

The Blood Smear (What, Why and How)

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Accurate hematologic data is essential for diagnosis of many disorders. It is the role of the Veterinary Technician/Nurse to collect the sample, handle and store the sample, prepare and examine the blood sample.

The Study of Blood

Hematology is the study of blood and the tissues that form, store, or circulate blood cells. The circulatory system is responsible for the transport of nutrients, oxygen, and hormones to cell throughout the body and removal of metabolic wastes (carbon dioxide, nitrogenous wastes, and heat). Along with the function of regulation of body temperature, fluid pH, and water content of cells. White blood cells circulating in the blood defend and protect the body against foreign microbes and toxins. Clotting mechanisms, called platelets, are also present that protect the body from blood loss after injuries.

Blood Formation

Hematopoiesis is the process that produces the formed elements of blood. Hematopoiesis takes place in the red bone marrow found in the epiphyses of long bones, flat bones, vertebrae, and the pelvis. All stages of development are present in the red bone marrow, including stem cells, which are stimulated to develop into many types of blood cells. Most blood cell formation is from mitosis and produces various blast cells. Each of these cells divides and differentiates into red blood cells (RBCs), white blood cells (WBCs), and platelets. (See *hematopoiesis* handout)

Basic Blood Physiology

Blood consists of cells and cell fragments (*erythrocytes, leukocytes, thrombocytes*) and water with dissolved molecules (*plasma*). Cells make up approximately 45% of blood and 55% is plasma.

Erythrocytes, or red blood cells (RBCs), transports oxygen (O₂) and carbon dioxide (CO₂) in the blood. RBCs contain the protein hemoglobin to which both O₂ and CO₂ attach. Life span of a normal erythrocyte is only 120 days. Degenerated (damaged) erythrocytes are broken down in the spleen and liver by macrophages.

Leukocytes, or white blood cells (WBCs), are cells that protect the body from foreign microbes and toxins. Leukocytes are classified into two groups, *granulocytes* and *agranulocytes*, based upon the presence or absence of granules in the cytoplasm and the shape of the nucleus. *Granulocytes* contain numerous granules in the cytoplasm and have nucleus that is irregular shaped with lobes. There are three types of granulocytes called *neutrophils, eosinophils, and basophils*. *Neutrophils* are the first to arrive at a site of infection. By phagocytosis, they actively engulf bacteria, then are usually destroyed in the process, contribute, together with other dead tissue, to the formation of pus. *Eosinophils* actively phagocytize complexes formed by the action of antibodies on antigens (foreign substances). They increase numbers during parasitic infection and allergic reactions. *Basophils* release histamine (inflammatory response) in response to tissue damage and pathogen invasion. *Agranulocytes* do not have visible granules in the cytoplasm and the nucleus is not lobbed. There are two types of these leukocytes; lymphocytes and monocytes. *Lymphocytes* regulate the immune system response producing T lymphocytes and B lymphocytes depending on their role in the immune response. *Monocytes* leave the bloodstream and move into tissues, they enlarge and become macrophages, which engulf microbes and cellular debris.



Platelets (thrombocytes) are fragments of huge cells called megakaryocytes. Platelets adhere to damaged blood vessel walls and release enzymes that activate hemostasis, the stoppage of bleeding. Average life in circulation is approximately 10 days.

Plasma is the straw-colored, liquid portion of the blood. It consists of 92% water, 8% proteins and trace amounts of other materials. Of the 8% proteins, 60% is albumin, which is produced in the liver and serves to preserve osmotic pressure (passive movement of fluid across a membrane) between blood and tissues. 36% is globulin which transports fat-soluble vitamins and lipids in the blood, with gamma globulins functioning to assist the immunity. And 4% fibrinogen and prothrombin, which is utilized in, clot formation.

Collection and Handling of Blood sample

The Blood Smear consists of several components, beginning with proper collection of the blood sample. In the case of improper collection and handling may lead to unreliable and misleading results. Blood collection should start with selection of the venipuncture site. Any events or circumstances occurring at the time of collection that may potentially influence the result, especially stress, excitement, exercise, apparent disease signs, and extreme weather conditions, should be noted. An anticoagulant, a substance that prevents a clot from forming, is needed in storing the collected blood sample. Since we are concerned about analyzing the cellular appearance and/or changes, we need to prevent the formation of a clot in the suspension. There are several different types of anticoagulant but EDTA is by far the most common anticoagulant used in evaluating blood cellular components. The mechanism of action of almost all anticoagulants is binding of calcium ions. EDTA “purple top” is excellent in preserving for up to 6 hours. Other anticoagulant like heparin “green top” and sodium citrate “blue top” are also excellent but have several disadvantages. Heparin may cause clumping of WBCs and is unsuitable for blood smears and also interferes with stainability of WBCs. Sodium citrate interferes with many chemical tests, prevents clotting for only a few hours, and causes the cells to shrink.

Avoid these common errors in blood collection and handling

- Rapid or forceful aspiration of the blood, especially through a needle less than 22g.
- Traumatic aspiration of blood
- Spraying the blood through the needle into a second vial. The needle and tube stopper should be removed and the blood runs gently down the inside of the vial.
- Water present in the syringe, needle, or tube, causing osmotic damage to the cells.
- Collecting too little or too much blood for the amount of anticoagulant present, resulting in dilution error or direct cellular damage from concentration anticoagulant.
- Slow collection or delayed transfer to anticoagulant, allowing clumping of platelets and clot formation.
- Improper or incomplete mixing of anticoagulant. The tube should be gently but thoroughly rotated by hand or on an automatic tube rotator or it may be rolled across a flat surface.
- Excessive physical force, such as shaking, jarring, or dropping.
- Allowing the sample to overheat or freeze.
- Excessive time at room temperature between collection and utilization, allowing degeneration of cells (autolysis). If sample cannot be prepared within an hour of collection, the sample should be refrigerated for optimum results.



Adapted from: *Hematology Techniques and Concepts for Veterinary Technicians*, Gregg L. Voigt, DVM

Monitoring Cellular Components

There are a number and types of cellular, serologic, and chemical tests that may potentially be run on a blood sample. Routinely there are only a few of these tests that are used. A Complete Blood Count (CBC) should include a minimum of a packed cell volume (PCV), total protein (TP), hemoglobin concentration, and a differential leukocyte count. Other advanced tests that might be requested are a total leukocyte count a total erythrocyte count, and reticulocyte count.

When collecting a PCV and TP place sample either directly into a heparinized microhematocrit tube or from an EDTA tube place in non-heparinized tube. Plug one end with clay, centrifuge for 5 minutes at 10,000-12,000 rpm or 3 minutes at 15,000-16,000 rpm will usually show complete separation and packing of cells. The PCV is the percentage of whole blood composed of red blood cells. When preparing PCV the sample must be read within 6 hours of collection or the RBCs will swell and may increase PCV due to the enlarged size of the red cells. It is read using a special grid, placing the bottom of the red cell column (not including the clay) on the lower, or 0 line. The tube is then moved across the reader until the top of the plasma is even with the 100 mark. The tube must remain perpendicular to the grid. The line that crosses the tube at the top of the packed red cells (not the buffy coat-which consists of WBCs and platelets) is the percentage of red blood cells in the blood sample. The plasma or the straw-colored, liquid portion of the blood consists of about 8% mixture of many protein molecules, the majority of which are classified as albumin, globulin, and fibrinogen. Most of these proteins are manufactured in the liver, with the notable exception of the immunoglobulins, which are the antibodies produced by cells of the immune system. The total protein (TP) is usually measured with a refractometer. The design of this instrument is based on the principle of light waves passing through a liquid. The light will be bent from their original direction (refracted) by any solids present in the solution and the degree of this refraction is a function of the amount and type of solid present. To measure the TP, place a drop of the plasma on the refractometer. A horizontal line between white and blue should appear across several scales. The measure of protein is measured in gram per deciliter.

Almost all hospital these days have an automatic cell counter. These machines usually give a WBC, RBC count and a 2 part differential (Lymph/Mono and segmented Neutrophils). A blood smear evaluation must always be performed along with the automatic analysis to determine the true distribution of the different types of cells in the blood stream as well as the maturity level and morphology. Along with the blood smear evaluation a manual WBC count may need to be performed if the automatic cell counter is unable to interpret the results. A hemacytometer and unopette method is usually the method of choice. This is usually performed by the Veterinary Technician/Nurses.

Performing a Blood Smear Differential

Examination of a blood smear is an integral part of The Blood Smear. It allows us to quantify different types of leukocytes, estimation of the platelet count, and detection of morphologic abnormalities. To obtain the full value of the blood smear it is important to have a well-prepared, well-stained blood smear.

After the sample as been aseptically collected by venipuncture with a 22-20g needle and placed into an EDTA tube the next step is making the blood smear. Place a small drop of blood near the end of a microscope slide. Using another slide (spreader slide), bring the edge of the other slide just in contact with the drop and allow the drop to flow evenly across the spreader



slide. The angle between the two slides should be around 30-40 degrees. When the blood has spread along most of the width of the spreader slide, now you push the spreader slide forward with a steady, even, motion. Sometimes a few practice runs will help in getting down the angle and an even motion. The blood smear should be nice and thin with an even distribution of cells. The idea of spreading the blood is to make a layer of single cells (monolayer) so that each cell can be examined. The monolayer is sometimes referred to as the feathered edge. It should be shaped like a rainbow, not flat or jagged. After you have successfully made a blood smear it should be stained to identify the cells and their morphology. Most of the current stains for routine blood work are of the Romanowsky type (Diff-Quik), which incorporates basic (blue/purple) and acidic (red) dyes. This will stain some structures red and others blue depending on the chemical nature of the cellular structure. Diff-Quik, along with other stains, must be kept in good staining quality. The caps to the stains should be kept on tight when not in use. This prevents evaporation, minimizes contamination of solutions, and prevents water from the air getting into the fixative. Also do not “top-off” the solutions. When fluid levels drop or staining quality declines, empty, clean and dry the container, then refill with fresh solution. This will cut down on stain precipitate and water artifact. Smears should be made as soon as possible after the sample collection. Samples that are left sitting in the EDTA will cause the cells to break down altogether, rendering the specimen useless for analysis.

After the blood smear has been prepared by staining it is now time to observe the cells under the microscope. First scan the smear at low magnification (10x) to locate the optimal area for examination at higher magnification, and check the feathered edge for platelet clumping and microfilaria. Make sure the area you find to examine is an area of the smear in which the cells form a monolayer. Red cells are separated and barely touching, with little overlapping. By placing a drop of oil covered by a cover slip you can identify and count. Now you can start your differential count by moving back and forth across the smear in a pattern that avoids covering the same area twice. Using the high dry (40x) objective identify each leukocyte (WBC) that you see until 100 cells have been counted and sorted by type using a mechanical differential cell counter. The percentage of each cell type that results is termed the relative differential count. The absolute differential is derived by multiplying the percentage of each type by the WBC to get the number of each type of leukocyte/ μl . The WBC count comes from the electronic cell counter along with the RBC count, hematocrit, hemoglobin, etc...

Example:



===== HEMATOLOGY =====

HCT	HB	RBC	MCV	MCH
%	g/dL	mill/μL	fl	pg
MCHC	RDW	RETIC	NUCL RBC	WBC
g/dL	%	%	/100 WBC	thou/μL
SEG N	BAND N	LYMPH	MONO	EOS
thou/μL	thou/μL	thou/μL	thou/μL	thou/μL
BASO	PLAT SMR	S+IV PLAT	MPV	TP-REF
thou/μL		thou/μL	fl	g/dL

RBC MORPHOLOGY:
 PARASITES:
 WBC EXAM:
 PLASMA APPEARANCE:

1997 Cornell University; TW French & French JT Blue

Once the WBC count has been performed place a drop of oil on the cover slip and view the blood smear under oil immersion (100x) for morphology of cells.

Morphology of the blood cells can indicate certain disease along with possible blood parasite. Being able to identify these abnormalities will help in diagnosing. It is a vital skill in the diagnostic approach to curing the disease.

When performing the differential cell count, nucleated red blood cells (nRBCs) should be counted separately and reported as nRBCs/100 WBCs. This number is then used to correct the apparent WBC (which is actually just a count of nucleated cells, which is why avian CBC cannot be done on a machine).

$$\text{Corrected WBC count} = \frac{100}{100 + \#nRBCs} \times \text{WBC count}$$

There are several morphologic abnormalities in leukocytes and erythrocytes. Most WBC abnormalities are noted as in immature cells or toxic cells. With immature neutrophils, which are non-segmented, band cells or myelocyte are the most commonly observed. The presence of band or stab cells in blood is called a left shift. Inflammation is the most common cause of a left shift, but some other conditions, such as hemolytic anemia and primary disease of marrow, also can cause release of immature neutrophils. With lymphocytes a so-called “reactive” cell can be observed as a larger than normal cell with coarse chromatin, and deep blue cytoplasm. These “reactive” lymphocytes are sometimes called immunocytes and are associated with an immune response. These are fairly common in blood of younger animal recently vaccinated.

When looking at erythrocyte (RBC) there are numerous morphologic abnormalities that can be identified during the blood smear evaluation. Some of these abnormalities can be due to artifacts from specimen handling, preparation, aging of sample, or poor maintenance of staining solution. Like leukocytes, immature cells are also an abnormality. Reticulocytes are slightly immature, anucleate red cells that contain RNA. When examining a blood smear stained with Diff-Quik reticulocytes are larger than normal red blood cells and stain a blue-gray tint, called polychromatophilia. In most species, once the cell reaches the reticulocyte stage, it remains in the



marrow for about 2 days, and then released to complete its maturation. When reticulocytes are noted it usually a sign of some type of anemia. To assess the adequacy of the regenerative response in relation to the severity of the anemia a reticulocyte count is performed. To determine if the anemia is regenerative, the bone marrow is responding to the anemia by increasing erythrocyte production and releasing reticulocyte. Or if it is non-regenerative anemia, the bone marrow is unable to respond to the anemic state, and reticulocytes are absent on the blood smear.

Other RBC morphological changes or abnormalities can consist of different sizes, shapes, color, and numbers. There is a *handout provided* with definitions of hematologic changes. The morphology of a canine RBC is biconcave disc with prominent central pallor. The size is 6.0-7.0 μ . Rouleaux formation (stacking of coins) is moderate to none. The color of the cells are usually polychromaphils (bluish-red immature erythrocytes), polychromatophils correspond to reticulocytes in new methylene blue stained preparations. Howell-Jolly bodies are deeply staining nuclear remnants found in red cells. These inclusions are rare in normal dogs. The morphology of a feline RBC is biconcave disc with minimal central pallor. The size is 5.5-6.0 μ . Rouleaux formation is moderate to large. The colors of the cells are usually polychromaphils with occasional Howell-Jolly bodies, more common than in dogs.

Observing the RBC for parasite changes is a very important aspect of the Blood smear. As Veterinary Technician/Nurses it is very important that we be able to recognize different RBC parasite in our anemic patients. The automatic cells counters are unable to detect these parasites. In felines, *Haemobartonella felis* organisms are recognized on the red cell surface as either chains of small basophilic rods or ring forms. These parasites produce hemolytic anemia by causing RBCs to be phagocytosed by macrophages. Canines also carry a *Haemobartonella canis*, which looks very similar to the *H. felis*. With *H. canis* there are long chains of small basophilic cocoid organisms noted on the surface. *Babesia canis* and *Babesia gibsoni* are two known canine blood parasites. *Babesia* is a true intravascular hemolytic anemia, often characterized by hemoglobinemia and hemoglobinuria. It is recognized on a blood smear as intra-erythrocytic teardrop shaped organisms measuring approximately 1.5 (*B. gibsoni*) to 3 (*B. canis*) in length. Up to four organisms may be seen in a parasitized cell.

Artifacts can contribute to morphological changes. Crenation is the presence of red cells covered by short spiky surface projections. Crenation can be confused with acanthocytic changes. Crenation is differentiated from true poikilocytosis in that crenation affects all the red cells in a given area of the film whereas true poikilocytosis affects only scattered red cells on the blood film. Other changes are refractory "bubbles" on the surface of red cells. This is usually seen when the blood smear is stained while still wet. Staining precipitate can also affect the appearance of the blood smear. Blood smears that have been exposed to formalin fumes will have an overall greenish discoloration when stained. Smears that have been exposed too long to the basophilic (blue) component of Diff-Quik stain will be over-stained, making evaluation of polychromasia difficult to impossible.

Platelets are the last cells examined when looking at a differential blood smear. Platelets (thrombocytes) are a very important component of hemostasis. Hemostasis is a combination of three separate mechanisms, each with a different function, working in unison. These three factors are the response of the blood vessels to injury, the activation of platelets, and the stimulation of the clotting cascade. When a blood vessel is injured, platelets are exposed and activated to alter surface membrane properties and adhere to the vessel wall and each other. A platelet estimate is counted to determine the average per oil immersion field. By counting platelets in 5-10 fields on oil immersion and getting the average. At this magnification, each platelet is approximately equal to 15,000 platelets/ μ l. As in WBCs and RBCs, the morphology of the platelets is important as well. The presence of unusually large platelets should be noted, as it suggests early release from the bone marrow. Clumping of platelets should also be noted.



Conclusion

As you can tell by the extent of information, a differential blood smear is extremely important. As Veterinary Technician/Nurses it is our job to perform this analysis. Electronic counts are very helpful, but cannot take the place of Veterinary Technician/Nurses' eyes. Every CBC should be examined under the microscope to determine specific changes that the machine cannot pick up.

Remember: The Machine or the Veterinary Technician/Nurse?

Answer: Veterinary Technician/Nurse



Definition of Terms for Evaluating Red Blood Cells

Anisocytosis-Variation in the size of red blood cells

Acanthocytes-red cells with 2-10 blunt elongated finger-like surface projections

Burr cells-elongated red cells with ruffled margins (also termed echino elliptocytes)

Dacryocytes-tear-drop shaped erythrocytes

Eccentrocytes-red cells with hemoglobin concentrated at one pole with an unstained area at the other pole

Elliptocytes-oval erythrocytes

Poikilocytosis-Variation in shape of red blood cells

Polychromasis-Large blue non-nucleated red blood cells; would be a reticulocyte if stained with new methylene blue

Hypochromasia-Reduced hemoglobin concentration with increased central pallor
-ghost cells

Macrocytes-Larger than normal red blood cells

Microcytes-Smaller than normal red blood cells

Nucleated RBC-Immature red blood cell containing a nucleus

Reticulocyte-Immature cells having dark blue filaments and granulation when a supravital stain (New Methylene Blue)

Target Cells (codocyte)-Erythrocytes that are thinner than normal; when stained show a peripheral rim of hemoglobin with a dark central hemoglobin containing area

Schistocytes-irregular shaped, often roughly triangular, red cell fragments

Spherocytes-Small, dark erythrocytes lacking central pallor (only in dogs); observed in autoimmune and isoimmune hemolytic anemias and following transfusions; removed prematurely from circulation

-small

-round

-stain intensely

-lack of central pallor

Stomatocytes-cup-shaped erythrocytes

Keratocytes-a red blood cell with notches that results in projections that look like horns. Keratocytes occur when fibrin is being deposited within blood vessels as in disseminated intravascular coagulation.

Inclusion Bodies

Basophilic Stippling-Red blood cell containing dark blue staining granules (seen with Wright's Stain)

Howell Jolly Bodies-Nuclear remnant in red blood cells dot

Heinz Bodies-Refractile inclusions of denatured hemoglobin in the cat that may protrude causing bulging of erythrocytes



Reticulocyte Index Calculation

1. Take 2 drops of whole blood anticoagulated with EDTA (purple top) and place it in a vial.
2. Add 2 drops of New Methylene Blue (NMB) to the vial, let stand for 10 minutes.
3. Take a drop of the incubated blood and make a smear onto a slide, let dry.
4. Read the smear on 100x (oil immersion) in the monolayer. Count a total of 1000 RBCs, keeping track of how many are reticulocytes vs. non-reticulocytes.
5. Divide the number of reticulocytes counted by 1000 then multiply by 100 to obtain the reticulocyte count%.
6. This % must then be corrected in terms of the animal PCV. You then take your reticulocyte count and multiply it by the patients PCV. Take this number and divide it by the normal PCV expected for that patient (found on Reticulocyte Index Chart). These numbers are different for varying species, sex and ages of animals and are printed on a chart. This result is know as the Corrected Reticulocyte Count.
7. To obtain the reticulocyte Index, take the Corrected Reticulocyte Count and multiply it by 1/MF (also obtained from Reticulocyte Index Chart). This final result is the Reticulocyte Index.

Here is an example as a guideline:

A 18 year old, M/N, DSH with chronic renal failure (CHF) has a PCV of 10%. On the smear prepared with NMB stain out of 1000 RBCs counted, 20 of them were reticulocytes. Remember cats have two types of reticulocytes, aggregate and punctate. Count only the aggregates. Punctate are not counted do to the fact that they circulate for at least several days (7-10 days) in the blood stream and do not reflect the most recent bone marrow response.

$$\frac{20}{1000} \times 100 = 2.0\%$$

$$2.0 \times \frac{10}{34.6} = 0.58\% \text{ Corrected Reticulocyte Count}$$

$$0.58\% \times 0.36 = 0.2 \text{ Reticulocyte Index}$$



Reticulocyte Index Chart

Feline Packed Cell Volume at Different Ages:

Sex	0-12mnths Average	Average	1-5yrs	Average	6+ yrs
Male	24.0-37.5	31	26.9-48.2	37.6	34.6
Female	23.0-46.8	31.5	25.3-37.5	31.4	30.8

Canine Packed Cell Volume at Different Ages:

Sex	0-12mnths Average	Average	1-7yrs	Average	7+ yrs
Male	22.0-45.0	31	35.2-52.8	37.6	34.6
Female	25.8-55.2	31.5	34.8-52.4	31.4	30.8

Measuring Reticulocyte Index:

Percent(%) Reticulocyte counted x Measured Hct/Normal Hct = Corrected Reticulocyte Count

Corrected Reticulocyte Count x 1/Maturaton Factor = Reticulocyte Index

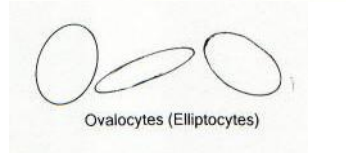
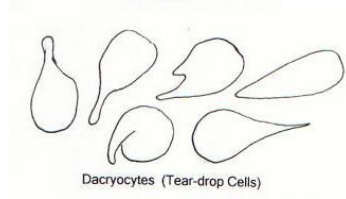
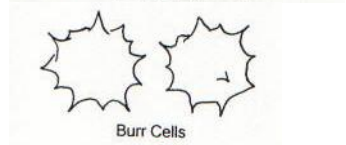
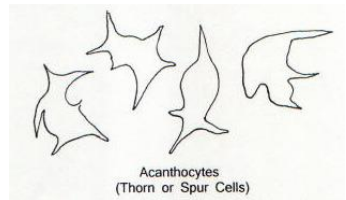
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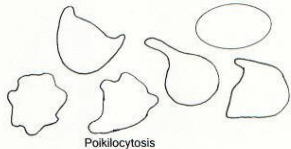
- Anatomy and Physiology, CliffsQuickReview
- Laboratory Procedures for Veterinary Technicians, Pratt, Second Edition
- Pictures in presentation from Interactive, Learning through participation, Human Body, CD, MegaSystems
- Hematology Techniques and Concepts for Veterinary Technician, Gregg L. Voigt, DVM
- Small Animal Clinical Diagnosis By Laboratory Methods, Willard, Tvedten, Turnwald, 2nd Edition
- Article: Laboratory Testing in the Anemic Patient, Karen M. Young, VMD, PhD, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison
- www.web.vet.cornell.edu/public/popmed/clinpath/CPmodules/heme1/micro.htm (Pictures)
- www.funsci.com/fun3_en/blood/blood.htm



Maturation Factor (MF)

Measured Hct	MF	1/MF	Measured Hct	MF	1/MF
10	2.75	0.36	33	1.60	0.63
11	2.70	0.37	34	1.55	0.65
12	2.65	0.38	35	1.50	0.667
13	2.60	0.39	36	1.45	0.69
14	2.55	0.40	37	1.40	0.71
15	2.50	0.41	38	1.35	0.74
16	2.45	0.42	39	1.30	0.77
17	2.40	0.43	40	1.25	0.80
18	2.35	0.43	41	1.20	0.83
19	2.30	0.44	42	1.15	0.87
20	2.25	0.45	43	1.10	0.91
21	2.20	0.46	44	1.05	0.95
22	2.15	0.47	45	1.00	1.00
23	2.10	0.48	46	0.95	1.05
24	2.05	0.49	47	0.90	1.11
25	2.00	0.50	48	0.85	1.18
26	1.95	0.51	49	0.80	1.25
27	1.90	0.53	50	0.75	1.33
28	1.85	0.54	51	0.70	1.43
29	1.80	0.56	52	0.65	1.54
30	1.75	0.57	53	0.60	1.66
31	1.70	0.59	54	0.55	1.81
32	1.65	0.61	55	0.50	2.00





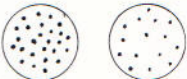
Poikilocytosis



Schistocytes (Schizocytes)



Stomatocyte (Mouth Cell)



Basophilic Stippling



Howell-Jolly (Ho-Jo) Bodies



Crescent Cells



Helmet Cells



Blister Cells



Keratocytes

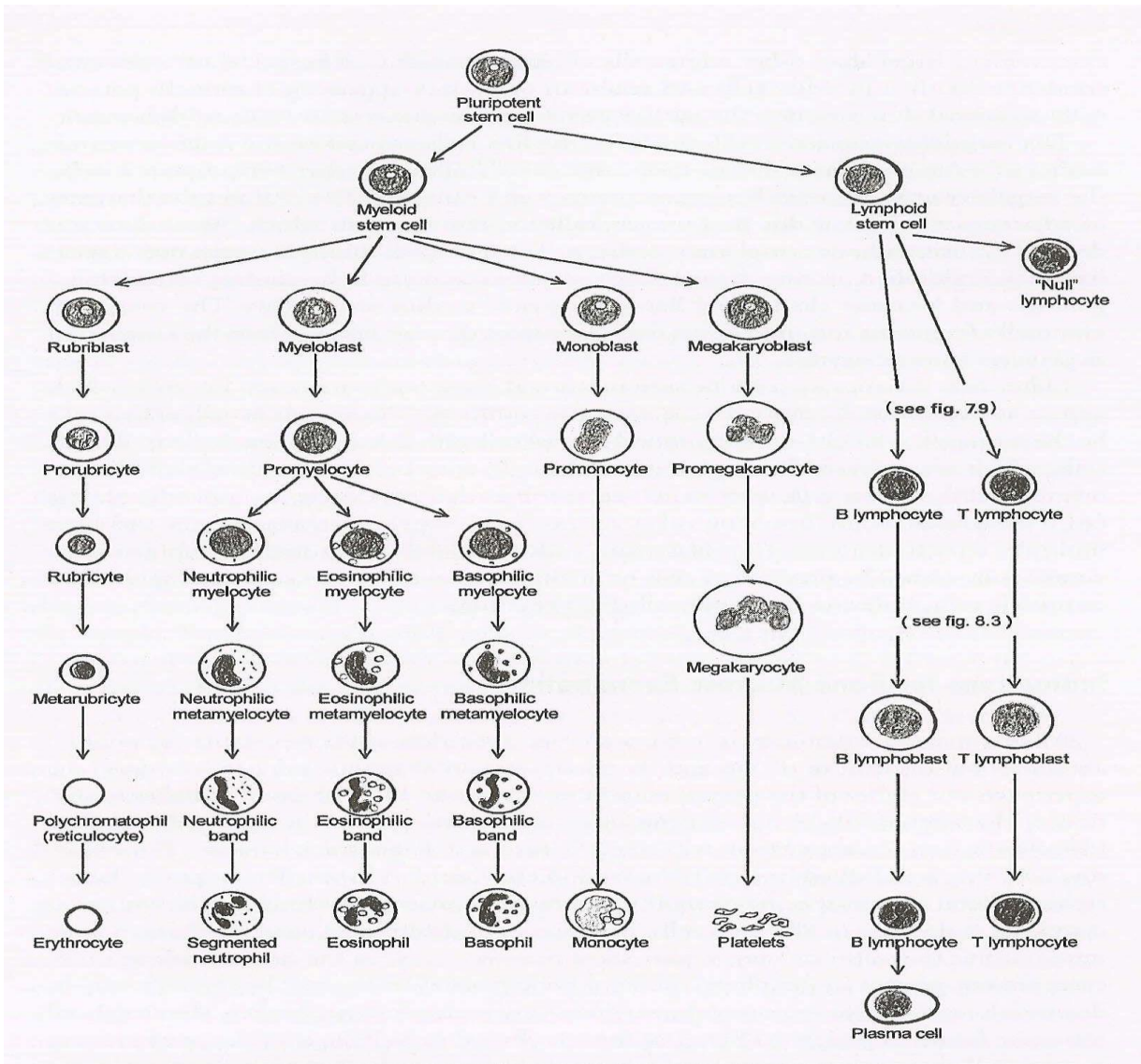


Fig. 13.1. Developmental stages of cells in hematopoiesis.