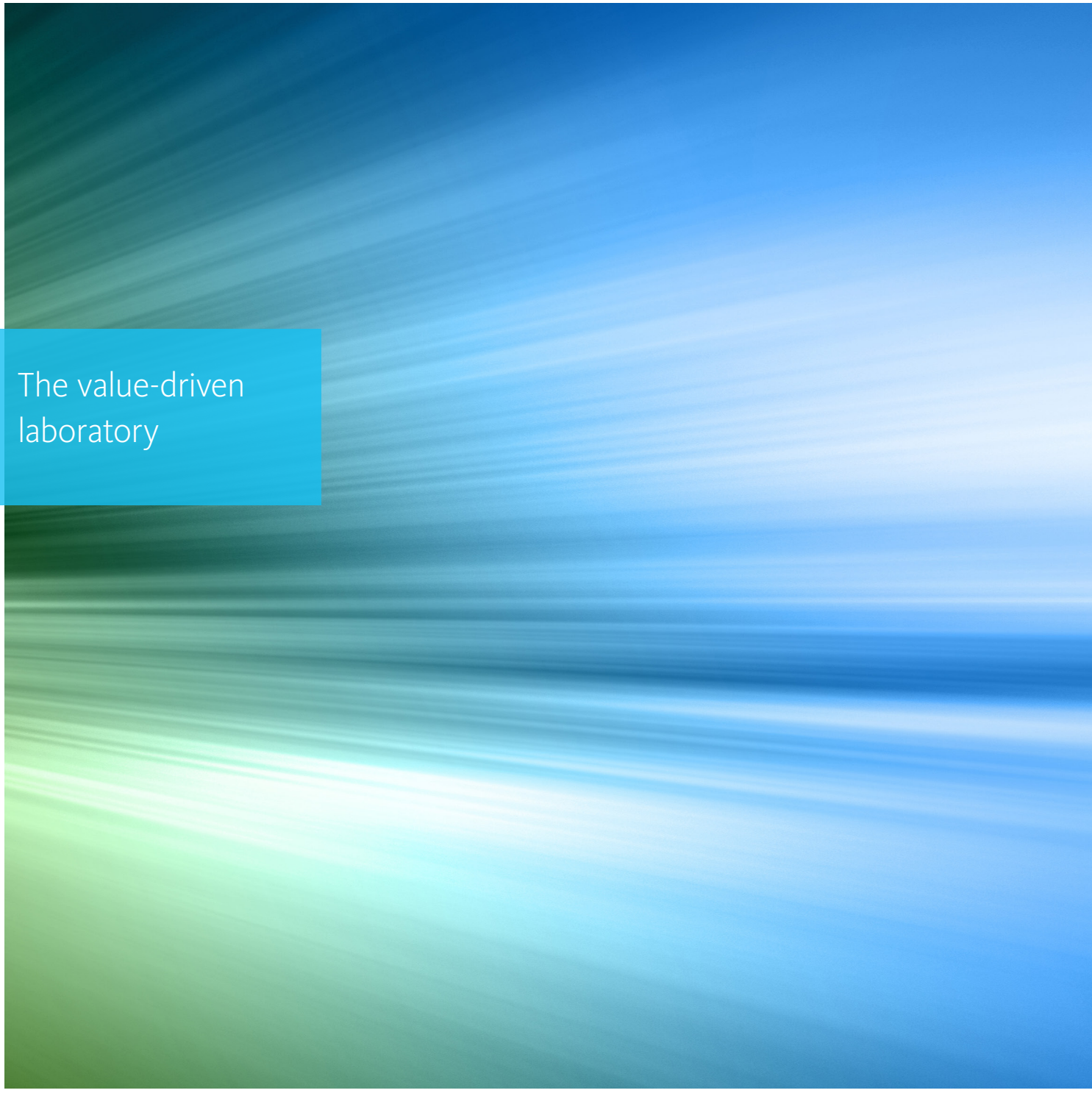
WHITE PAPER

THE INFLUENCE OF RBC COUNTING TECHNOLOGY ON MCHC RESULTS



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The Influence of RBC Counting Technology on MCHC Results

The manufacturers of hematology analyzers use varied technologies to count and size red blood cells. Technological differences, including the detection system, diluent reagent chemistry variation and parameters being directly measured or calculated, can all have an impact on results. It is important to be familiar with the technology used on a hematology analyzer and how it can impact red blood cell counting and sizing measurements in both normal and abnormal samples.

RBC Indices: A Historical Perspective

Maxwell Wintrobe invented the first reliable hematocrit measurement around 1929. Dr. Wintrobe subsequently investigated the relationship of normal red cell measurements (RBC, hematocrit and hemoglobin) and developed the red blood cell indices – MCV, MCH, and MCHC. The mathematical calculations used to obtain the red blood cell indices can be found in any hematology textbook. At the time, because of the lack of calculators and analyzers that calculate the indices automatically, laborious manual calculations needed to be performed to determine the red blood cell indices. Because of the known relationship between normal red cell measurements, a quick shortcut that could easily be done in one's head was developed. This quick shortcut became known as the "Rules of Three".

The "Rules of Three" were:

Hemoglobin x 3 = Hematocrit +/- 3%

RBC x 3.3 = Hemoglobin +/- 1.5 g/dL

RBC x 9 = Hematocrit +/- 3%

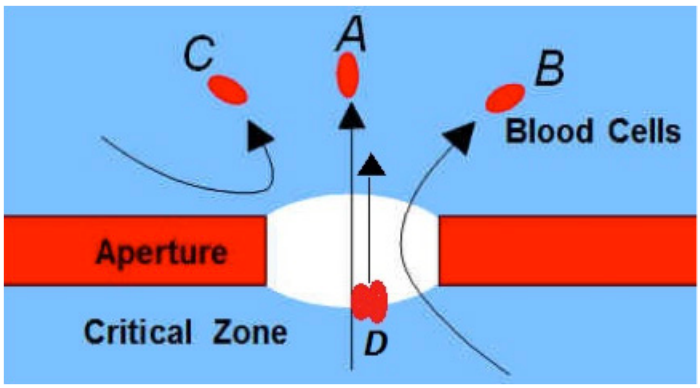
For many years the "Rules of Three" were used to compare the RBC, hemoglobin and hematocrit measurements because it was easier than calculating the indices. The "Rules of Three" helped to determine if the red blood cells were normal in size and hemoglobin content, abnormal in size and hemoglobin content or if there was an analytical error with one of the three measurements. Unfortunately, some hold onto the "Rules of Three" concept even though current hematology instrumentation automatically calculate and report the red blood cell indices. Some individuals erroneously expect every sample to follow the "Rules of Three". What is often lost in the history of the "Rules of Three" is that they only work when the red blood cells are normal. That is exactly what Dr. Wintrobe discovered: there is a relationship between red cell measurements when the red cells are normal in size and hemoglobin content. Thus, patients with microcytic or macrocytic RBCs cannot be expected to follow the "Rules of Three". The "Rules of Three" were developed because RBC indices were not readily available. RBC indices are routinely reported by automated hematology analyzers and it is time to leave the "Rules of Three" to the history books.

Detection System

Cell counting has its origins in electrical impedance. Classic impedance counting pulls diluted cells through the sensing zone of an aperture by applying a vacuum force to the back of the aperture. There are well documented limitations inherent in classic impedance counting which can affect cell counting and sizing. These limitations include:

- Coincidence (two or more cells passing through the aperture simultaneously)
- Non-axial passage (a cell that does not pass straight through the aperture)
- Recirculation (a cell which recirculates after counting, back into the aperture sensing zone being recounted)
- Deformation of the cell as it is pulled by vacuum through the aperture. (This is especially problematic with abnormal red blood cells. This may result in artificially decreased hematocrit results and artificially increased MCHC results.)

Classic Impedance Counting



The above diagram illustrates the issues inherent with classic impedance counting.

Example A:

Most cells are pulled through the center of the aperture and counted and sized accurately.

Example B (Non Axial Passage):

Some cells are pulled through the aperture near the edge of the aperture sensing zone rather than through the center of the aperture. When this occurs, the cells spend more time in the sensing zone and appear larger than their actual size. While the cell is counted correctly, it is not sized correctly.

Example C (Recirculation):

After passing through the aperture, some cells recirculate back into the sensing zone. These cells are erroneously counted again, and because they have spent little time in the sensing zone, are erroneously measured as very small cells.

Example D (Coincidence):

Multiple cells can pass through the aperture simultaneously. This is a well-known limitation referred to as coincidence. When two or more cells pass through the aperture together, they are erroneously measured as one big cell. Coincidence is a statistically predictable phenomenon related to the cell count – the higher the cell count, the more likely cells will go through the aperture two or more cells at a time. Because coincidence is statistically predictable, the cell count can be mathematically corrected and an accurate result obtained. Although the cell count can be corrected mathematically for coincidence, cell sizing cannot be corrected.

Various methods have been devised to decrease the impact of these limitations on the accuracy of red cell counting and sizing. These include:

- Mathematical correction for coincidence
- Pulse height editing (Eliminating aberrant data, such as with examples B, C and D, from the RBC size determination)
- Eliminating cell recirculation by passing a stream of fluid or placing a physical barrier behind the aperture to keep cells away from the sensing zone.
- Smaller aperture size to force more cells toward the center of the sensing zone.

While these methods provide some improvement to classic impedance counting, they cannot completely overcome the limitations associated with correctly sizing the red blood cells.

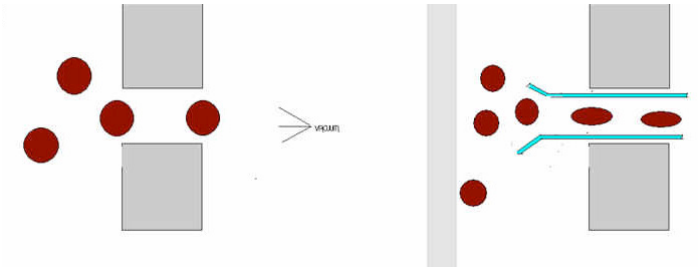
Optical particle counting is a more recent technology. To count cells optically, it is necessary to control the number of cells and their orientation as they are measured as well as to modify the cell for optimal detection. Controlling the orientation of cells as they are measured is done using the principle of hydrodynamic focusing. With hydrodynamic focusing, a stream of sheath reagent forces the cells to enter the measurement zone in a single file pattern. Hydrodynamic focusing allows for cells to be analyzed individually without the limitations associated with classical electrical impedance counting such as coincidence, non-axial passage, recirculation or cell deformation. Modifications which enhance optical detection include staining of cells or sphering of cells by the diluent reagent to increase the amount of light they scatter.

Sysmex hematology analyzers use electrical impedance with hydrodynamic focusing to count and size red blood cells and overcome the limitations associated with classic impedance counting. Rather than pulling cells through the aperture with vacuum, cells are surrounded by a stream of sheath fluid. The sheath fluid forces the cells to pass through the aperture single file by guiding them individually. This eliminates the error associated with coincidence, nonaxial passage and recirculation of cells after counting. Cells are maintained close to their natural size and appearance in the plasma and not deformed by vacuum as they pass through the aperture or from sphering of cells to enhance detection. This allows for accurate determination of hematocrit and MCHCs in both normal and abnormal samples.

Hydrodynamically Focused Impedance Counting

Impact of Hydrodynamic Focusing and RBC Deformability on RBC Counting and Sizing.

Normal Red Blood Cells

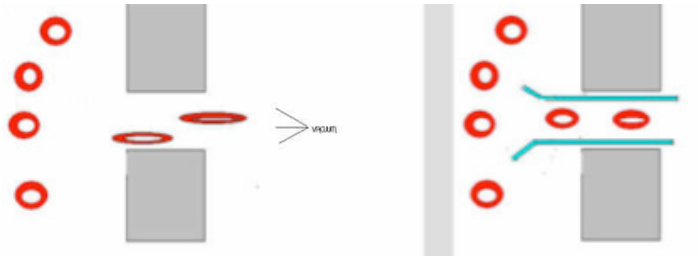


Classic Impedance | Hydrodynamically Focused Impedance

Normal RBCs, when pulled through an aperture, deform a predictable amount. They become a little more streamlined and pass through the aperture at a predictable rate. Since RBC size measurements are related to the time a cell takes to pass through the sensing zone, the predictable rate is important. This is why non-axial passage causes normal cells to appear abnormally large. The cells remain in the sensing zone longer than they should be.

Hydrodynamic focusing assures all the cells pass through the center of the aperture. Hypochromic RBCs pulled through an aperture and exposed to the same shear force will deform more than normal RBCs because they lack the normal amount of hemoglobin. Hypochromic RBCs pass through the aperture more quickly which results in an underestimation of their size. Since the size of the cells is underestimated, the hematocrit will be decreased and the MCHC increased.

Hypochromic Red Blood Cells



Classic Impedance | Hydrodynamically Focused Impedance

The opposite is true with hyperchromic RBCs. Because hyperchromic RBCs deform less than normal RBCs, they take longer to pass through the aperture. This results in an overestimation of cell size because they passed through the sensing zone more slowly than they should have. The resultant hematocrit will be increased and the MCHC decreased.

Hydrodynamic focusing controls the shear force and speed of passage of cells through the aperture, minimizing cell deformability regardless of the hemoglobin content. Thus the cell size is accurately measured in both normal and abnormal red blood cells. This allows an MCHC determination that reflects the true physiologic range seen.

Diluent Reagent Chemistry Variation

For electrical impedance counters to function properly, the diluent reagent must conduct electricity and stabilize the cells during the counting process. Manufacturers employ diluent reagents with varying osmolality and components to stabilize the cells during the counting process. These varying reagent chemistry characteristics can impact the final cell sizing results, particularly in abnormal samples or old blood.

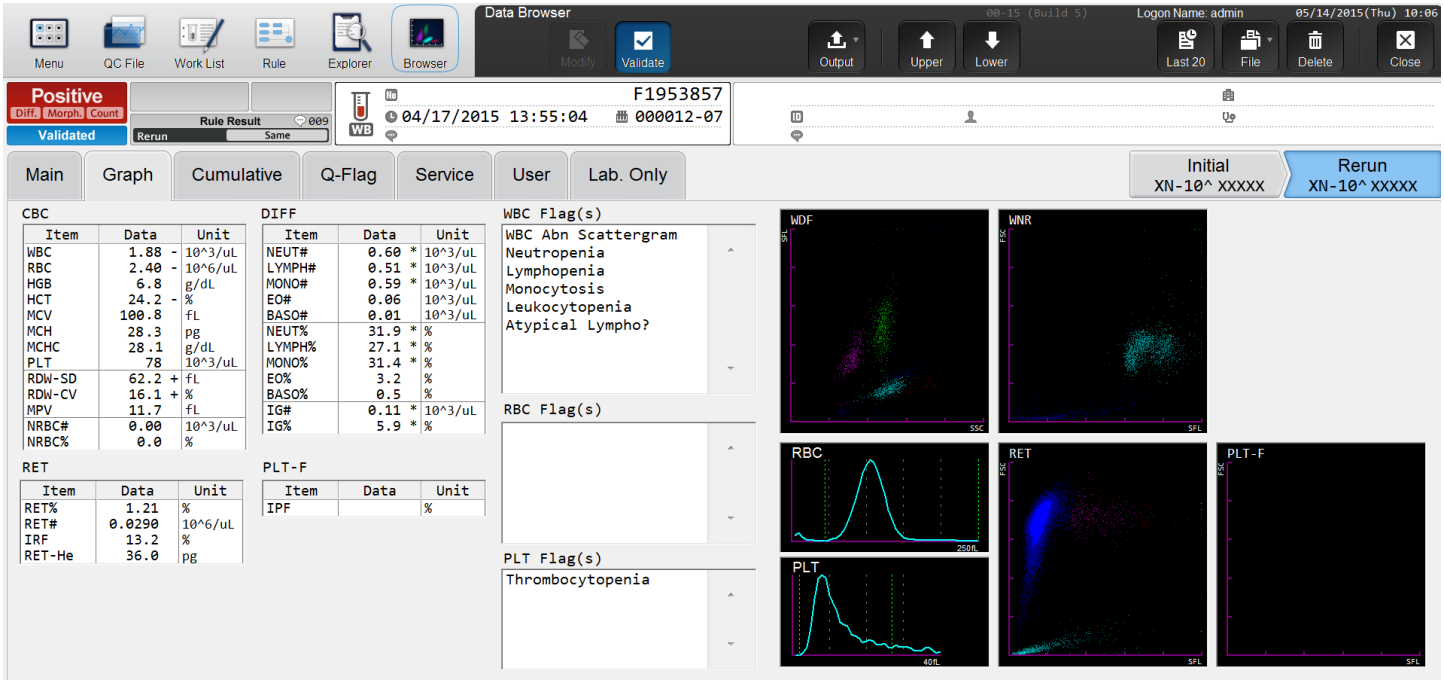
Sysmex CELLPACK™ DCL has an osmolality of approximately 250 mOsm/kg. This means a normal cell, which has a slightly higher osmolality, takes water in. The MCV rises, but the rise is insignificant. This slight rise in cell volume can be compensated for in the calibration of the hematocrit. The advantage of this method is better separation of the RBC and PLT populations during the counting and sizing process.

Other vendors can employ diluents with higher osmolality than normal plasma. When cells are exposed to this more hypertonic diluent, they will lose water and shrink slightly. While this effect is compensated for by calibration, the compensation is in the opposite direction compared to that of Sysmex analyzers.

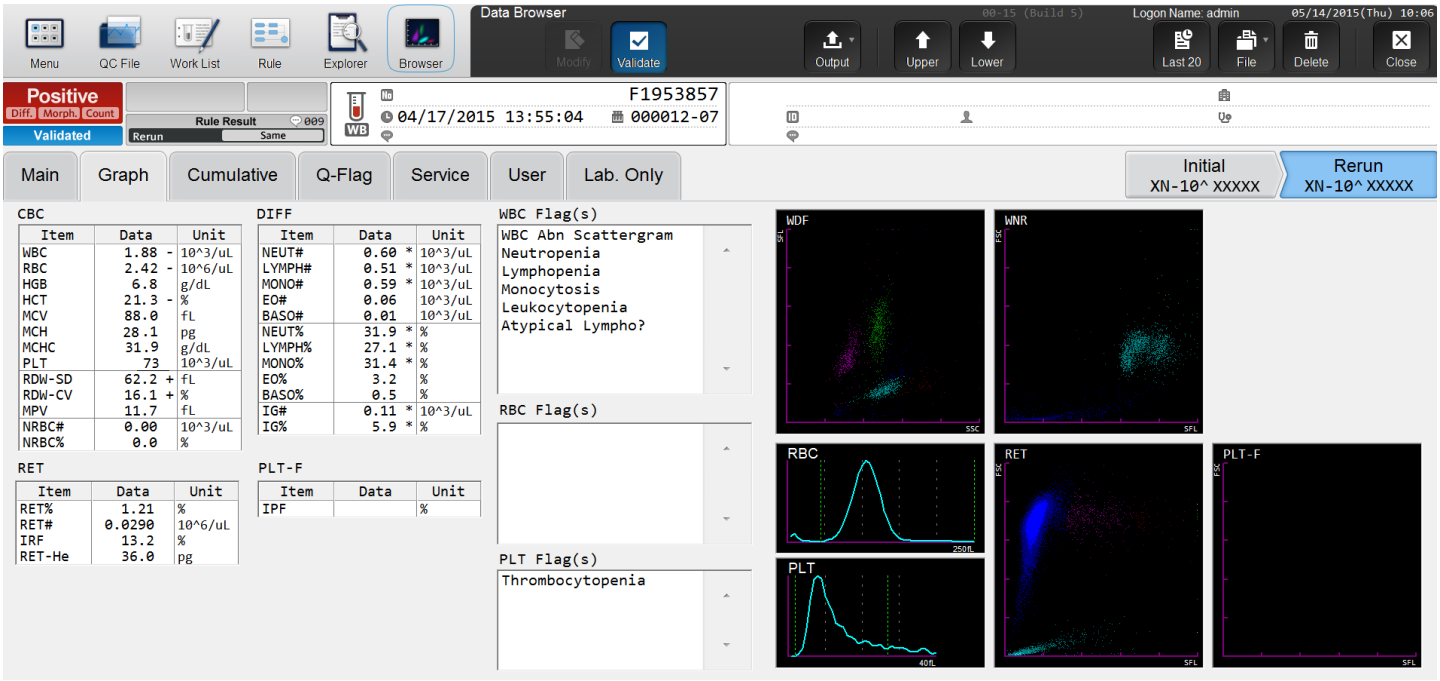
If the osmolality of the sample is pathologically high (>350 mOsm/kg) from electrolyte imbalances, hyperglycemia or from drugs which impact plasma osmolality, the MCV may be falsely increased and the MCHC falsely decreased due to the relative hypotonic solution compared with the hypertonic cell concentration. In samples where this interference is suspected, dilution of the sample with CELLPACK DCL followed by room temperature incubation for ten to fifteen minutes prior to reanalysis is helpful. This allows the cells to equilibrate with the CELLPACK DCL so correct MCV and MCHC results can be obtained.

Impact of Electrolyte Abnormalities on MCHC Determination

Example A1 shows the initial run of a sample from a patient with severe hypernatremia. The MCV is 100.8 fl and the MCHC is 28.1 g/dL. While anemias may have microcytic/hypochromic cells or microcytic/normochromic cells, they generally do not have macrocytic/hypochromic cells. Thus the indices on this patient do not make sense. When pre-diluted with CELLPACK DCL diluent and allowed to equilibrate for 15 minutes, the RBC measurements change dramatically. The pre-diluted/equilibrated results (Example A2) show an MCV of 88 fl and an MCHC of 31.9 g/ dL.



Example A1: Hypernatremia (Initial Run)



Example A2: Hypernatremia (Following pre-dilution with CELLPACK and 15 minute incubation)

Why does this occur? RBCs must adjust to their environment to survive. When a patient has a severe electrolyte imbalance, the plasma environment is no longer normal. For instance, when the plasma sodium is high, the RBCs must adjust to this environment by expelling water and concentrating their sodium content to match the in vivo environment. So neither the RBCs nor the plasma is what one would call 'isotonic'. When these RBCs are put in a near isotonic environment such as CELLPACK DCL, they quickly adjust to the new environment. The new environment is no longer a high sodium environment and the cells rapidly take in fluid to adjust and lower their sodium content. However, because cell counts on automated analyzers are performed rapidly, the cells do not have time to completely equilibrate before being counted. This causes the MCV and HCT to be artificially elevated and the MCHC to be decreased. Pre-diluting the sample with CELLPACK DCL and giving the RBCs time to equilibrate to the more normal environment allows the cells to stabilize to their actual size.

Hyponatremia will have the opposite effect. The RBCs take in water to dilute their internal content and equilibrate to the in vivo environment. When these RBCs are put in a near isotonic environment such as CELLPACK DCL, they quickly try to adjust to the new environment. The environment is no longer a low sodium environment, so the cells rapidly expel fluid to adjust and increase their sodium content and equilibrate with the now isotonic environment. Again, because of the speed of the analyzer cell analysis, these RBCs do not have time to fully equilibrate. This results in an artificially decreased MCV/HCT and an elevated MCHC. Again, pre-diluting the sample with CELLPACK DCL and allowing the RBCs time to equilibrate will allow the cells to stabilize to their actual size.

A patient's state of hydration can also impact the MCHC result. The fact that many patients are fasting when their blood is drawn adds the complication that they may be somewhat dehydrated. Red blood cells from patients who are severely dehydrated, common in patients who present to the Emergency Department, can cause the MCHC to approach or go slightly beyond 36.0 g/dL

Measured Versus Calculated Parameters

Hematocrit is a function of the number of red blood cells and the size of the red blood cells. Most hematology analyzers calculate hematocrit using the following equation:

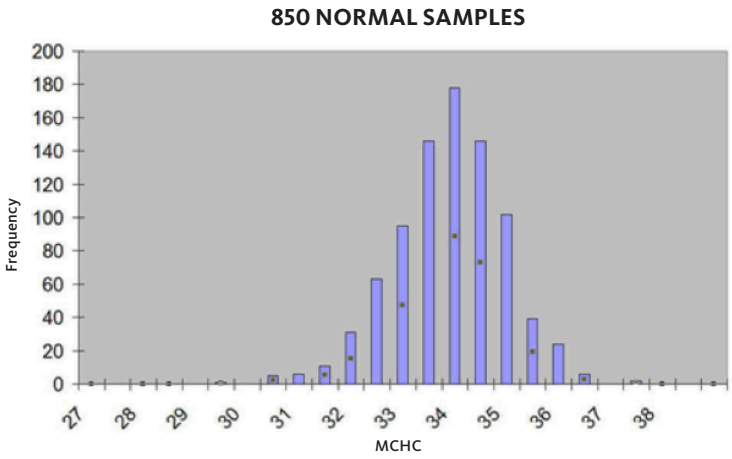
HCT% = (RBC x MCV)/10

Sysmex hematology analyzers directly measure hematocrit by adding up the cumulative number and heights of the pulses determined during the RBC counting process. This correlates more closely with the reference micro-hematocrit method than other methods which calculate the hematocrit.

MCHC Reference Range Information

A reference range study conducted on a Sysmex analyzer with hydrodynamically focused impedance technology using samples from 850 normal, healthy blood showed a range of 32.0 to 36.4 g/dL (central 95%). As this range was determined from the central 95%, 5% of these normal healthy individuals had MCHC results of less than 32.0 or greater than 36.4 g/dL.

Sysmex recommends that each laboratory establish its own expected reference intervals based upon the laboratory's patient population. Individuals that have an MCHC slightly higher than 36.0 g/dL often tend to be healthy young males who have a hemoglobin result that is near the high end of the reference range.



MCHC Reference range study Mount Sinai Medical Center, Milwaukee, WI (1996)

Summary

The use of electrical impedance with hydrodynamic focusing, using diluent closer to normal plasma osmolality and directly measuring hematocrit will result in a wider dynamic range for MCHC results on Sysmex systems when compared to systems that use classic electrical impedance. This is more apparent with abnormal samples because the cells are not being distorted during the counting process. The net effect is hematocrit and MCHC results that more closely correlate to reference methods regardless of patient condition.

References

1. Sysmex XN-L Series Flagging Interpretation Guide, February 2019.
2. XN-Series Automated Hematology Systems Flagging Interpretation Guide, February 2019.
3. Bessman, J.D., Automated Blood Counts and Differentials, A Practical Guide, Johns Hopkins University Press, 1986.
4. Wintrobe, M., Greer, J., Wintrobe's Clinical Hematology, 12th edition, Lipinncott, Williams & Wilkins, 2008.
5. Mohandas, N., Clark, M.R., "Inaccuracies associated with the automated measurement of mean cell hemoglobin concentration in dehydrated cells", Blood, Volume 56, 1980.

Evaluation and Troubleshooting of MCHC Results

Pattern of Results	Encountered in
Low MCV Low MCHC	• Microcytic, Hypochromic Anemia
Low MCV Normal MCHC	• Hemoglobinopathies (i.e., thalassemia)
Normal or High MCV Low MCHC (<30 g/dL)	• Plasma electrolyte abnormalities (i.e., high sodium) affecting hematocrit results • High glucose affecting hematocrit results ***Refer to Troubleshooting Chart***
Low or Normal MCV High MCHC (>37.5 g/dL)	• Hemolysis • Plasma electrolyte abnormalities (i.e., low Sodium) affecting hematocrit results • Severe lipemia • Icterus • Severe leukocytosis affecting hemoglobin measurement • Abnormal plasma protein precipitation affecting hemoglobin measurement ***Refer to Troubleshooting Chart***
High MCV High MCHC (>37.5 g/dL)	• RBC Agglutination • Rouleaux ***Refer to Troubleshooting Chart***

Troubleshooting Chart
Always follow your local laboratory procedure for repeat testing or rejection of samples

Electrolyte Abnormalities or High Glucose Affecting Hematocrit?	RBC Agglutination?	Severe Lipemia, Icterus, Abnormal Protein or Leukocytosis Affecting Hemoglobin Measurement?
1. Perform a 1:5 dilution of sample with CELLPACK 2. Allow the dilution to equilibrate for ten to fifteen minutes 3. Rerun after equilibration	1. Prewarm at 37°C for fifteen to thirty minutes then rerun 2. Severe cold agglutinins or rouleaux may require dilution or plasma replacement with CELLPACK 3. For severe cold agglutinins, additional incubation at 37°C may be necessary following dilution or plasma replacement	1. Perform a 1:5 dilution of sample with CELLPACK 2. Repeat diluted sample Lipemia or Icterus Only Perform a plasma replacement procedure

* Taken from XN-Series flagging interpretation guide, Rev 5

Sysmex America, Inc.
577 Aptakisic Road, Lincolnshire, IL 60069, U.S.A. · Phone +1 800 379-7639 · **www.sysmex.com/us**
Sysmex Canada, Inc.
5700 Explorer Drive Suite 200, Mississauga, ON L4W0C6 Canada · Phone +1 905 366-7900 · **www.sysmex.ca**

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