

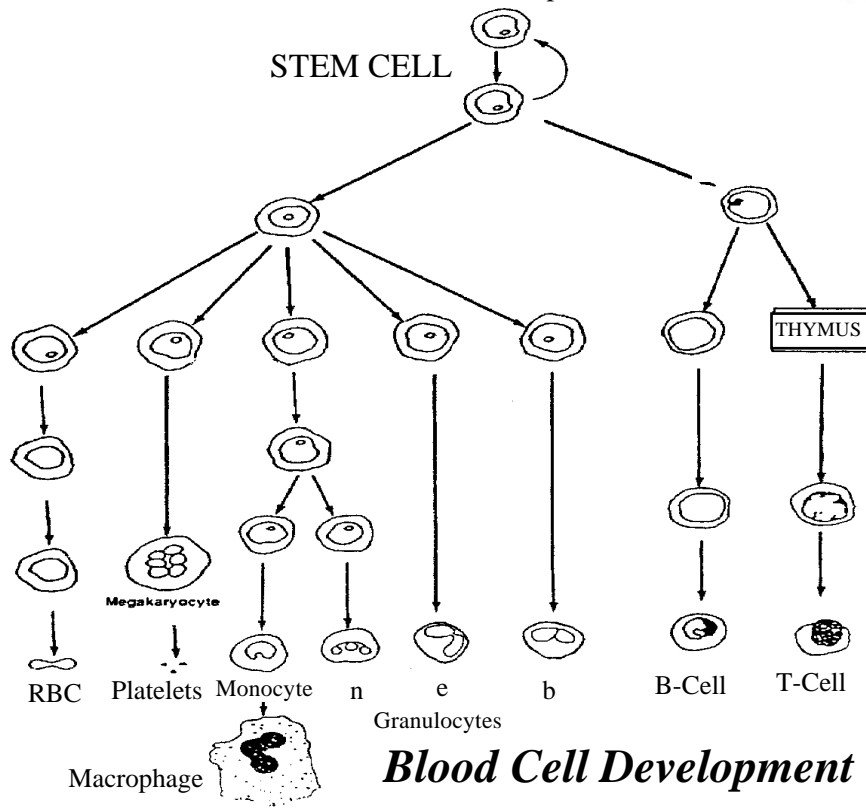
BLOOD TESTS

Jan 2001

Information compiled and edited by Barb Hauser for the IWMF

WHOLE BLOOD is comprised of plasma (the liquid part) and the formed elements (**red blood cells, white blood cells, and platelets**).

The process by which all formed elements of the blood are produced (hematopoiesis), occurs mostly in the bone marrow, where cells mature from a primitive



stem cell.

Billions of red, white blood cells and platelets are produced per kilogram of body weight daily. Factors important in regulating blood cell production include the environment of the bone marrow, interactions among cells, and secreted chemicals called growth factors.

Patients with Waldenstrom's Macroglobulinemia experience a reduced capacity to produce several types of whole blood cells in the bone marrow (myelosuppression) because the overproduction of immature WM cells suppresses production of the other blood cell types.

Chemotherapeutic agents (which destroy fast growing cells of the

body) also contribute to lowered blood cell production.

NOTE: As used in this paper, "Normal" is not what you might presume. Nobody really knows what "normal" means, and it varies with gender, age, nutritional environment and methods of testing. Laboratories compare your readings to a "Normal" or "Reference Range" but that range is **not a national standard**, but a comparison with all of their other tests. We collected "ranges" from 18 major national clinics and labs, and all of the numbers differed. The numbers you see here will give you some rough idea of "normal" but what is more important is how your numbers change over time, more than the absolute value. It is for this reason that it is important to always have your blood tests done at the same laboratory.

A **COMPLETE BLOOD COUNT** (CBC) is a measurement of the blood cells in a specific volume of blood. Common components of a blood count that are important to WM are as follows:

RBC

Name: Red Blood Cells (erythrocytes).

Normal Range: 4.7-6.1 million/cu.mm

WM Abnormality: Low RBC count (anemia) diminishes the body's ability to carry oxygen to the tissues.

Function/Test Purpose: Formed in the bone marrow, a typical red blood cell lives for about 120 days. These cells transport oxygen to all parts of the body. This test is done to support other tests in diagnosis of anemia and to supply figures for computing the erythrocyte indices, which reveal RBC size and hemoglobin content.

Test Mechanism: Red Blood Cells are counted with an instrument called a Coulter Counter. The sample is diluted in an electrically charged solution and moves slowly through an aperture across which a specific voltage passes. As each cell passes through, the voltage changes, creating a pulse. The voltage magnitude varies also with cell size. This way the cells are counted, and sized. Particles greater than 36 fL are counted as RBCs.

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HCT -

Name: Hematocrit

Normal Range: 42-51%

WM Abnormality: Lowered hematocrit.

Function/Test Purpose: The volume occupied by the packed red blood cells in a given volume of centrifuged blood. It is used in determination of anemias, and is usually expressed as a percentage of the volume of the whole blood sample.

Test Mechanism: This is a calculated value from the values of the red cell count and the Mean Corpuscular Volume. $HCT = RBC \times MCV$

HGB

Name: Hemoglobin

Normal Range: 14-18 g/100 ml.

WM Abnormality: Lowered hemoglobin.

Test Purpose: Hemoglobin is the pigment in red blood cells that contains iron and transports oxygen to the tissues. This is the main component of the red blood cell. The test coordinates with other red blood cell data.

Test Mechanism: An instrumental method, using a spectrophotometer, measures the intensity of light which passes through the blood sample. Less light transmittance equates to more hemoglobin.

ERYTHROCYTE INDICES below provide information about the size, hemoglobin concentration and hemoglobin weight of an average RBC:

MCV

Name: Mean Corpuscular Volume

Normal Range: 80-96 fL

WM Abnormality: Varies; however, MCV may be elevated due to erythrocyte aggregation.

Function/ Test Purpose: This is the average volume of the red blood cells, the ratio of hematocrit to the RBC count. The Mean Corpuscular Volume expresses the average size of the erythrocytes, and indicates whether they are undersized, oversized or normal.

Test Mechanism: Measured with a Coulter Counter. The cell volume is derived from the amount of voltage variance as each cell is counted. $MCV = \frac{HCT \times 10}{RBC \text{ count (millions/}_l)}$

MCH

Name: Mean Corpuscular Hemoglobin

Normal Range: 20-33 pg

WM Abnormality: Varies; hemoglobin will be depressed if RBC count is lowered.

Function/Test Purpose: Mean Corpuscular Hemoglobin is the content of hemoglobin in the average red blood cell, the hemoglobin/RBC ratio. It expresses the weight of hemoglobin in an average RBC.

Test Mechanism: A calculated value using RBC count, hemoglobin and hematocrit.

$$MCH = \frac{\text{Hemoglobin (g/L)} \times 10}{RBC \text{ count (millions/}_l)}$$

MCHC

Name: Mean Corpuscular Hemoglobin Concentration

Normal Range: 32-36%

WM Abnormality: Varies; if hemoglobin is lowered, hematocrit is also lowered.

Function/Test Purpose: The ratio of hemoglobin weight to hematocrit.

This test defines the concentration of hemoglobin in 100 ml. of packed red blood cells. It helps distinguish the normally colored cells from paler cells to help classify different anemias and aid in determining cause.

Test Mechanism: A calculated volume using RBC count, hemoglobin and hematocrit.

$$MCHC = \frac{\text{Hemoglobin (g/dl)} \times 100}{HCT (\%)}$$

ESR

Name: Erythrocyte Sedimentation Rate

Normal Range: 0-30 mm/hr in people over 50

WM Abnormality: Elevated rates are usually found in people with WM because of the presence of excessive amounts of the macroglobulin IgM.

Function/Test Purpose: This is a determination of erythrocyte and/or plasma abnormalities. It aids in the ability to distinguish among diseases with similar symptoms.

Test Mechanism: This test establishes the distance that erythrocytes have fallen in a vertical column of anticoagulated blood under the influence of gravity. The test procedure itself is easily influenced by a number of environmental factors, including blood cell size and shape, temperature, handling. This test does not stand alone as a good diagnostic tool.

PLT

Name: Platelets (thrombocytes)

Normal Range: 150,000 - 500,000/cu.mm

WM Abnormality: Low platelet count (thrombocytopenia) and function. Platelets become coated with IgM molecules, decreasing their clotting capacity.

Function/Test Purpose: Some of the same stem cells that produce the red blood cells in the bone marrow develop into large specialized cells called Megakaryocytes; each of these is packed full of as many as 4000 platelets (discs with a sticky surface substance); the large cells erupt and the separate platelets circulate throughout the bloodstream to assist in clotting, surviving for about ten days.

Test Mechanism: During the Coulter Counting cycle, as the platelets and RBCs pass through the apertures, the particles which are between 2 & 20 fL in size are counted as platelets.

MPV

Name: Mean Platelet Volume

Normal Range: 7.2-11.1 %

WM Abnormality: Varies; if platelets are depressed, platelet hematocrit will be lowered.

Function/Test Purpose: The ratio of Platelet Hematocrit to Platelet Count; aids in determining platelet volume and size.

Test mechanism: This is a calculated value. $MPV = \frac{\text{Platelet Hematocrit}}{\text{Platelet Count}}$

WBC

Name: White Blood Cells (Leukocytes) are composed of Basophils, Eosinophils and Neutrophils, which are collectively called Granulocytes. Other white blood cells are Lymphocytes and Monocytes.

Normal Range: 4.8-10.8 thousand/cu.mm

WM Abnormality: Lowered WBC count (leukopenia)

Test Purpose: Primarily formed in the bone marrow; white blood cells may also be produced in organs of the lymphatic system such as spleen, thymus and lymph nodes. They serve to fight off infections and protect the body from disease. This test serves to monitor disease progression and/or response to chemotherapy.

Test Mechanism: White Blood Cells are counted with an instrument called a Coulter Counter. The sample is diluted in an electrically charged solution which is passed slowly through an aperture across which a specific voltage passes. As each cell passes through, the voltage changes, creating a pulse. The voltage magnitude varies also with cell size. This way the cells are counted, and sized.

WHITE BLOOD CELL DIFFERENTIAL:

The following percentages are part of the White Blood Cell Differential, and are part of the Complete Blood Count.. They evaluate the distribution of the various types of white blood cells based on size distribution as determined by the Coulter Counter. The sizes are then graphed to show numbers of each type. They are compared to the normal range for a healthy person and abnormalities are flagged. The differential is done to evaluate the body's capacity to resist and overcome infection.

% NEUT

Name: Percent Neutrophils

Normal Range: 7.4-10.4 thousand/cu.mm (50-70% of WBC's)

WM Abnormality: Lowered count and percentage, from disease or chemotherapy. However, if other white blood cell counts also fall, percentage may not vary.

Function/Test Purpose: Neutrophils are the most numerous white blood cells; they are mobile cells that capture foreign particles and bacteria that have entered the body, engulf and digest them. These cells are often referred to as **Phagocytes**. The test is performed to relate Neutrophil count to other blood counts.

Test Mechanism: These are among the Granulocytes which are identified by Coulter Counter at 160-450 fL. They are then differentiated from other Granulocytes by light scatter based on cell shape and plotted.

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% LYMPH

Name: Percent Lymphocytes

Normal Range: 20-30% of white blood cells

WM Abnormality: Change will depend on status of disease and chemotherapy. Direct counts may fall; percentage may remain the same. Most patients do not have large numbers of circulating WM cells in the blood.

Function/Test Purpose: Originating as immature stem cells in the bone marrow, they mature in the lymphatic tissues to B cells (20% of the lymphocytes) which release antibodies (**Immunoglobulins IgD, IgE, IgA, IgG, IgM**) for defense against infectious agents, and to T cells (80% of the lymphocytes), which provide cell mediated immunity. As the lymphocyte matures it develops antigens (specific proteins) on its own cell membrane. These are referred to as markers (in WM, the CD20 marker is identified) which stimulate the production of the immunoglobulins. Once the cell fully matures, and is referred to as a plasma cell, it usually no longer exhibits these antigens. It is these antigens which stimulate the excess production of IgM in the rapidly multiplying B Lymphocytes of WM patients. Lymphocytes are responsible for specific recognition and immune responses to viruses, cancer cells, and other foreign substances within the body. The antibodies produced by the B cells of a healthy person coat the foreign antigens, marking them for attack by the Neutrophils.

In WM, the excess antibody doesn't find enough foreign antigens to coat, so it tends to coat the blood platelets, inactivating them, and it deposits in body tissues. Both B and T cells are long lived, (about four years) and travel throughout the body.

The test is performed to relate count to normal, and to monitor disease progression.

Test Mechanism: Are nucleated cells of 35-90 fL as measured by Coulter Counter.

% MONO

Name: Percent Monocytes

Normal Range: 1.7-9% of WBC's

WM Abnormality: Direct count may fall from disease or chemotherapy; if other white blood cell counts fall, percentage may not be affected.

Function/Test Purpose: Monocytes are a type of white blood cell produced in bone marrow, arising from the same stem cell as the Neutrophil. They are mobile in the bloodstream or in body tissues where they mature to cells called **Macrophages**. Monocytes capture and destroy bacteria and other foreign substances, remove dead cells from the body, participate in iron metabolism, and process information about foreign antigens for the lymphocytes. The test is performed to relate count to normal, and to monitor disease progression.

Test Mechanism: 90-160 fL as measured by Coulter Counter.

% EOSIN

Name: Percent Eosinophils

Normal Range: 0-7% of WBCs

WM Abnormality: Direct count may fall from disease or chemotherapy; if other white blood cell counts fall, percentage may not be affected.

Test Purpose: Eosinophils are a type of white blood cell that engulfs antibody labeled substances and functions in inflammatory reactions. The test is performed to relate count to normal, and to monitor disease progression.

Test Mechanism: These are among the Granulocytes which are identified by Coulter Counter at 160-450 fL. They are then differentiated from other Granulocytes by light scatter based on cell shape and plotted.

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% BASO

Name: Percent Basophils

Normal Range: Less than 1% of WBCs

WM Abnormality: Direct count may fall from disease or chemotherapy; if other white blood cell counts fall, percentage may not be affected.

Test Purpose: Basophils are a type of white blood cell which secrete chemicals to promote inflammatory reaction in the body. The test is performed to relate count to normal, and to monitor disease progression.

Test Mechanism: These are among the Granulocytes which are identified by Coulter Counter at 160-450 fL. They do not show up by light scatter, but demonstrate a different conductivity from other Granulocytes and are that way differentiated.

ABS NEUT

Name: Absolute Neutrophil Count

Normal Range: 7.4-10.4 thousand/cu.mm

WM Abnormality: Lowered Neutrophil count (Neutropenia); puts the patient at greater risk from infectious agents both inside and outside the body.

Test Purpose: Neutrophils are a type of mobile white blood cells that capture foreign particles and bacteria that have entered the body, engulf and digest them; these cells are often referred to as phagocytes. The test is performed to identify abnormal Neutrophil count and relate to disease progression.

Test Mechanism: This is a calculated value:

$$\text{Absolute Neutrophil Count} = \frac{\text{Total white blood cell count} \times \% \text{ neutrophils}}{100}$$

VISCOSITY

Name: Serum Viscosity Test

Normal Range: 1.0-1.8

WM Abnormality: Increased blood viscosity (hyperviscosity)

Function/Test Purpose: Viscosity is the property of blood that resists a force causing it to flow. It is compared to the viscosity of distilled water at room temperature, and is a function of the concentration of protein in the blood. High serum viscosity is most often caused by an overabundance of IgM.

Test Mechanism: Blood is allowed to clot. Then the serum is separated by centrifuging and warmed. The serum is allowed to flow down through a narrow tube (viscometer), and its rate of flow is timed. It is compared to the rate of flow for distilled water.

$$\text{Serum Viscosity} = \frac{\text{Flow time of blood serum}}{\text{Flow time of distilled water}}$$

IMMUNOGLOBULINS

Blood serum total proteins are grossly separated into **Albumin** and **Globulins**.

Albumin is the protein of highest concentration in the serum and is important for maintaining oncotic blood pressure (keeps the blood from leaking out into the tissues).

Globulins are divided into alpha, beta, and gamma globulins, based on the spacing zones into which they are graphed when separated, and include the types D, E, G, A & M (called immunoglobulins). Those of interest in WM are the immunoglobulins A, G and M.

Name: Immunoglobulins A, G, & M

Normal Range: Immunoglobulin A: 160-260 mg/dl

Immunoglobulin G: 950-1550 mg/dl

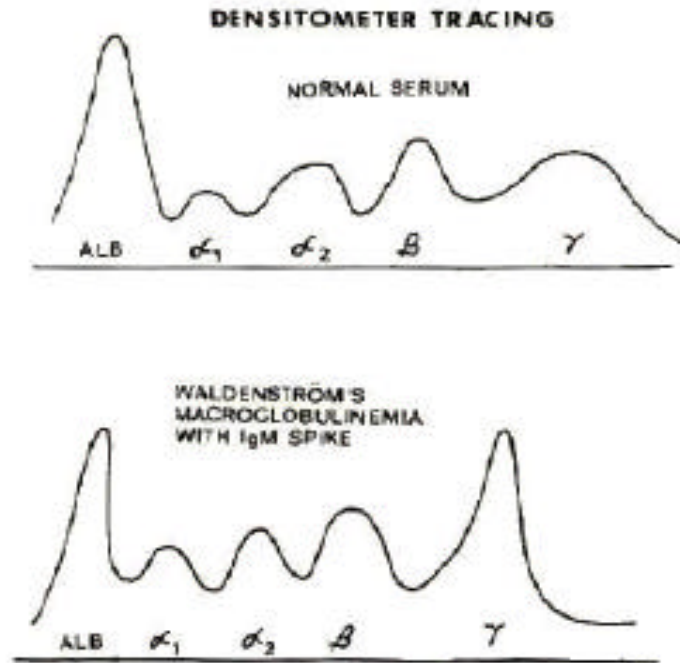
Immunoglobulin M: 50-300 mg/dl. This molecule is approximately six times larger than the others.

WM Abnormality: IgM count is extremely elevated; can be up as much as 10,000 mg/dl, and is the major blood abnormality which leads to diagnosis of this disease. IgA and IgG count may be depressed.

Function/Test Purpose: Immunoglobulins are found in the blood serum; they are antibody proteins produced by the B white blood cells at an immature stage as part of the immune function to mark foreign antigens for destruction. The test is performed to determine globulin count and to monitor disease progression.

Test Mechanism: The laboratory test to separate and quantify the globulins is called Serum Protein Electrophoresis. This is an analysis of the proteins present in blood serum based on size and electrical charge. The blood serum (fluid portion of blood) is placed on specially treated cellulose acetate paper, saturated in an electrostatic fluid, and exposed to a continuous electric current. The various proteins

migrate (move on the paper) to form bands that indicate the relative proportion of each protein fraction. The proteins are then stained and a densitometer can determine and graph the amount of each protein present by the intensity of stain.



BONE MARROW BIOPSY

WM Abnormality: Marrow shows a proliferation of immature B Lymphocytes and depression of other cell types. The result is reported as "% involvement of nucleated marrow cells" and diagnosis is confirmed at greater than 30%.

Function/Test Purpose: A bone marrow biopsy is an examination of the soft tissue within the bones (bone marrow); it provides reliable diagnostic information about blood disorders. It is used to diagnose anemias, thrombocytopenia, to evaluate the effectiveness of chemotherapy and help monitor suppression of blood cells of the bone marrow. In WM it is also used to identify infiltration of bone marrow by the WM cells.

Test Mechanism: A local anaesthetic is administered, and sometimes a sedative. Patient will feel pressure on needle insertion, and a brief pulling pain on removal of the marrow. Marrow specimen is placed on a series of slides and sent to the laboratory for microscopic examination. Special stains are used to obtain count of different types of marrow cells for identification of disease cells.

CD20 MARKER

Name: Cell Marker Identification

WM Abnormality: WM disease cells may exhibit CD20 marker

Function/Test Purpose: This test aids in diagnosis and monitoring accumulation of WM cells at specific sites. It is performed by a technique called Flow Cytometry which is used for defining and enumerating lymphocytes. It can detect, count and identify characteristic molecules on groups of cells.

Test Mechanism: Individual cells are first tagged with fluorescing antibody chemicals. These chemicals will bind to a specific cell surface protein, a marker which identifies that cell. Once the antibody-marker complex is formed, the cells are now considered labeled. The sample is then forced through a nozzle creating a fine stream of liquid containing cells spaced singly at intervals. The stream of cells then passes through a laser beam. They scatter the laser light and the dyes on the labeled cells fluoresce. Phototubes pick up this light. This provides information on the size and characteristics of the cell and on the binding

of the antibodies, expressing the surface markers. Cells can then be separated by a cell sorter: as each cell passes through the nozzle the light sends a signal to the computer which generates an electric charge characteristic of that particular cell. The charges can be deflected and grouped - a specific charge representing each of the cells with the same antibody-marker complex. Thus an entire grouping of cells expressing the CD20 marker has been identified and tracked.

UNIT LABEL CODE

g = gram
mg = milligram (one thousandth of a gram)
pg = picogram (one trillionth of a gram)
L = liter
dl = deciliter (one tenth of a liter)
_l = microliter (one millionth of a liter)
fL = femtoliter (one quadrillionth of a liter)
cu.mm = cubic millimeter

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