1. Summary

The mean corpuscular volume (MCV) is one of the standard red blood cell "indices". It is the volume of the "average" red blood cell, stated in femtoliters (fL) and it has been available as a reliable, directly measured value, for more than 40 years. Diagnosis of different types of anemia may be assisted by the mean corpuscular volume.

2. Definition and principles MCV measurement

Definition:

MCV represents the average size or volume of the red blood cells. It is very stable and intra-individual variations are symptomatic of altered physiology or spurious results in reference to XM and XB analysis controls*. It was originally obtained from the blood film but, nowadays with the practice of automated hematology analyzers, it is a very reproducible measure, even more than Red Blood Cell (RBC) count. Since the MCV is derived from the entire populations of RBC with a life span of about 120 days, it takes some time to be altered by an acquired anemia (such as state of iron deficiency for example).

To calculate the MCV in femtoliters (fL or 10^{-15}L), the following formula is used

\[
MCV = \frac{\text{Hematocrit} \times 10}{\text{RBC count (millions/mm}^3\text{ blood)}}
\]

Hematocrit is the percentage by volume of packed red blood cells in a given sample of blood after centrifugation

*XB monitors instrument performance by tracking the MCV, MCH, and MCHC parameters of all patient samples. It is a very sensitive indicator of instrument problems.

*XM analysis monitors instrument performance using an Exponentially Weighted Moving Average of CBC, Diff, and Reticulocyte parameters of all patient samples. It compares these averages with target means and limits.
Principle and explanation of the measurement

Despite its steadiness during patient life span, the MCV is the measured red cell parameter that varies the most among technologies.

The MCV measurement is obtained automatically by two methods:
- Aperture impedance:
- Or by light scattering technology

**Measurement by Impedance**

Impedance measurement (Coulter principle) was historically the first automated principle of measurement. This is based on the measurement of changes in resistance during cell passage through a small defined opening between two electrodes. The cells have lower conductivity than the diluent. The resulting electrical impulse is proportional to the cell volume. The sum of the impulses from all cells in a fixed measurement volume is evaluated with a histogram. The principle of hydrodynamic focusing was an important development of this concept. This almost totally prevented coincidence and gave a clean Gaussian curve for the erythrocytes. It also improved the separation of small erythrocytes and platelets. The development of hydrodynamic focusing enabled to avoid duplicated cell counting by coincidence. The sample stream is coated with a coat stream fluid (sheath stream). This reduces the diameter of the sample stream to cell size and isolates the individual cells. The cells are then passed through the electric field like a string of pearls and cannot be washed back by turbulence in the area of measurement.

**Measurement by light scattering technology**

The measurement is performed with erythrocytes transformed to a spherical form (sphered erythrocytes; Patent Technicon, Bayer, Siemens) or treated with a surface active diluent to optimize their shape. There are three preconditions for exactly reproducible scatter signals when measuring and evaluating erythrocytes: i) formation of isovolumetric spheres or optimization, ii) a monochromatic light source, and iii) isolation of the blood cells in the measurement cell by hydrodynamic focusing.

The frequency of coincidence passages is statically predictable based on the number of cells. This correction is done automatically by the analyzers.
Horiba measurement on Pentra series

The RBC volume is measured by an electronic impedance variation principle. MCV is calculated directly from the RBC histogram.

Traceability chain for Hematocrit measurement on Horiba analyzers (2)

The controls and calibrators associated with HORIBA analyzers comply with the requirements of the standard ISO 17511 : 2003, relating to the metrological traceability of values attributed to calibration agents and control equipment (*in vitro* diagnostic medical devices) for the measurement of quantities in biological samples.
Competitors measurement

Siemens Advia 2120 and Abbott Cell Dyn Ruby use optical technology for measuring MCV whereas Sysmex XE/XN series, Coulter BCI LH series/Dx800 series, Abbott Cell-Dyn Sapphire measure MCV by Impedance technology. (3) (4) (5)

3. Analytical Performances of Medical analyzers

Precision (Repeatability)

These imprecision studies of Horiba Medical instruments were performed using normal fresh whole blood whose values did not exceed the normal reference interval for the laboratory as recommended by CLSI H26-A2 document (§5.9) (2)

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Specifications</th>
<th>Normal level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HORIBA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentra 60 series</td>
<td>&lt;1.5% (6)</td>
<td>0.26%</td>
</tr>
<tr>
<td>Pentra 80 series</td>
<td>&lt;1.5% (6)</td>
<td>0.42%</td>
</tr>
<tr>
<td>Pentra 120 serie</td>
<td>&lt;1% (6)</td>
<td>0.51%</td>
</tr>
<tr>
<td><strong>COMPETITORS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sysmex XE 2100</td>
<td>≤1.5% (6)</td>
<td>0.25% (5)</td>
</tr>
<tr>
<td>BCI LH 750</td>
<td>&lt;0.8% (6)</td>
<td>0.3% (4)</td>
</tr>
<tr>
<td>BCI DxH 800</td>
<td>≤1.0% (6)</td>
<td>≤1.0%</td>
</tr>
<tr>
<td>Abbott CD 3700</td>
<td>≤1.0% (6)</td>
<td></td>
</tr>
<tr>
<td>Abbott CD Sapphire</td>
<td>≤1.0% (6)</td>
<td>0.2% (7)</td>
</tr>
<tr>
<td>Siemens Advia 2120</td>
<td>0.78% (6)</td>
<td>0.2% (8)</td>
</tr>
<tr>
<td>Mindray BC 6800</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Accuracy evaluation of laboratories participating in periodic blind surveys (External Quality Assessment EEQ ProBioQual)

ProBioQual statistic histogram distribution for MCV measurement illustrate the variability of MCV measurement among technologies showing the differences between different medical analysers.

MCV Precision (CV)
4. Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation (9)

Dr. Carmen Ricos provides scientific community with a comprehensive database of biologic variation: the data includes the observed between- and within-subject biologic variation for these quantities, as well as desirable specifications for imprecision, bias and total allowable error.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Biological Variation</th>
<th>Desirable specification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CVw</td>
<td>CVg</td>
</tr>
<tr>
<td>MCV</td>
<td>1.3</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Note on abbreviations:
- CVw = within-subject biologic variation
- CVg = between-subject biologic variation
- I = desirable specification for imprecision
- B = desirable specification for inaccuracy
- TE = desirable specification for allowable total error

5. Reference range (10)

Reference intervals for red cell measurement vary with gender, age and environmental factors. Each laboratory is strongly advised to establish its own set of normal ranges according to the local population.

Normal values range for pediatric to adult specimens (10):

<table>
<thead>
<tr>
<th>Age</th>
<th>Male (fL)</th>
<th>Female (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-10</td>
<td>76-88</td>
<td>76-89</td>
</tr>
<tr>
<td>10-14</td>
<td>78-90</td>
<td>79-91</td>
</tr>
<tr>
<td>14-18</td>
<td>80-95</td>
<td>80-95</td>
</tr>
<tr>
<td>18-25</td>
<td>81-97</td>
<td>81-96</td>
</tr>
<tr>
<td>25-35</td>
<td>83-97</td>
<td>80-97</td>
</tr>
<tr>
<td>35-45</td>
<td>83-98</td>
<td>77-98</td>
</tr>
<tr>
<td>45-55</td>
<td>84-98</td>
<td>76-97</td>
</tr>
<tr>
<td>55-65</td>
<td>84-100</td>
<td>81-99</td>
</tr>
</tbody>
</table>

Table of MCV cut-off from different sources:

Low or high values of MCV refer to abnormalities or disease and suggest action limits. The table below summarizes different cut off obtained from several studies:

<table>
<thead>
<tr>
<th>Units</th>
<th>ANAES (11)</th>
<th>Failace-Pranke (12)</th>
<th>Chapman M (13)</th>
<th>CLSI H26-A2 (2)</th>
<th>Buttarello (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Min</td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>MCV fL</td>
<td>N/A</td>
<td>N/A</td>
<td>75</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>
6. Clinical evidence: MCV in anemia

Erythrocytes that have a normal size or volume (normal MCV) are called normocytic.

Patients with normal or hypoproliferative reticulocyte counts and normocytic, normochromic anemia generally requires bone marrow evaluation. A peripheral blood smear may provide valuable clues for the differential diagnosis.

| Curve lies between the lower and upper limits |
| When the MCV is low (under 70 fL), RBC are termed microcytic. |

**Microcytic anemia**

Microcytic anemia is the most commonly encountered anemia in general medical practice. Nutritional iron deficiency (ID) and β thalassemia trait (BTT) are the primary causes in pediatrics, whereas bleeding disorders and anemia of chronic disease are common in adulthood.

| Curve lies near or just under the lower limit |
| When the MCV is high (up to 100 fL), RBC are called macrocytic. |

**Macrocytic anemia** is less common than normocytic or microcytic anemia. Macrocytic anemia may be subdivided into cases with a normal RDW (principal those caused by bone marrow failure, such as aplastic anemia and myelodysplasia), and those with a high RDW (caused by either deficiencies of vitamin B12 or folic acid; by autoimmune hemolysis or cold agglutinins).

| The peak of the Curve rises to the upper limit |

**MCV Inaccuracies resulting from pathology**

Literature identified several clinical conditions that affect the MCV analysis to different degrees on all hematology analyzers. Briefly, falsely high MCVs can be caused by high white blood cell (WBC) counts or the presence of agglutinins. These interferences are corrected by removal of the buffy coat and replacement with isotonic salt solution and repeat estimation. Changes in plasma osmolarity also affect the MCV analysis. RBCs from patients with severe hypernatremia or severe hyperglycemia are hyperosmolar. When such blood is run on an automated hematology analyzer, RBCs swell rapidly because water is moving in faster than electrolytes, glucose, or urea can move out, resulting in an artificially increased MCV. Such phenomena have been observed in hypernatremic dehydration, severe uremia in renal failure, and hyperglycemia due to uncontrolled diabetes or intravenous feeding with concentrated carbohydrates. The opposite effect is seen in hypo-osmolar states such as hyponatremia in chronic alcoholics and patients with inappropriate secretion of antidiuretic hormone. Dilution of the blood sample in an isotonic solution before analysis allows red cells to equilibrate to normal external conditions and results in more accurate MCVs. Falsely low MCVs can also be generated when significant numbers of large platelets are present.
Common blood disorders: a primary care approach

- **HGB**
  - **MCV**
    - **RDW**
      - **Microcytic** (MCV < 80 fl)
        - **Normal RDW**
          - Anemia of Chronic Disease (ACD)
          - Thalassemia minor
          - Hemoglobinopathies
        - **HbA2**
          - Hemoglobin analysis
      - **High RDW**
        - Iron deficiency
        - Hemoglobinopathies
        - (HbS/Thal/HbH)
        - Mechanical hemolytic anemia
        - Sideroblastic anemia
      - **Iron binding capacity testing**
    - **Normocytic** (MCV 80 - 100 fl)
      - **Normal RDW**
        - Anemia of Chronic Disease (ACD)
        - Hereditary spherocytosis
        - Renal chemotherapy
        - Aplasia
        - Heterozygote hemoglobinopathy
        - Acute blood loss
      - **High RDW**
        - Early iron deficiency
        - Chronic hepatobiliary disease
        - Myelotoxic chemotherapy
        - Acute lymphocytic leukemia
        - Chronic myelocytic leukemia
        - Hemolysis-G6PD
        - Sickle cell disease
      - **MCH**
      - **Normal or decreased RET**
    - **Macrocytic** (MCV > 100 fl)
      - **Normal RDW**
        - Bone Marrow failure (Aplastic anemia, myelodysplasia)
        - Refractory anemia
      - **High RDW**
        - Acute nonlymphocytic leukemia
        - Myelotoxic chemotherapy
        - Liver and Thyroid disease
        - Hereditary spherocytosis
        - Cold agglutinine disease
        - B12 deficiency
        - Folic acid deficiency
        - Alcohol
        - Autoimmune hemolytic anemia
      - **Elevated RET**
      - **Positive COOMBS**
7. Bibliography

7. Younhee Park, M.D., Jaewoo Song, M.D., Sungwook Song, M.D., Kyung Soon Song, M.D., Mee Suk Ahn, M.T., Mi-Sook Yang, M.T., Il Kim, M.T., and Jong Rak Choi, M.D. Evaluation of the Abbott Cell-Dyn Sapphire Hematology Analyzer. 2007.