Drucker Diagnostics
Innovative Solutions for a Healthier World

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Quantitative Buffy Coat Analysis

A Pictorial Review and Reference Guide

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A History of Buffy Coat Analysis

The buffy coat has long interested physicians, as "phlegm" was one of the four humors that dominated the medical thought of the ancients. In 1933, in a paper entitled "Macroscopic Examination of the Blood", Wintrobe1 was perhaps the first to suggest that quantitative examination of the buffy coat in centrifuged blood would provide useful information. He stated that its color and thickness were good guides for estimating the platelet and leukocyte counts. Subsequently Bessis2 described a way of separating the buffy coat into three layers. His work was amplified by Davidson3 and by Zucker and Cassen4.

Wardlaw, Levine and Massey in 1977 developed the technique of expanding the buffy coat by using a high-precision plastic float moving in a precision-bore capillary tube. Their work5 was reported in 1983 in a paper entitled "Quantitative Buffy Coat Analysis." The QBC™ Centrifugal Hematology System, manufactured by QBC Diagnostics, Inc. was developed based upon their work.

The Significance of Absolute Counts

Absolute leukocyte counts, which are obtained by simple multiplication, provide a more accurate and more readily comprehensible picture than the total white blood count with the percentage leukocyte differential count. Although the information is similar in both cases, presenting it in the traditional percentage format may mask abnormalities in the absolute counts.

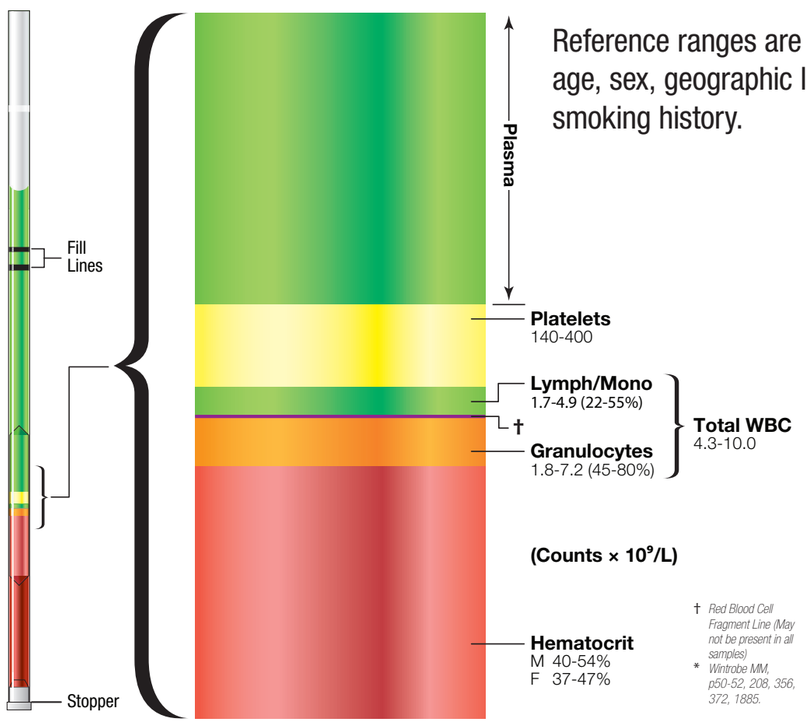
The importance of the absolute counts is apparent when it is realized that the various leukocyte cell populations are separate entities and under separate control mechanisms; increases in one line may not be paralleled by increases in another. Thus, using the absolute counts as indicators of a particular process offers a more sensitive means than conventional data presentation and also enhances the user's awareness of the pathophysiology.

Note: The QBC system is designed to provide immediate data which can be used as a CBC in many circumstances. The data is not complete, however, and when clinically indicated or when samples show values outside of the approved ranges, the results should be augmented or confirmed by an alternate method, including examination of a stained peripheral blood film.

See package insert and operator's manual. For technical assistance, call 866-265-1486.

1. Wintrobe MM, Macroscopic Examination of the Blood, Am J Med Sci 185:58-71, 1933.
2. Bessis M. Une Methode Permettant L'isolement des Differents Elements Figures du Sang, Sang 14: 262-264, 1940.
3. Davidson E. The Distribution of Cells in the Buffy Layer in Chronic Myeloid Leukemia, Acta Hematologica 23: 22-28, 1960.
4. Zucker RM, Cassen B, The Separation of Normal Human Leucocytes by Density and Classification by Size, Blood 34: 591-600, 1969.
5. Wardlaw SC, Levine RA, Quantitative Buffy Coat Analysis, JAMA 249: 617-620, 1983

EXPECTED NORMAL HEMATOLOGICAL VALUES

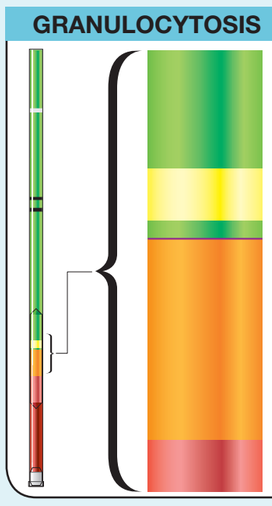


† *Red Blood Cell Fragment Line (May not be present in all samples)*

* *Wintrobe MM, p50-52, 208, 356, 372, 1885.*

Reference ranges are a function of age, sex, geographic location and smoking history.

GRANULOCYTOSIS

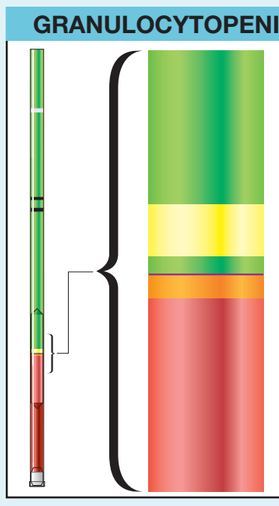


Granulocytes > 7.2 × 10^9/L

Possible causes of granulocytisis:
1. Acute bacterial infection, especially coccal. Also in certain non-bacterial infections.
2. Inflammation with tissue damage (myocardial infarction, gout, burns, postoperative state).
3. Toxins, acidosis, uremia.
4. Acute hemorrhage.
5. Malignancy. (Under some circumstances)
6. Physiologic neutrophilia due to exercise or stress.
7. Steroid or epinephrine administration.

* Wintrobe MM, p1285-1287, Table 54-1.

GRANULOCYTOPENIA

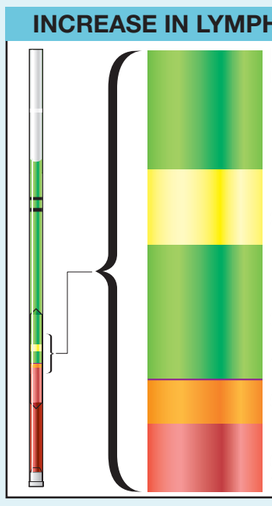


Granulocytes < 1.8 × 10^9/L

Possible causes of granulocytopenia:
1. Chemotherapy.
2. Certain infections such as typhoid, paratyphoid, measles, infectious hepatitis, malaria and most rickettsial diseases.
3. Overwhelming infection.
4. Adverse drug reactions, such as from aminopyrine, phenothiazines, sulfonamides, antithyroids, gold.
5. Cachexia or debilitated state.
6. Anaphylaxis and early stages of reaction to foreign protein.

* Wintrobe MM, p1287-1291, Table 54-2.

INCREASE IN LYMPHOCYTES/MONOCYTES

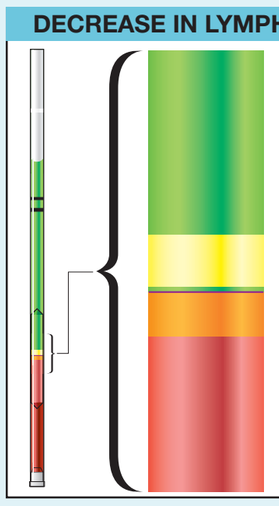


Lymphocytes/Monocytes > 4.9 × 10^9/L

Possible causes of increase:
1. Infectious Mononucleosis: Total WBC is 10-20 × 10^9/L in 60-70%; may exceed 20 × 10^9/L in 15%. Increase is usually due to normal and atypical lymphocytes. Peripheral smear usually shows atypical lymphocytes; heterophile or spot serology may be positive after the first week of illness.
2. Chronic Lymphocytic Leukemia: Most patients have between 20-100×10^9/L lymphocytes. Peripheral smear usually shows small, uniform "normal" lymphocytes. Mild anemia or thrombocytopenia may be present.
3. Certain acute infections; infectious hepatitis, pertussis, cytomegalovirus infection.
4. Certain chronic infections; tuberculosis, brucellosis, secondary congenital syphilis.
5. Primary hematopoietic disorder and lymphomas. (In acute leukemia the blasts will enlarge the lymph/mono layer.)
6. Certain protozoal infections.

* Wintrobe MM, p1301-1303 & 1368-1372. Tables 54-5, 54-6.

DECREASE IN LYMPHOCYTES/MONOCYTES

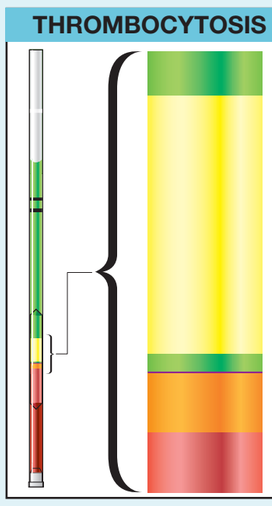


Lymphocytes/Monocytes < 1.7 × 10^9/L

Possible causes of decreased lymphocytes/monocytes:
1. Acute infections.
2. Certain malignancies.
3. Collagen vascular diseases.
4. Acquired Immunodeficiency Syndrome (AIDS).
5. Steroid administration/

* Wintrobe MM, p1288,1302,1304.

THROMBOCYTOSIS

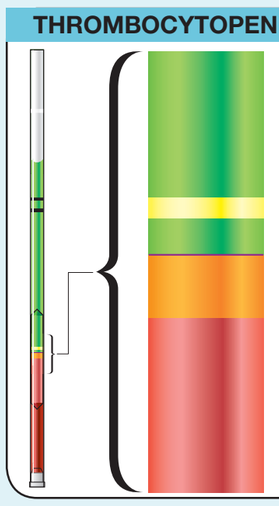


Platelets > 400 × 10^9/L

Possible causes of thrombocytosis:
1. Physiologic: result of stress and epinephrine release.
2. Myeloproliferative syndromes.
3. Regeneration after hemorrhage.
4. "Rebound" thrombocytosis following thrombocytopenia.
5. Asplenic state.
6. Many acute and chronic infections.
7. Many neoplasms.
8. Trauma and surgery.

* Wintrobe MM, p1128-1134, Table 48-1

THROMBOCYTOPENIA

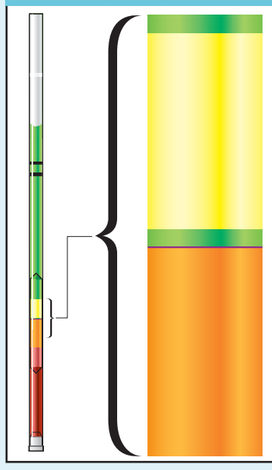


Platelets < 140 × 10^9/L
(Bleeding is uncommon unless platelet count drops below 50× 10^9/L.)

Possible causes of thrombocytopenia:
1. Chemotherapy.
2. Drug reactions; diuretics, oral hypoglycemics, ethanol, some antibiotics, anti-depressants, gold, non-steroidal anti-inflammatory agents.
3. Idiopathic Thrombocytopenia (ITP).
4. Portal hypertension.
5. Acute viral infections.
6. Bacterial septicemia.
7. Disseminated Intravascular Coagulation (DIC).
8. Massive blood transfusion.

* Wintrobe MM, p1090-1127. Tables 47-2, 47-3.

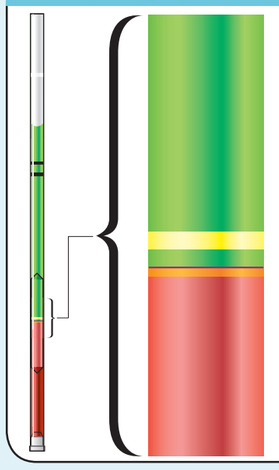
MYELOPROLIFERATIVE SYNDROME



Uncontrolled or disturbed release of cells from the bone marrow.
1. Polycythemia Vera: Increase hematocrit not in response to stimuli such as hypoxia, etc. Frequently associated with elevations in granulocyte and platelet counts.
2. Idiopathic Myelofibrosis: Increased platelets early in disease. Anemia with "tear drop" RBCs and nucleated RBCs seen on peripheral smear Granulocytes may or may not be increased/

* Wintrobe MM, p1596-1630

PANCYTOPENIA



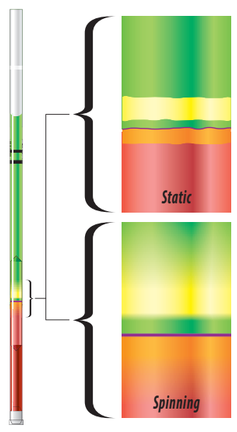
Decrease of all elements below reference limits. This is not a disease entity but a triad of findings that may be due to a number of causes.
1. Disorders associated with bone marrow infiltration; neoplasm, myelofibrosis.
2. Chemotherapy.
3. Toxic exposure; benzene and derivatives, ionizing radiation.
4. Drug reactions; chloramphenicol, quinacrine, antithyroids, phenylbutazone, gold compounds.
5. Idiopathic aplastic anemia.
6. B_{12} or folate deficiency.
7. Overwhelming infection.

* Wintrobe MM, p698-700 & 1304-1305. Tables 28-1, 54-7

Important observations

Inspection of QBC tube may yield other valuable clinical information.

NON-SEPARATION OF RBCs/GRANS

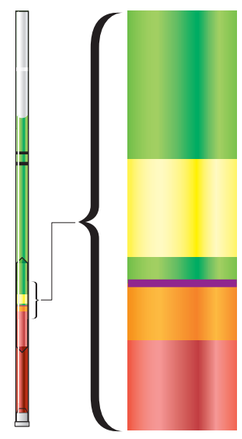


Blurred or absent red cell/granulocyte interface may occur when:
A. Granulocyte adhesiveness increases; may occur with severe infection or inflammatory disorders.‡ Sample incubation at 43°C/ 5 min. destroys adhesiveness, may improve separation.
B. Densities of red cells and granulocytes overlap. Occurs in ~3% of ambulatory, up to 30% of hospitalized patients. Some causes are:
   1. Hemoglobinopathies or diseases associated with microcytosis; e.g., iron deficiency anemia, thalassemia trait. Red cell fragmentation Syndromes, including microangiopathic anemia. In many of these conditions float sinks deeper into RBC layer.
   2. I.V. fluid or drug administration.
   3. Immediate postpartum state.

Note: Rarely, density overlap may be so great as to suggest the absence of granulocytes.

‡ Garvin JE: Factors influencing the adhesiveness of human leukocytes and platelets in vitro. J.Exp Med 114 51-52, 1961

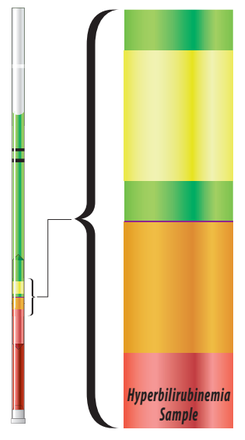
RED BLOOD CELLS FRAGMENT LINE



Red blood cells that have been damaged tend to be lighter than the granulocytes and may form a small dark band between the granulocyte and lymph/mono layers.

This band may be particularly large (up to the thickness of a normal lymph/mono layer) in asplenic patients and those who have recently received I.V. drugs that can damage red blood cells.

OTHER SAMPLE ABNORMALITIES



Hyperbilirubinemia.
Inspection of the plasma layer may show increased plasma bilirubin. Generally, bilirubin >3 mg/dl can be observed as a brownish coloration of the plasma; but, the yellow fluorescent dye in the tube may mask it for some observers. Increased bilirubin causes the fluorescence of the granulocyte and platelet bands to appear green tinged. This does not affect the accuracy of the measurement.

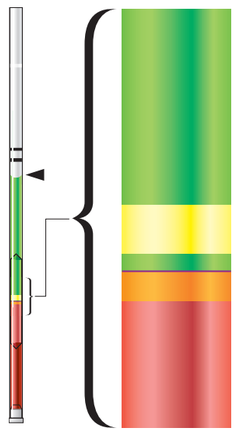
Hyperlipidemia.
Increased lipids may show as an opalescence of the plasma or as a small pellicle at the top of the plasma. This also has no effect on the measurement.

Cryoglobulinemia.
Cryoglobulins may precipitate or form a gel at 0-15°C and may show as opalescence of the plasma. A distinct layer of precipitated cryoglobulins may appear in the plasma layer.

Proper technique ensures accuracy

Inspection of QBC tube prior to reading is an important step in quality assurance.

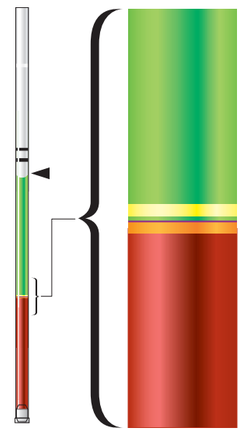
INCORRECT FILLING/LEAKAGE



Technique error: Incomplete filling of the tube or specimen leakage during centrifugation will adversely effect the results.

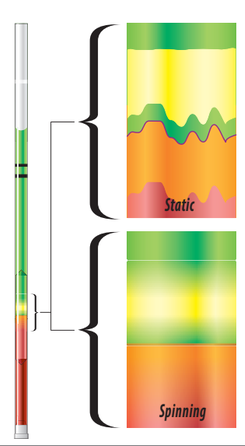
After centrifugation, **ALWAYS** check the plasma level to ensure that it is within the fill lines.

OMISSION OF THE FLOAT



Technique error: Omission of the float causes a lack of expansion and a marked apparent reduction of the plasma level. The tiny buffy coat layers should not be mistaken for thrombocytopenia and leukopenia.

WAVY BUFFY LAYERS



Technique error: Wavy interfaces between the buffy coat bands occur when they are disturbed following separation. The most common cause is slippage of the closure, which can be prevented by carefully drying the end of the tube prior to attaching the closure. Overheating the centrifuge and operation in ambient temperatures over 32°C (90°F) will also cause waviness.

NOTE: Because the tube is rotated, the waviness will be seen as an enlarged, blurred lymph/mono band. The waviness can be seen if the rotation is momentarily stopped.

* Wintrobe MM, et al: *Clinical Hematology*, ed. 8, Philadelphia, Lea & Febiger, 1981.

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