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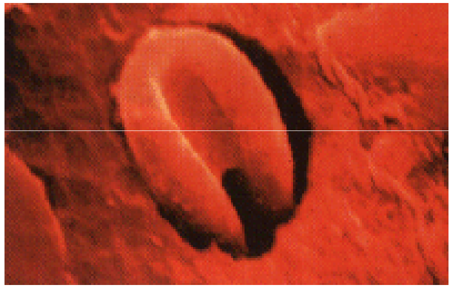
MED

I. Red blood cell count. Estimation of haemoglobin concentration. Calculated parameters of red blood cells
II. Estimation of blood group by slide method

Physiology I – practice
Autumn, weeks 4-6

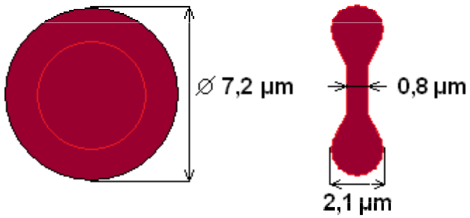
Red Blood Cell Count

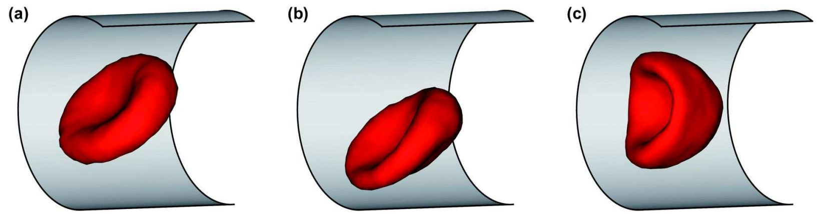
Red Blood Cell (RBC) – erytrocyte



- Anucleated cell, the most abundant blood cell
- **Shape**:
  - biconcave disc – surface increased by 30%
  - the shape is ensured by a protein named spectrin
  - the ability to deform inside the capillaries is necessary

- **Functions**:
  - Transport of oxygen to tissues (bonded mainly to haemoglobin)
  - Role in acid-base balance and CO_2 transport
- **Size**:
  - Normocyte: 7.5 µm
  - Microcyte: ≤ 7 µm
  - Macrocyte: ≥ 9 µm
  - Megalocyte: ≥ 20 µm
  - The width is around 2.5 µm peripherally and 1 µm centrally





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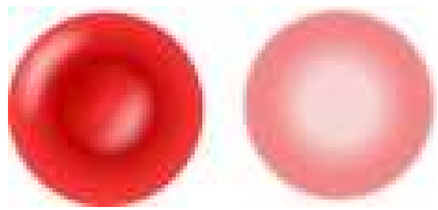
Reticulocyte

– Immature RBC
– In peripheral blood: 1% ± 0.5% of RBC
  – reticulocytosis: increased ratio of reticulocytes in peripheral blood, occurring mainly after blood loss or blood donation
– No nucleus, but residues of membrane organelles in the cytoplasm (substantia granulo-filamentosa)
– Within 48 hrs. maturation to RBC

retikulocyte



mature RBC

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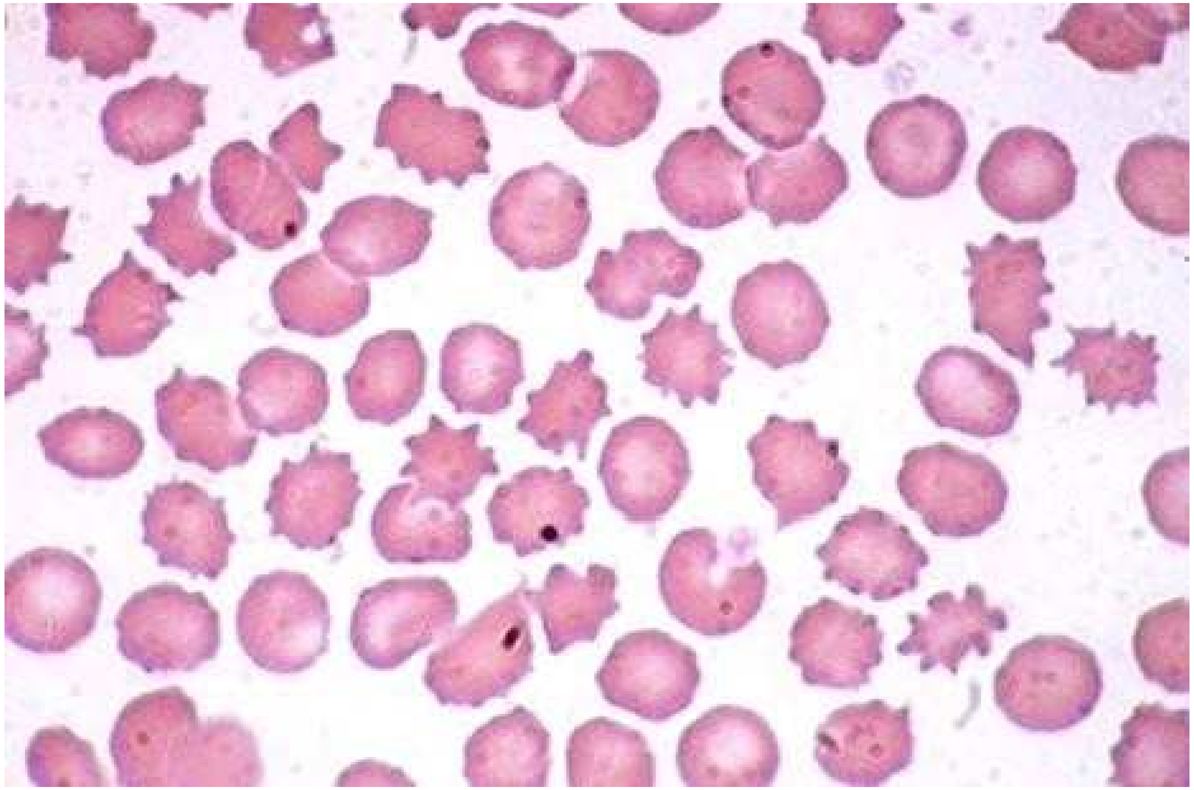
Poikylocytosis: irregular shape of RBCs

– Usually in anaemia



RBC count

- Number of RBC (red blood count)
  - Men: 4.3-5.3 * 10^{12} / l
  - Women: 3.8-4.8 * 10^{12} / l
  - Newborns: 4.4-7.0 * 10^{12} / l
- Intersexual differences:
  - Men: testosterone (male sex hormone) stimulates releasing of erythropoietin
  - Women in fertile period: relative erythrocytopaenia due to menstruation (and lower levels of testosterone)
- Alterations of RBC count
  - Polyglobulia – increased number of RBCs, increased blood viscosity
    - polycythemia vera, dehydration, adaptation to high altitude
  - Erythrocytopaenia – decreased number of RBCs, decreased blood viscosity

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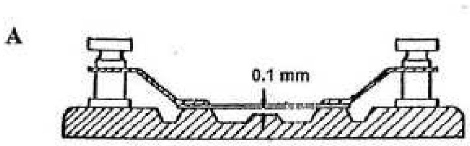
Estimation of RBC count

- Automatic methods
  - Electrical impedance – RBC count is determined based on the increase in electric resistance and the decrease in electric current while passing through the capillary from the storage container to the smaller container. RBC has a lower conductivity than diluent. It allows us to find out the size of the RBC, small = higher current, large = lower current.
  - Photo-optical – when passing through the capillary, a light beam falls on the RBC and the RBC causes the dispersion of light which we capture.
- Classical method:
  - Bŭrker‘s chamber + Hayem‘s solution for dissolution
  - 4950 μl of Hayem‘s solution and 25 μl of blood – dilution *198, or
  - 4975 μl of Hayem‘s solution and 25 μl of blood – dilution *199

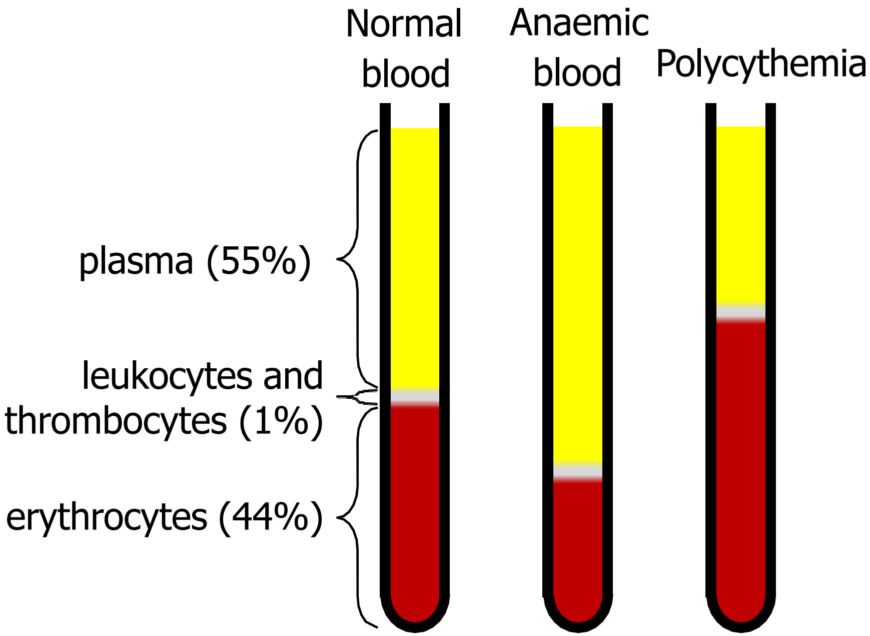


Haematocrit

- The volume percentage of red blood cells in a blood sample (erythrocyte volume fraction)
- Estimation after centrifugation of **anti-coagulated** blood*
  - Plasma
  - Buffy coat – white non-transparent layer above RBCs consisting of leukocytes and thrombocytes making up 1% of the whole volume
  - RBC
- Hct (hematocrit)
  - Men: 42-52%
  - Women: 37-47%

  - •centrifugation of coagulated blood results in blood serum (which differs from plasma by the absence of coagulation factors)

Hematocrit



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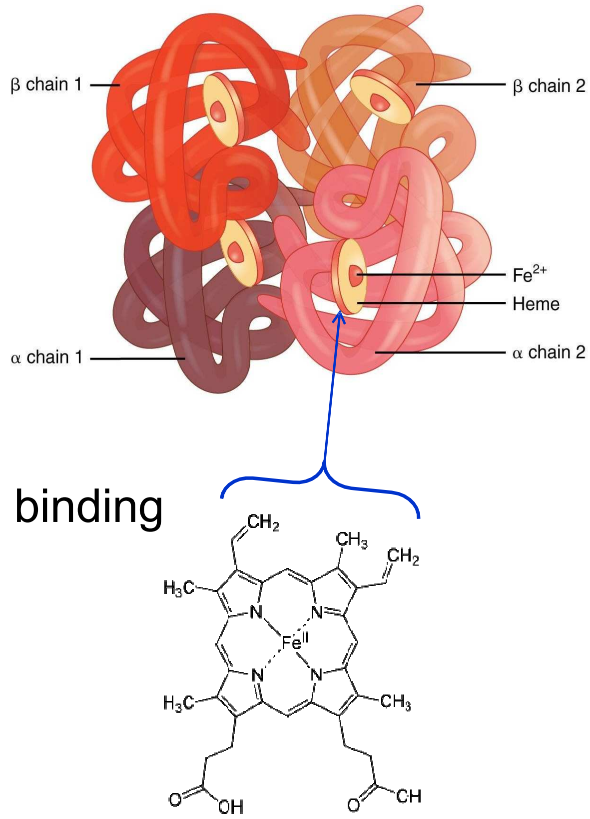
Hemoglobin



- Structure:
  - Adult haemoglobin is comprised of α and β globin subunits
  - Fetal haemoglobin consists of α and γ globin subunits

    higher affinity to oxygen than in adult hemoglobin
  - A heme group contains an iron ion (Fe^{2+}) which is the site of oxygen binding
- Hemoglobin concentration (HGB)
  - Men: 140-180 g/l
  - Women: 120-160 g/l
  - Newborns: 160-240 g/l
- Spectrophotometric determination of HGB
  - An addition of the transformation solution to the blood causes the lysis of erythrocytes and the release of haemoglobin and its transforms into cyanohaemoglobin. Measurement of light absorption follows.

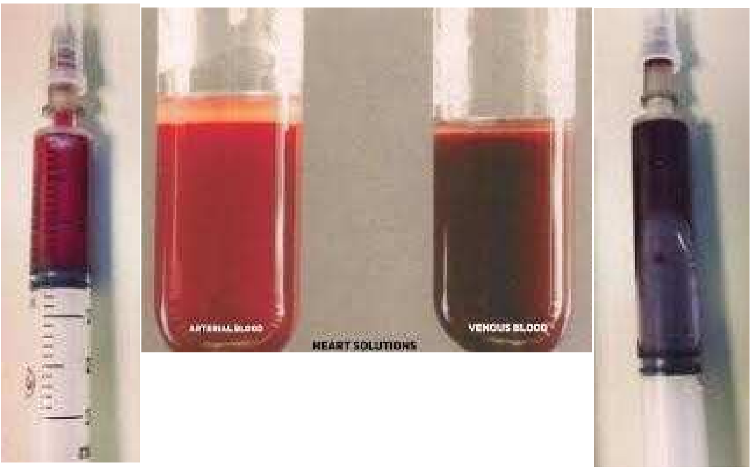
Hemoglobin – derivates

- **Oxyhemoglobin** – hemoglobin + O_2
  - Bright red color
- **Carboxyhemoglobin** – hemoglobin + CO
  - cherry red color, CO saturation higher than 50% results in a loss of consciousness
- **Carbaminohemoglobin** – hemoglobin + CO_2
  - dark red color, CO_2 binds to an amino group at the N end of the protein chain. The CO_2 concentration in inhaled air higher than 5% may lead to an impaired consciousness because of the disrupted binding of O_2 to hemoglobin
- **Methemoglobin** = hemiglobin with oxidized Fe (Fe^{3+})
  - dark "chocolate" brown, it does not bind O2, 1-3% levels are normal, 70% levels are fatal
- Glycated hemoglobin
  - a molecule of glucose binds to the protein chain – it reflects long-term blood sugar levels (glycemia). Normal levels are up to 4 mmol/l, increased levels are in uncompensated diabetes

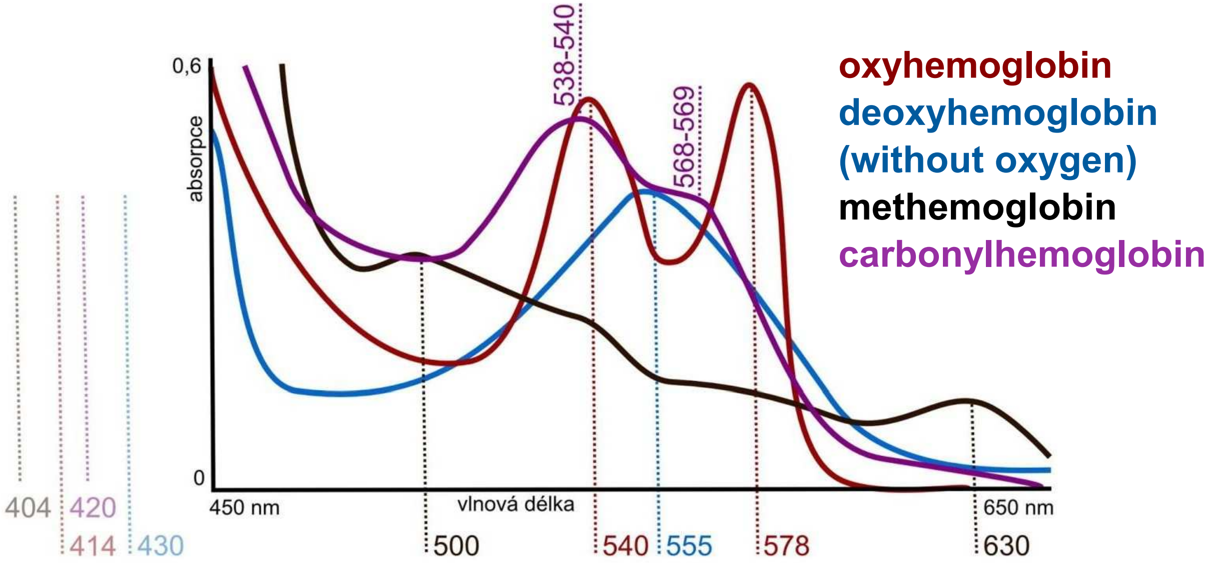
Oxy and deoxyhemoglobin

arterial oxygenated blood

venous deoxygenated blood



Absorption spectra of hemoglobin derivates



Překreslil Petr Menzel, 2011.

Spectra of oxyhemoglobin and carboxyhemoglobin are similar at the wavelength that evaluates the saturation of hemoglobin with oxygen → hypoxia caused by CO poisoning cannot be detected this way unless the sensor is specialized for it

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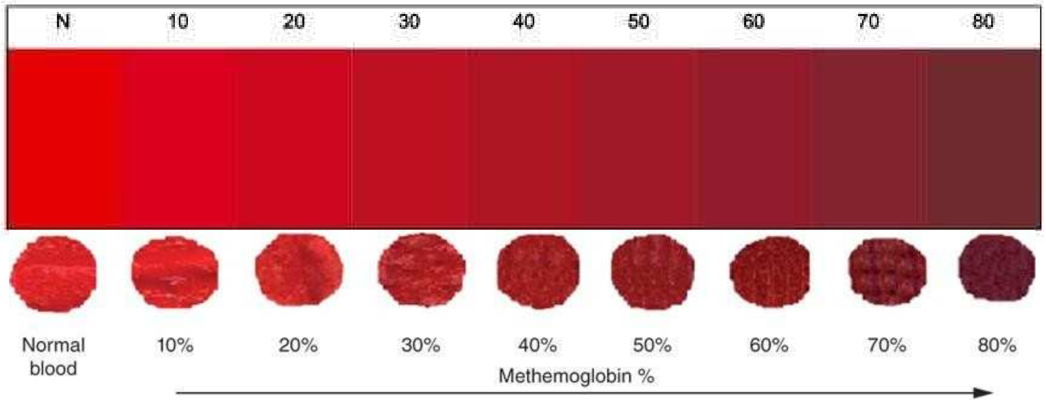
Hemoglobin derivates – methemoglobin

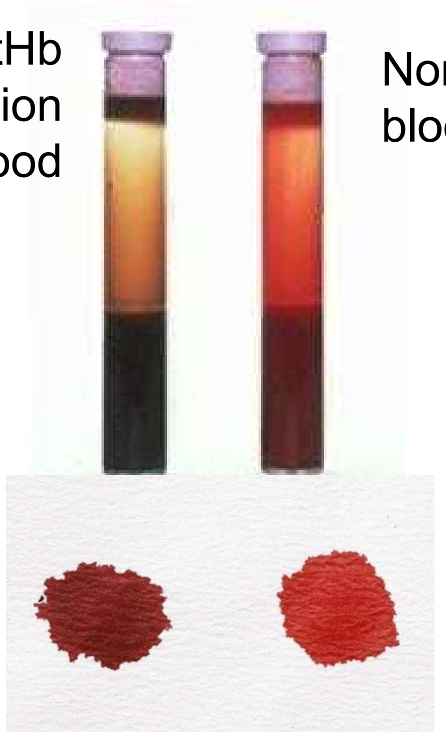
Higher methemoglobin concentration leads to the darker coloration of the arterial blood

Color spectrum for evaluation of metHb concentration



Oxygenated blood is used for the evaluation

High metHb concentration in blood



Normal blood

Hemoglobin derivates – methemoglobin

- Formation of metHb – nitrite reacts with the blood to create metHb
- A blood enzyme named methemoglobin-reductase reduces Fe^{+3} back to Fe^{2+}
  - Furthermore, non-enzymatic reduction via vitamin C is possible
- Infants have insufficiently developed reduction mechanisms
  - (+ fetal hemoglobin is easily oxidized)
  - → it is necessary to reduce intake of nitrites in the infant diet (use of infant water)
- Methemoglobinemia – congenital or acquired causes
  - Congenital – impaired synthesis of enzymes
  - Gained – increased intake of nitrates and nitrites (fertilizers), poisoning (nitrobenzene, aniline), drugs (benzocaine, also phenacetin, sulfonamides)
  - Therapy – administration of reducing agents such as methylene blue or ascorbic acid

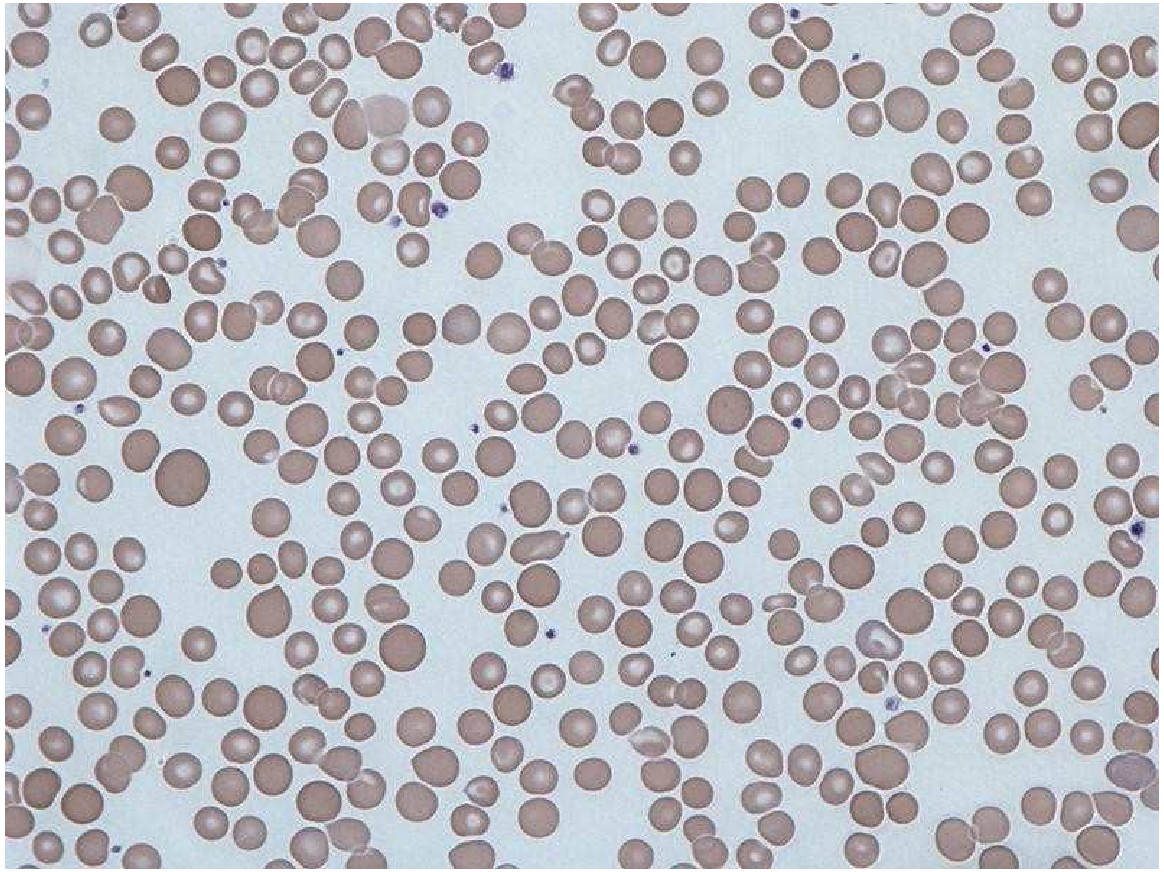
MetHb values	Symptoms
0–2%	normal values
<10%	cyanosis
<35%	cyanosis a others (headache, dyspnoea)
>70%	fatal

Calculated parameters of RBCs

- The average volume of one RBC (**MCV**, mean corpuscular volume)
  - **MCV = HCT/RBC** (hematocrit/red blood count) = 80-95 fL
- The average weight of Hb in one RBC (**MCH**, mean corpuscular hemoglobin)
  - **MCH = HGB/RBC** (hemoglobin/red blood count) = 28-32 pg
- The average concentration of Hb in one RBC (**MCHC**, mean corpuscular hemoglobin concentration)
  - **MCHC = HGB/HCT** (hemoglobin/hematocrit) = 310-360 g/L
- Red cell distribution width (**RDW**) = 11,5-14,5%
  - Variation of RBCs size
  - ↑RDW – anisocytosis

Anisocytosis: varying size of RBCs

Mild anisocytosis is physiological



Calculated parameters of RBCs

↑MCV → macrocytosis
↓MCV → microcytosis

HCT

hematocrit
men: 42-52%
women: 37-47%

MCV = HCT/RBC
RBC volume
80-90 fL

MCHC = HGB/HCT
Hb in RBC
310-360 g/L

RBC

number of RBCs
men: 4.3-5.3 * 10^{12}/L
women: 3.8-4.8 * 10^{12}/L

HGB

hemoglobin concentration
men: 140-180 g/L
women: 120-160 g/L

MCH = HGB/RBC
Hb concentration in RBC
28-32 pg/RBC

↑MCH → hyperchromic RBC
↓MCH → hypochromic RBC

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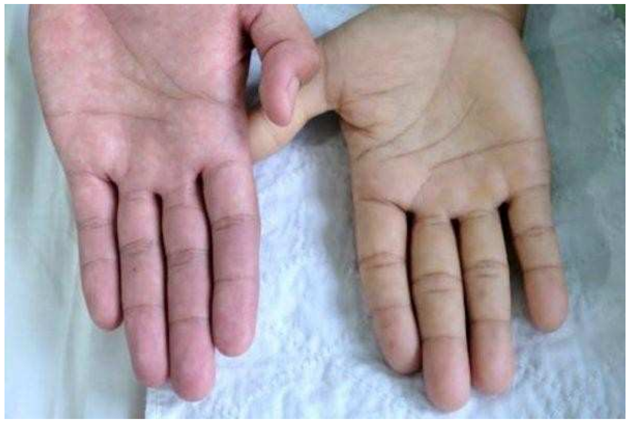
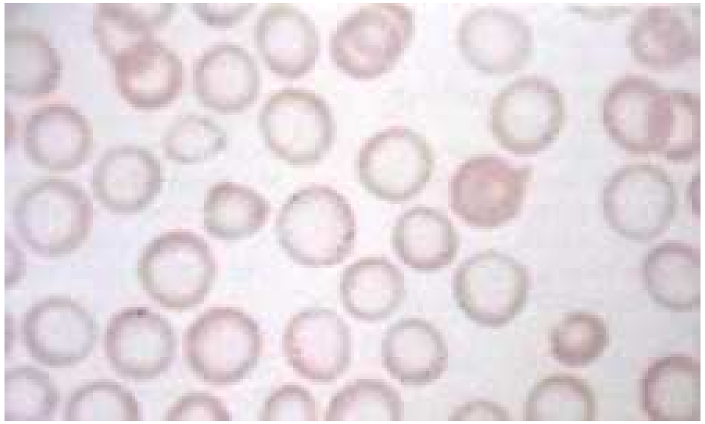
Anemia

- = Decreased concentration of functional Hb in the blood
  - A small number of RBCs or low hematocrit or unfunctional Hb
  - Impaired RBC production (proliferation or differentiation), increased RBC destruction or blood loss
  - RBCs are one of the fastest proliferating cells within the body thus early signs of malnutrition may be seen in RBC count
  - Anemia has various causes (congenital or acquired)
- Symptoms (anemic syndrome)
  - Tissue hypoxia: pale skin and mucose membranes (best seen in the conjunctiva), fatigue, weakness
  - Tachycardia
  - Dyspnoea in mild physical activity



Sideropenic anemia

- Cause: deficiency of Fe^{2+} → decreased production of hemoglobin → hypoxia stimulates releasing of erythropoietin → increased production of RBCs with insufficient Hb levels
- **Microcytic hypochromic anemia**
  - ↓MCV and ↓MCH, RBC count increased or unchanged
- RBCs are small and pale with Hb only peripherally



Megaloblastic anemia

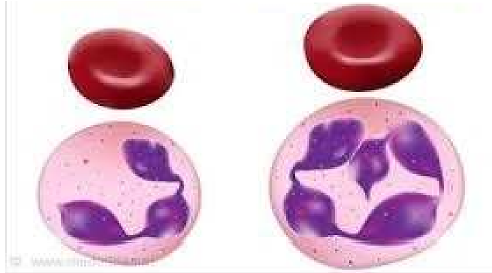
- Megaloblasts (oversized immature RBCs) in the bone marrow and a reduced number of reticulocytes in the peripheral blood
- **Macrocytic hyperchromic anemia** (↑MCV, ↑MCH, ↓RBC)
  - A small number of large erythrocytes with an increased hemoglobin content
  - It occurs due to a lack of vitamins B12 and folic acid which are important for the methylation of uracil to thymine
  - ↓ B12 and folic acid → ↓DNA formation, while RNA and protein synthesis is intact
  - B12 supply - years; folic acid – months → manifestations of hypovitaminosis occur with time delay
- **Pernicious anemia** – the most common type of megaloblastic anemia
  - Autoimmune disease interfering with B12 absorption
  - Autoimmunely damaged parietal cells of the gastric mucosa do not form intrinsic factor, which is necessary for the absorption of B12 (a disorder of factor formation can also be due to gastric surgery)

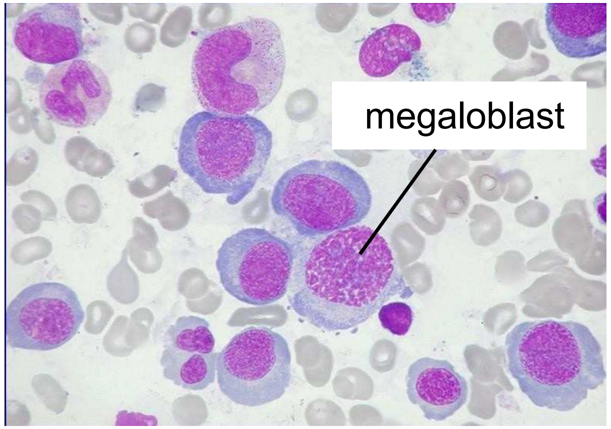
Megaloblastic anemia

Normal blood | Megaloblastic anemia

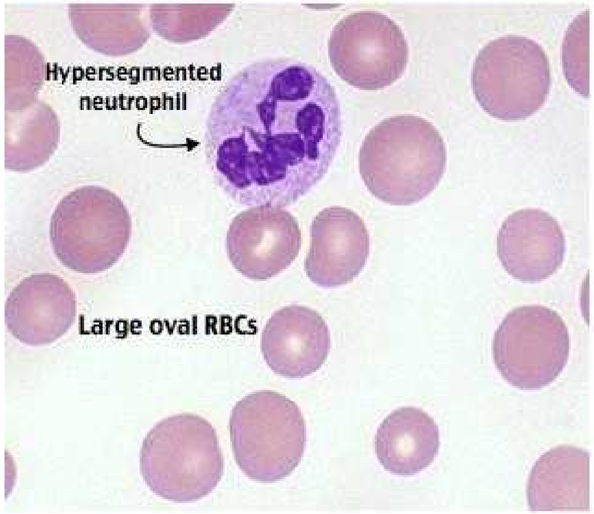
RBC

neutrophil

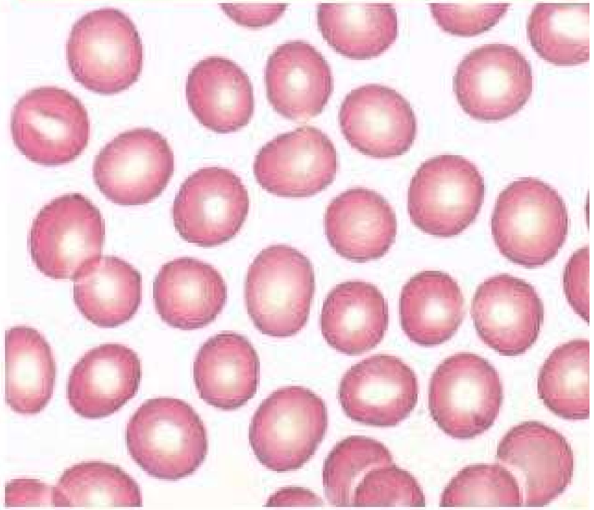




Megaloblastic anemia



Norrmal blood smear



RBCs are larger, hyperchromic, their centre is less pale than in normal RBCs
decreased RBC count (+presence of hypersegmented neutrophils)

Blood groups (types)

Antigens and antibodies in blood groups

- Blood groups are determined by antigens on the RBC membrane
  - These blood traits are inherited and remain unchanged throughout life
- A light introduction to the general principles of immunization
  - An antigen is a "recognition mark" by which the immune system determines whether it is a body's cell or a foreign one
  - When the immune system first encounters a foreign erythrocyte in the blood, it begins to produce antibodies against the foreign antigens on the erythrocyte (but not against those antigens that are the same as on the body's erythrocytes). It remembers the foreign antigen and creates antibodies faster the next time it encounters it.
  - If there are antibodies against a certain blood type in the blood, the erythrocyte of this blood type triggers an immune reaction → destruction of the foreign blood cell (hemolysis)

Antigens and antibodies in blood types

- The most important blood groups are AB0 and Rh
  - Antibodies against AB0 antigens are in the blood from the first months of life
  - Antibodies against Rh factor and other blood types form only after immunization with incompatible blood – unwanted immune response during repeated transfusions of not fully compatible blood
  - There are dozens of other systems that need to be considered from an immunological point of view (MNS, Kell, Lewis, Kidd systems, ...)

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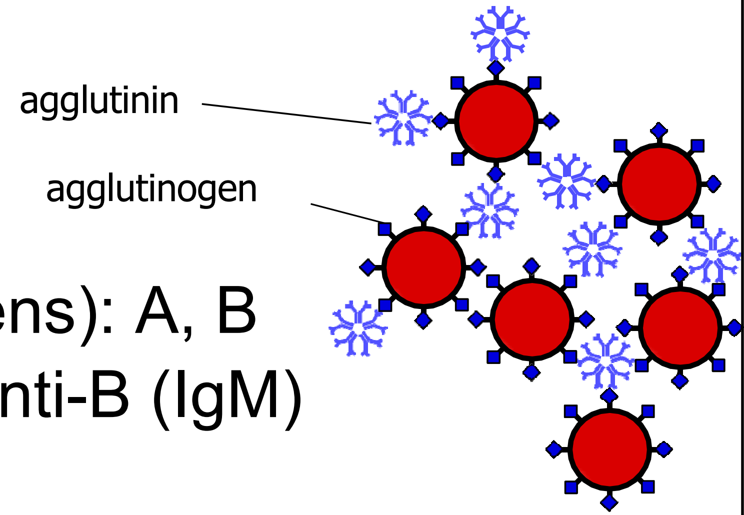
AB0 system



- Antigens on the surface of RBCs (agglutinogens): A, B
- Antibodies in the blood (agglutinins): anti-A, anti-B (IgM)
  - They do not cross the placenta
- Agglutination (clumping):
  - anti-A reacts with A, anti-B reacts with B
  - RBCs are connected by agglutinogens – clumps are formed
  - An immune response is triggered (complement activation) that destroys foreign erythrocytes via hemolysis

  - Immunization against A and B happens during the first months of life (these antigens are also in the diet) – agglutinins are then in the blood for the rest of the life
  - When incompatible blood is administered - strong activation of the immune system occurs, leading to hemolysis, anemia, hemoglobinuria (hemoglobin in the urine), kidney failure, ... death

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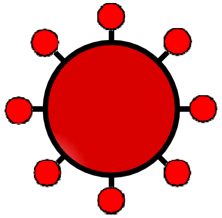
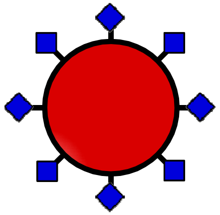
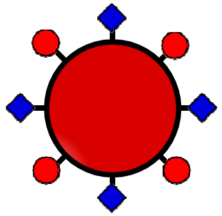
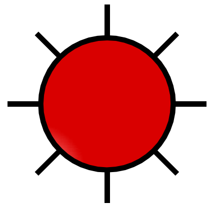
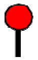
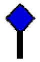
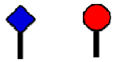
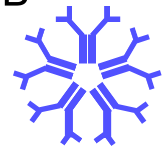
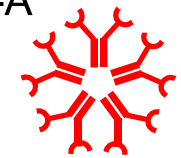
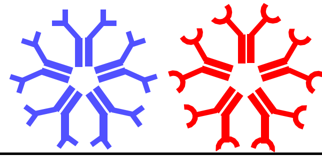
AB0 system

Blood groups	Group A	Group B	Group AB	Group 0
Prevalence in CZ	41%	18%	9%	32%
RBCs				
Antigens on RBCs	A 	B 	A a B 	none
Antibodies in the blood	anti-B 	anti-A 	none	anti-A + anti-B 

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Rh factor

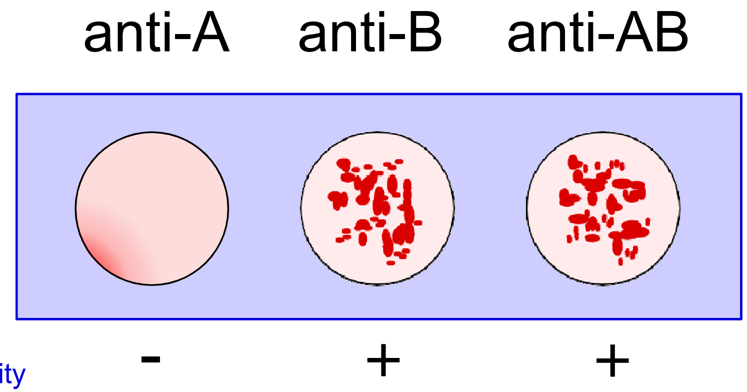
- Antigens D, d (also C,c, E, e, which are weaker) are only on RBCs
  - The strongest one is an antigen D – if present → Rh+ blood group
  - In recessive homozygotes (dd) → blood group Rh- (17% in Europe, <1% elsewhere)
- in Rh- blood, antibodies (anti-D, IgG) develop only after immunization (Rh- blood must meet Rh+ blood)
  - The first reaction is weaker, the next encounter with Rh+ blood will trigger a stronger immune response → hemolysis

Rh factor

- Causes of immunization:
  - Transfusion of incompatible blood – Rh- recipient receives Rh+ blood
  - Childbirth (miscarriage, abortion, bleeding from the placenta, invasive procedures) where the mother is Rh- and the fetus is Rh+
  - Immunization will manifest only during further pregnancies of this combination – IgG antibodies pass through the placenta and attack fetal RBCs → blood hemolysis → anemia → increased hematopoiesis → fetal erythroblastosis (more erythroblasts – immature RBCs), unconjugated hyperbilirubinemia (icterus), anemia, hydrops (tissue swelling due to hypoxia), death
  - Prevention – administration of anti-D after delivery (or other events) will eliminate Rh- RBCs before the mother's immune systém notices them

Estimation of blood groups by slide method

- Drop anti-A, anti-B and a combination of anti-A and anti-B antibodies onto the glass slide
  - Or blood group sera A (contains anti-B), B (anti-A) and 0 (anti-A, anti-B) are used – serum is blood plasma without coagulation factors, it contains the relevant agglutinins
- Mix the antibodies with a drop of blood (sera must not mix)
- A positive result occurs in serum with agglutination (clumping)



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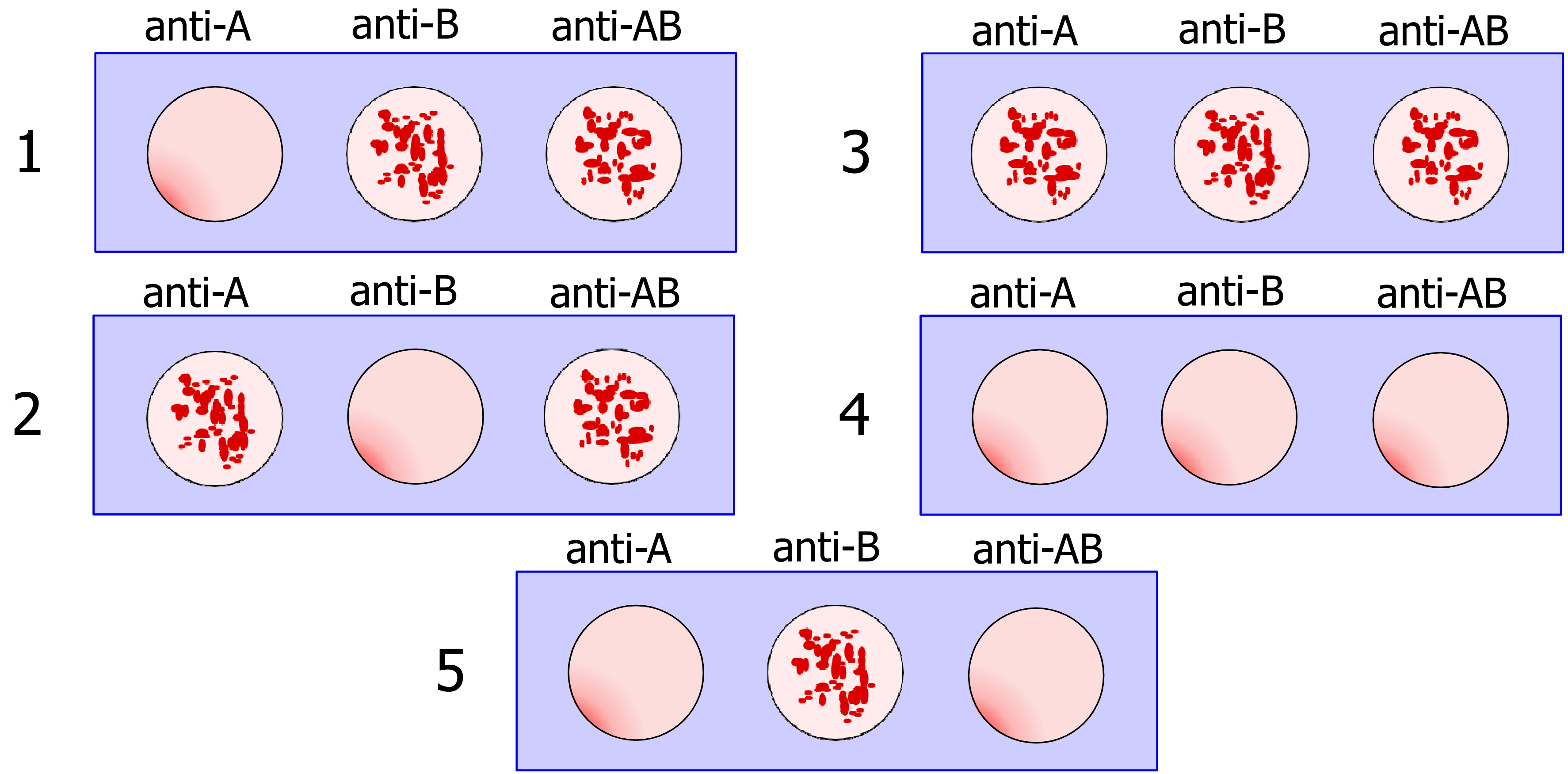
Fill correct blood groups to results



Correct results