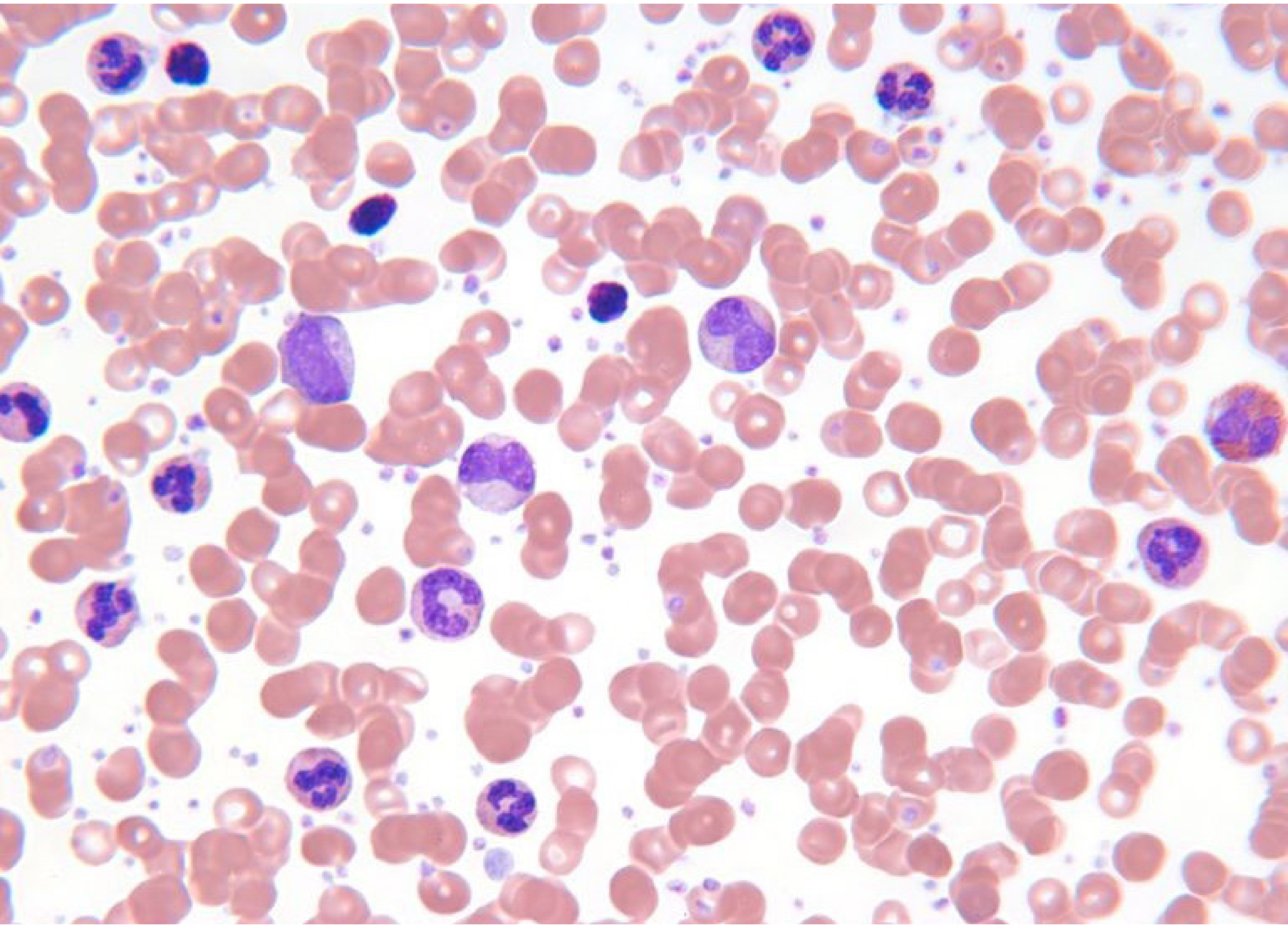
Application Note



Microscopic Examination of Blood

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Introduction

Blood is a body fluid that performs a variety of transport and regulatory functions. The heart pumps the blood around the body through the circulatory system (Figure 1). It consists of solid components and a liquid intercellular substance, blood plasma. This contains cellular solid particles formed in the bone marrow: the red blood cells (erythrocytes), the white blood cells (leukocytes), and the blood platelets (thrombocytes). These are responsible for specific functions within the body. The solid particles can be detected and identified with a light microscope. This procedure, referred to as complete blood count (CBC), is a standard, routine medical examination.

Blood Components and Their Formation

Blood formation in humans begins at the end of the fourth month of pregnancy [1] (Figure 2). All blood cells originate from what are known as pluripotent stem cells in the bone marrow. If hormones or growth factors (cytokines) act on these stem cells, the corresponding cell lineage develops. Initially, the cells divide and develop into progenitor cells for the two lineages of blood cells: myeloid cells and lymphatic cells. Through further division and, in the later stages of development, through maturation (differentiation), the various types of mature blood cells develop out of these progenitor cells through further intermediate stages, which are then released from the bone marrow and can perform their function in the body. In addition to the different functions, the cell lineages also have different maturation and survival times.

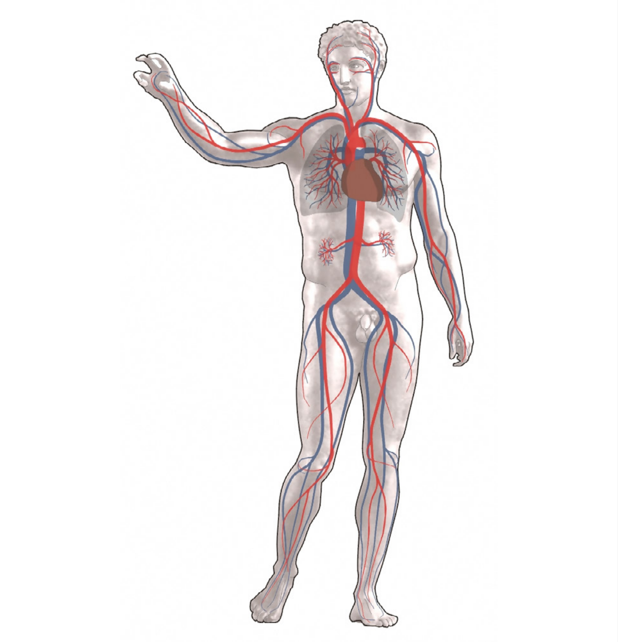


Figure 1 *The circulatory system, courtesy of: CC BY-SA 2.5, https://commons.wikimedia.org/w/index.php?curid=741255*

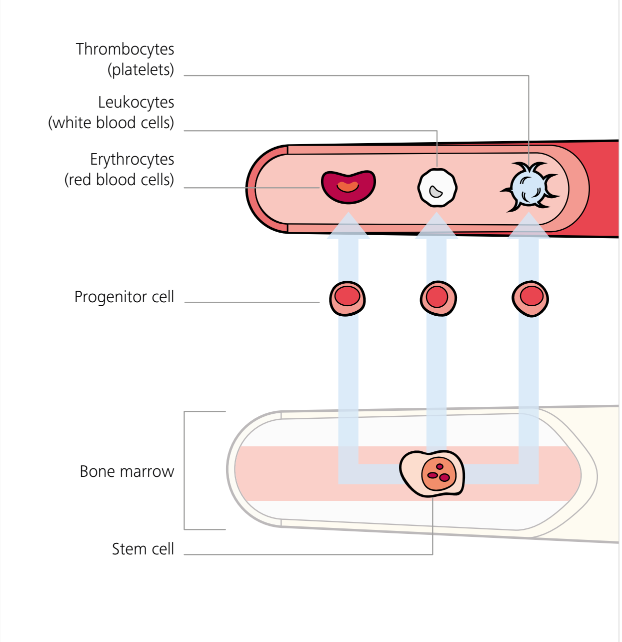


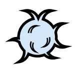
Figure 2 *Blood formation in the bone marrow, highly simplified*

Afterward, they are broken down in the spleen or die off. Due to blood cells' limited life span, the body must continuously form new blood cells – several billion cells per day, in fact [2].

Erythrocytes

- Red blood cells
- Diameter: 7.5 µm
- They require about 9 days to develop and have a life span of about 120 days in the peripheral blood before they are broken down in the spleen.
- Erythrocytes' red color comes from hemoglobin, which is also required to bind oxygen to the blood cells.
- They transport oxygen from the lungs into the various organs and tissues and are also responsible for transporting carbon dioxide from the tissues into the lungs.
- An insufficient number of red blood cells (low hemoglobin level) can cause paleness, fatigue, shortness of breath, and other symptoms. This is referred to as anemia.

Thrombocytes



- Blood platelets
- Diameter: 2–3 µm
- They have a life span of about 7 days in the blood before they are broken down in the spleen.
- Thrombocytes are responsible for blood coagulation and haemostasis: in the event of an injury, the walls of the blood vessels are sealed and platelets form at the injured site, which stops the bleeding. Later, the platelets disintegrate and release substances that activate coagulation.

Leukocytes

- White blood cells
- Diameter: 7–20 µm
- These occur in various types including granulocytes, monocytes, and lymphocytes.
- The granulocytes are the most important cells for the immune response to infections. These are divided into neutrophil, basophil, and eosinophil granulocytes.
- Monocytes are blood cells that migrate into the tissue and can absorb and eliminate pathogens, dead cells, etc. as macrophages (referred to as scavenger cells).
- Lymphocytes are divided into B cells, T cells, and "natural killer cells." Lymphocytes are necessary for a functioning immune response to infection, as they produce antibodies and can sometimes destroy pathogens and mutated cells themselves. They control the granulocytes and ensure that the body "remembers" pathogens to which it has previously been exposed.
- The different types of white blood cells have different life spans. Depending on the type of leukocyte, this can vary from a few days to years.
- Leukocytes play a role in the body's immune response. Depending on the type of leukocyte, a distinction is made between non-specific (phagocytosis) and specific (formation of antibodies) immunity.

In a microscopic image, cell types can be differentiated from each other by classifying them based on their characteristics.

Microscopic Blood Examination Procedure

Laboratory blood analysis is one of the most important routine diagnostic procedures in medicine. Different information about the quantity and composition of blood cells can be obtained from the complete blood count without differential, the differential blood count, or the complete blood count with differential.

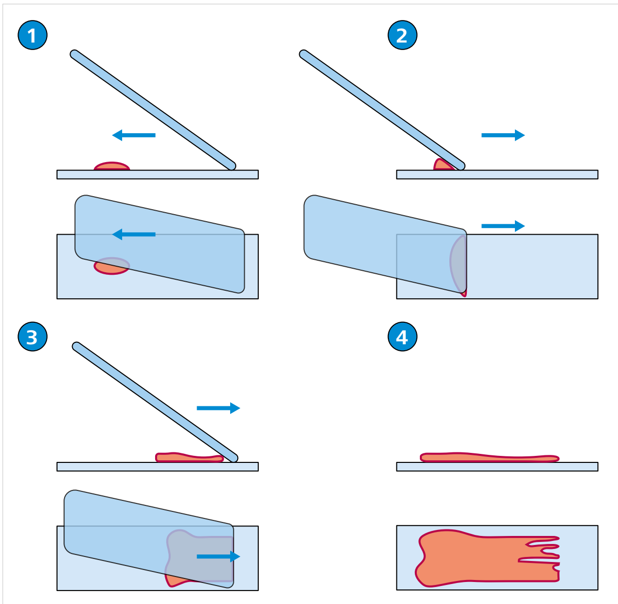
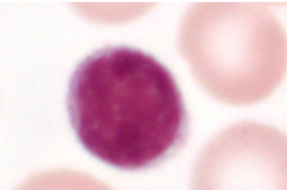


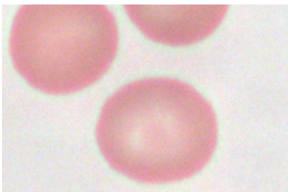
Figure 3 *Preparation of a blood smear*

Preparation and Staining

First, a blood smear (Figure 3) is prepared using a drop of blood from, for example, the fingertip. The smear is air-dried, fixed, and then stained using the Pappenheim method.

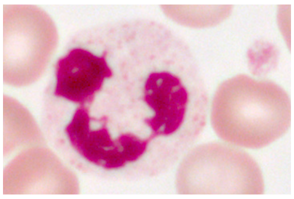


Lymphocyte

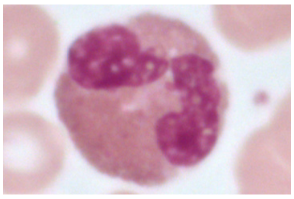


Erythrocyte

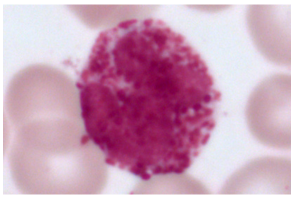
Monocyte



Segmented and banded neutrophils



Eosinophils



Basophils

Blood components	Coloration
Erythrocytes	Pink
Nuclei of leukocytes and nucleated erythrocytes	Reddish purple
Eosinophilic granules	Brick red to reddish brown
Basophilic granules	Dark purple to black
Neutrophilic granules	Light purple
Lymphocyte cytoplasm	Light blue
Monocyte plasma	Gray-blue

The blood smear is then examined under a microscope at a magnification of 1:40 or 1:100. In this process, 100 leukocytes are examined and separated from each other (200 visual fields in the case of strong anomalies). In addition, potential deformations or changes to the color of the erythrocytes are assessed and abnormalities of the thrombocytes recorded.

What Can You Learn from One Drop of Blood?

A drop of about 20 µl of blood contains:

- Approx. 80 million red blood cells (Rf: 4.0–5.0 million erythrocytes/µl)
- Approx. 140,000 white blood cells (Rf: 4,000–11,000 leukocytes/µl)
- Approx. 6,000,000 blood platelets (Rf: 150,000–450,000 thrombocytes/µl)

The aforementioned cells are contained in the blood plasma, which consists of 90% water. In addition, proteins, carbohydrates, lipids, enzymes, hormones, coagulation factors, minerals, and trace elements are dissolved in the blood plasma.

These substances dissolved in the blood plasma are transported via the bloodstream through the body to the organs and their metabolic waste products are transported to the excretory organs – the kidneys, liver, and intestines. Clinical chemical analyses and measurement methods can be used to determine their concentration (for example in mg/dl, mmol/l, U/l, kat/l). Deviations from the norm can be an indication of different diseases and/or provide the physician with important information for diagnosing diseases and monitoring their progression.

For example, abnormalities in the appearance of erythrocytes may indicate anemia. This is related to the hemoglobin that gives the erythrocytes their red color. Iron-containing molecules are located inside the hemoglobin, onto which vital oxygen can reversibly bind. If the body cannot produce enough hemoglobin due to iron deficiency (as a result of an unbalanced vegetarian diet, for example), the erythrocytes store less hemoglobin. This changes their normal color and shape – in a manner visible under a microscope – as an initial indication of the iron deficiency mentioned above.

The thrombocytes are examined under a microscope only in relation to their size, frequency, or aggregation. Thrombocyte aggregation is usually an artifact that can occur when venous blood is collected using EDTA tubes. [Footnote 4].

	Normal	Abnormal	Possible assessment
Leukocytes	4,000–11,000 cells/µl	15,400 cells/µl	Elevated – infection?
Erythrocytes	4.00–5.90 million cells/µl	3.26 million cells/µl	Low
Hemoglobin	13.0–18.0 g/dl	10.2 g/dl	Low – anemia?
Thrombocytes	150,000–450,000 cells/µl	125,000 cells/µl	Low – bleeding?

Table 1 *Normal blood count and abnormal blood count*

Microscopic Equipment

The stained blood smears are typically viewed and identified with an upright brightfield microscope such as ZEISS Axio Lab.A1. Typical overview magnifications include 10×/20×/40×. For precise identification of the blood cell, high-aperture oil lenses without cover glass correction with 63× or 100× magnification can be used. If the focus is on permanent specimens, lenses with cover glass correction should be used. In this case, 0.17 is indicated on the lens.

The distinguishing features of the leukocytes and erythrocytes mentioned above make it advisable to use high-quality optics, since the cell components should, of course, be displayed as accurately as possible. Precise medical drawings can still be found in use as reference images today.

Maximum optical performance can only be achieved through the interaction of all the optical components such as the lens, condenser, and eyepiece.

A microscope camera with a high dynamic range, precise image acquisition at the pixel level, and ideally, a cooling module should be used to document or archive the results.

Summary

The examination of blood under a microscope is a precise and equally complex procedure. The distinguishing features of the blood cells that are revealed must be clearly displayed in order to be classified correctly by the "eye of the trained observer" during daily routine activities. Even though the importance of a differential blood count seems to be universally well known, its relevance in daily routine should not be underestimated.

References:

[1] Wiley – VCH; Mahlberg, Gilles, Läsch; "Hämatologie – Therorie und Praxis für medizinische Assistenzberufe"; p. 23

[2] Typical diagram of lineages that originate from a pluripotent stem cell.

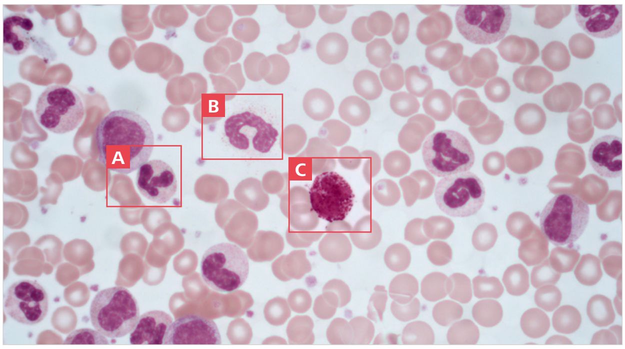
[3] Even though blood acts as the body's universal transport medium, it is not found in every tissue (blood-brain barrier). Blood also exhibits a rheological property, thixotropy. With increasing shear (e.g. in thin, peripheral veins) viscosity decreases so that the blood can flow through the veins.

[4] Footnote 4: EDTA is a salt that inhibits clotting and keeps the extracted blood fluid. It is typically used at a concentration of 1.8 mg/ml of whole blood, for example.

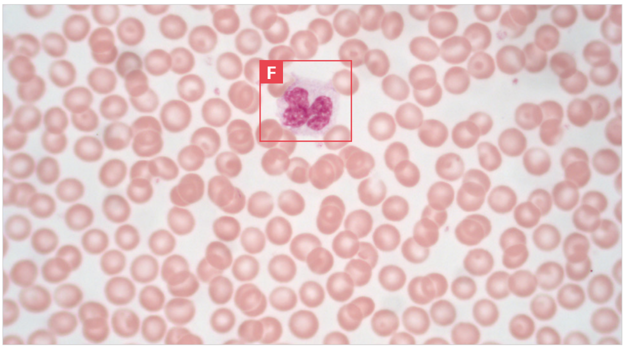
Questions a Medical Observer Should Ask When Screening a Blood Smear for Leukocytes

Question 1	How large is the blood cell being evaluated compared to a normal erythrocyte?
Question 2	What is the shape of the cell? Such as round, emarginated, etc.
Question 3	Nucleus type/shape: round/uniform/emarginated/one line/segmented
Question 4	Chromatin: aggregated/loose/coarse/translucent/fine
Question 5	Cytoplasm: colorless/grayish/blue/gray-blue/pigeon blue
Question 6	Granulation: azurophil/eosinophil/basophil
Question 7	Vacuoles: yes/no

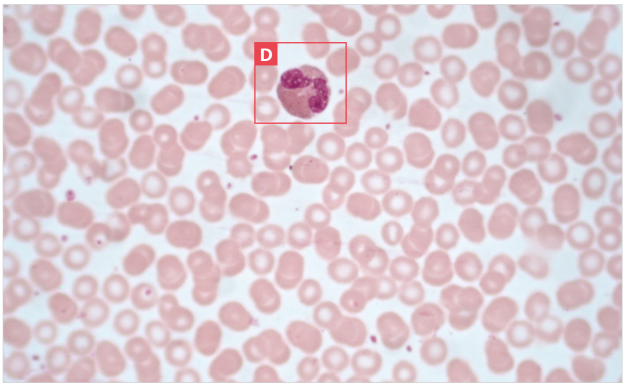
Teaching objective: Classification of the blood cells based on their specified characteristics. The students must identify the applicable characteristics.



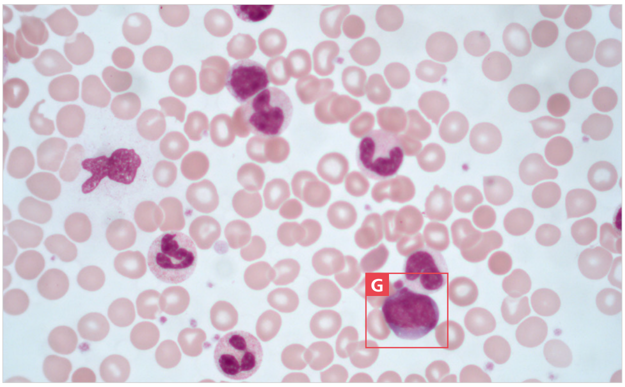
A) *Segmented granulocyte,* ***B)*** *Banded granulocyte,*
C) *Basophilic granulocyte*



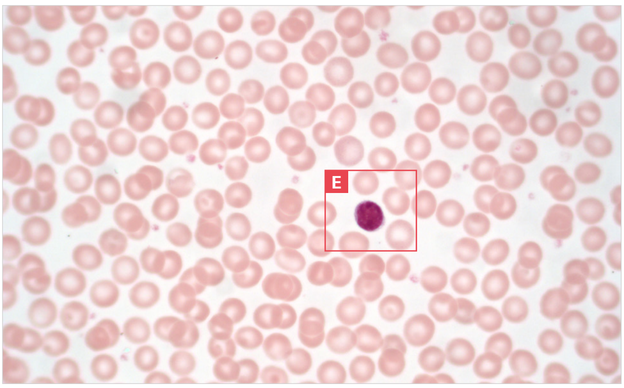
F) *Monocyte*



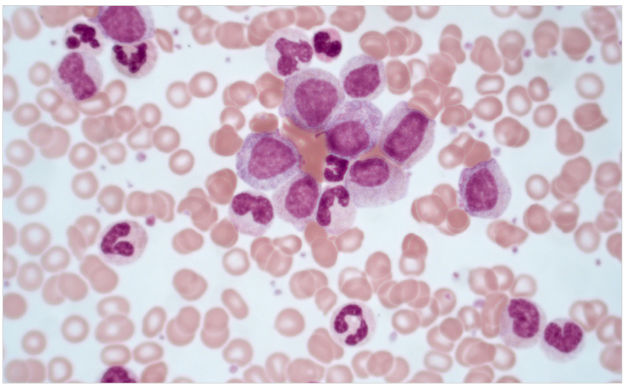
D) *Eosinophilic granulocyte*



G) *Blast, pathological*



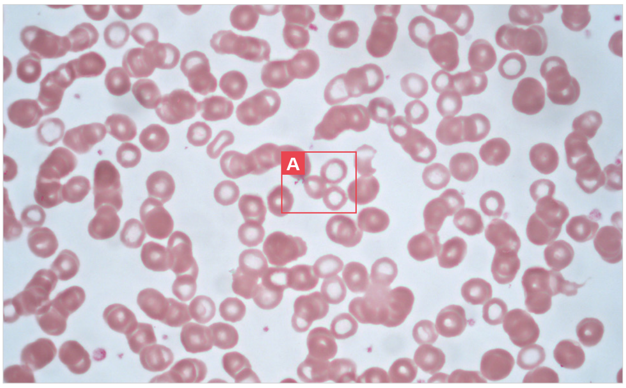
E) *Lymphocyte*



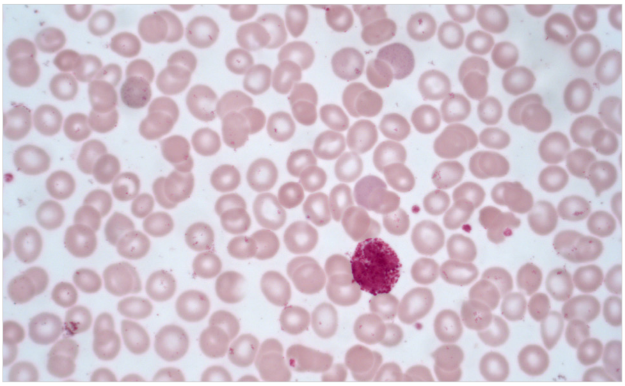
Pathological finding "AL" (acute leukemia)

Questions a Medical Observer Should Ask When Screening a Blood Smear for Erythrocytes

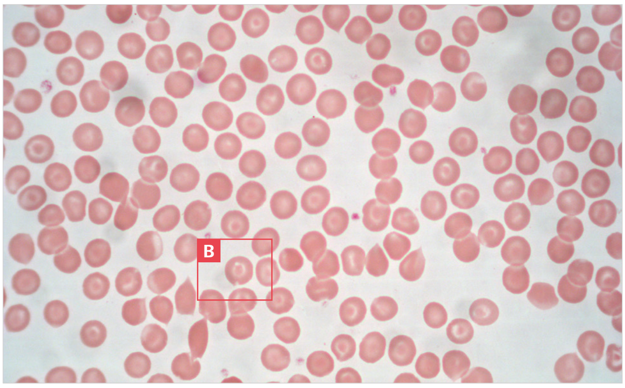
1) Have any colors changed?
2) Has the shape changed?
3) Are any inclusions visible?



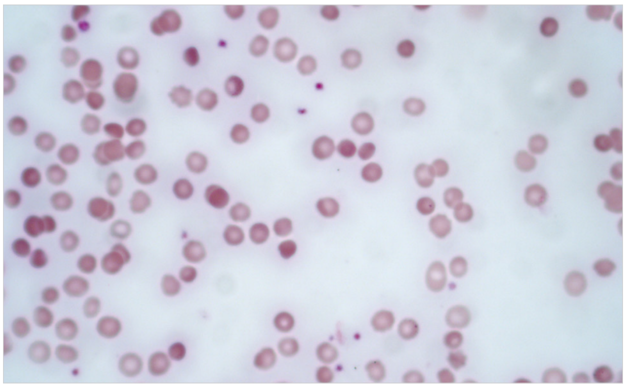
A) *Anulocyte in the event of iron deficiency*



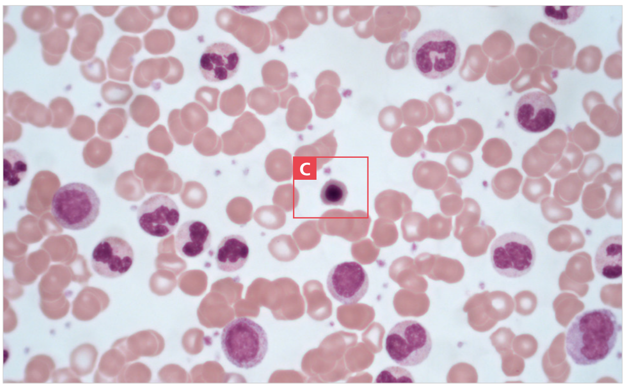
Basophilic granulocyte



B) *Target cell*



Spherocytes/sphere-shaped cells. Since these erythocytes lack the supporting structure, the typical dumbbell shape cannot develop.



C) *"Polychromatic erythoblast", pathological*

Footnotes to images on p. 6 and 7:

[1] In contrast to the histology, the interpretation of the images shown here depends on the combination of the observer's visual impression when viewed under the microscope and the subsequent classification of the characteristics identified (from the observer's visual memory) on a PC.

[2] "You only see what you know." Goethe

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