FIZIOLOGIJA ŽIVALI

Laboratorijske vaje

ANALIZA KRVI

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UP FAMNIT

ANALIZE KRVI

- kri prenaša topne snovi v celicah in izven celic v telesu
- krvne celice so pomembne tudi pri obrambi
- analiza krvi da pomembne informacije o tem, kako dobro se te funkcije izvajajo
  - hematokrit
  - stopnja sedimentacije eritrocitov
  - določanje Hb
  - določanje krvne skupine
  - določanje količine holesterola

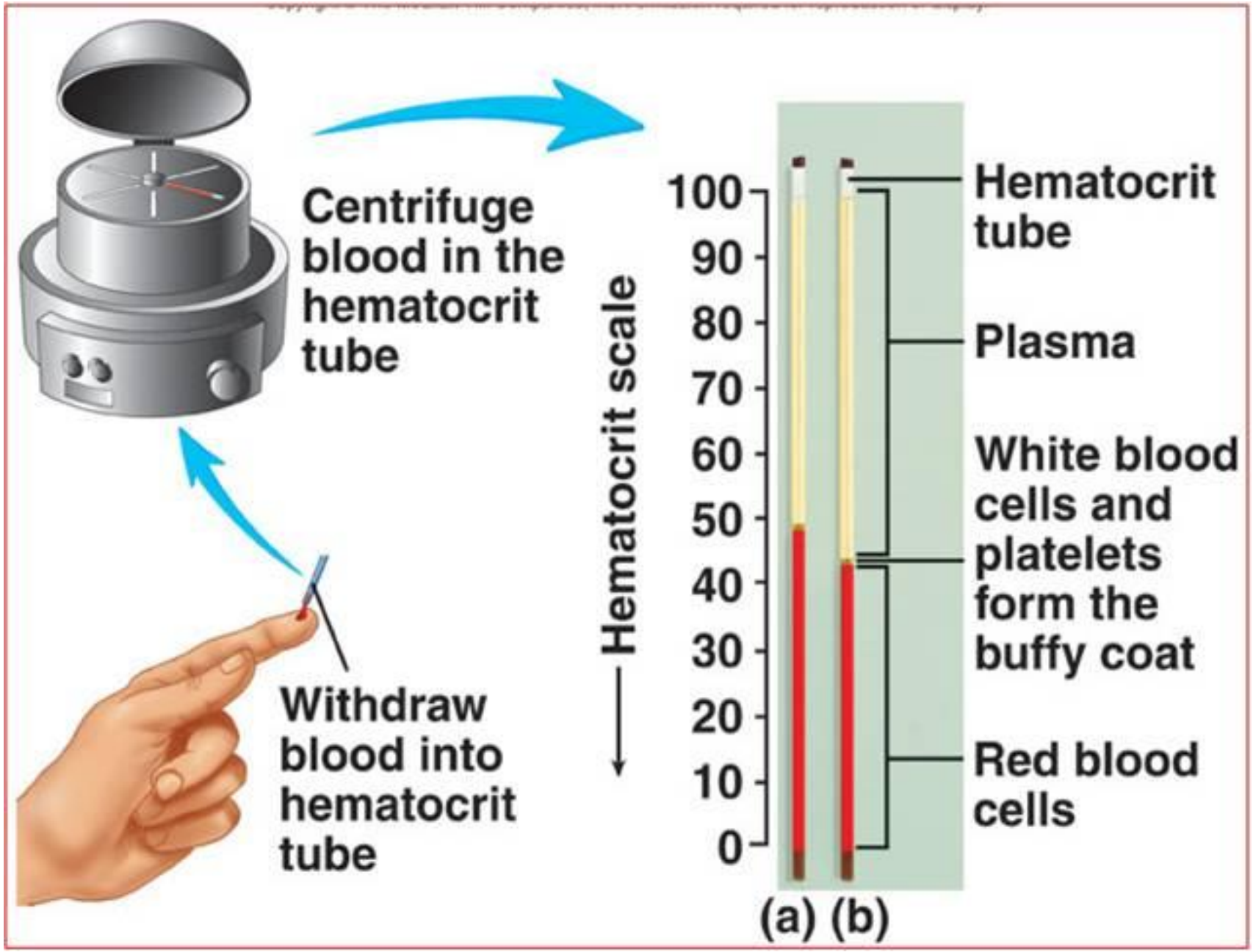
HEMATOKRIT

- hematokrit = odstotek rdečih krvnih celic (RCBs) v vzorcu krvi
- določimo s centrifugiranjem, določanjem višine rdečih krvničk v stolpcu - % rdečih krvničk
  - določimo tudi % belih krvničk – WBC – “buffy coat”
- višji → več celic za transport O2

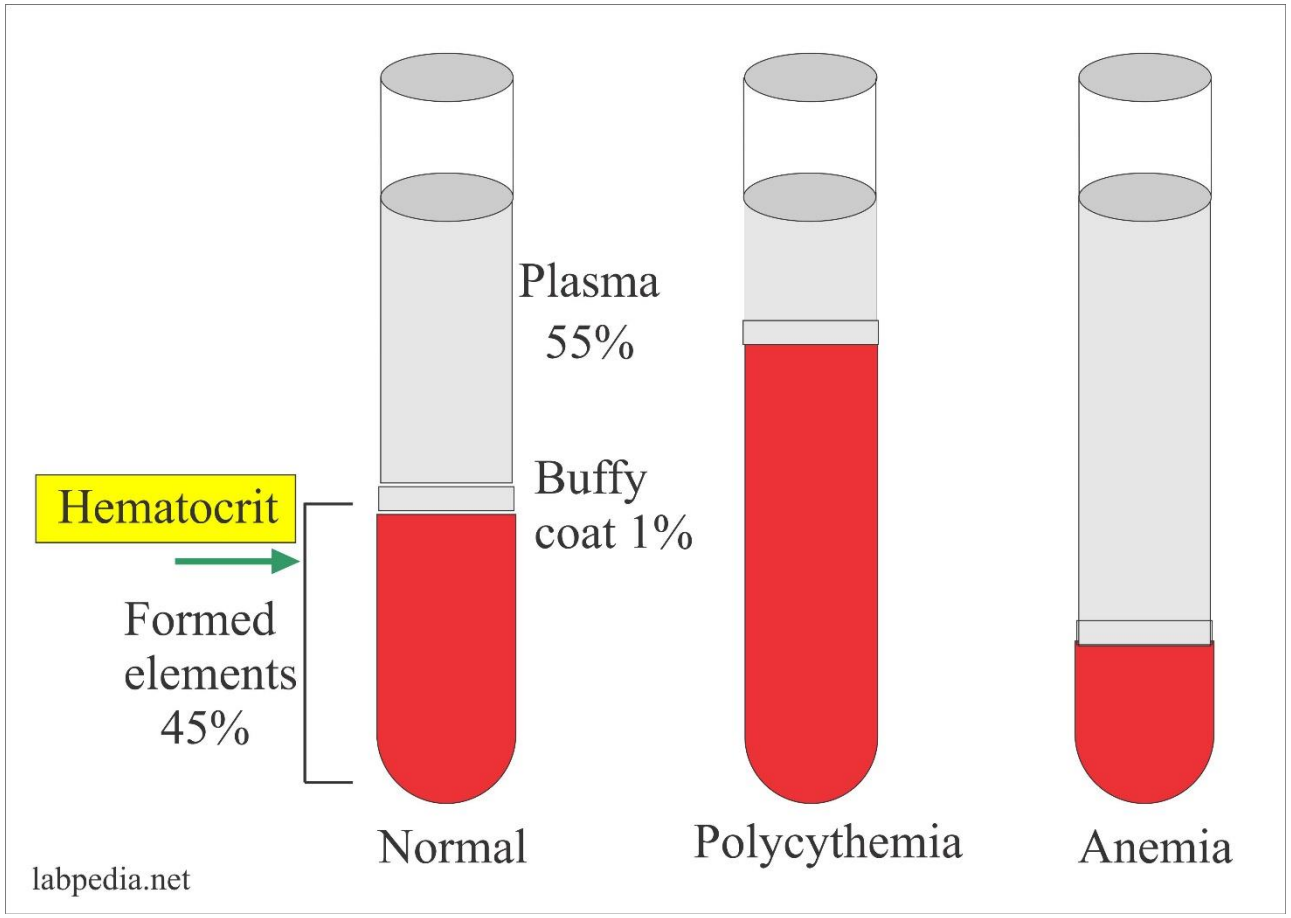
55% zgornja meja

- povprečni MOŠKI: 47%, ŽENSKE: 42%
- MOŠKI: testosteron stimulira izločanje hormona eritropoetina iz ledvic (stimulira sintezo eritrocitov)

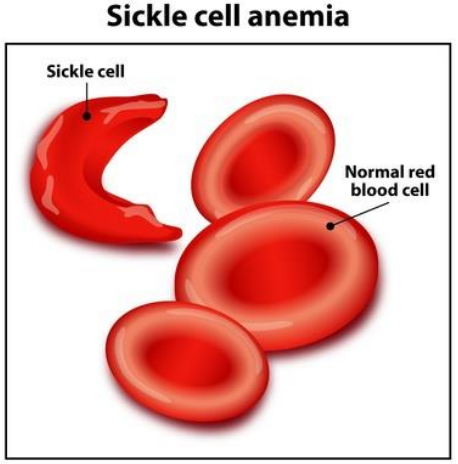
- odstopanja: anemija/policitemija


Centrifuge blood in the hematocrit tube
Withdraw blood into hematocrit tube
Hematocrit scale
100
90
80
70
60
50
40
30
20
10
0
(a) (b)
Hematocrit tube
Plasma
White blood cells and platelets form the buffy coat
Red blood cells

- višji – **policitemija (polycythemia)** - nenormalno delovanje kostnega mozga (tumor) ali posledica kronične hipoksije ali pomanjkanja kisika v vdihanem zraku, prilagajanje na višino



Anemija



- premalo O_2 se prenaša do celic v telesu
- razlogi: majhna vrednost eritrocitov, majhna vrednost Hb v celicah, nenormalno oblikovani eritrociti

- hem: vsebuje Fe atom za vezavo kisika

- če ni Fe – telo ne more sintetizirati Hb → *iron defieciency anemia* = **sideropenična deficitarna anemija - SDA**
- ***aplastična anemija*** – kostni mozeg ne sintetirira dovolj celic
- **hemolitična anemija** - zaradi povečanega razpada eritroc
- **anemija po krvavitvah, anemija kroničnega vnetja**
- ***anemija srpastih celic*** – dedna, proteinski del Hb se nepravilno oblikuje ob nizkih vrednostih O2, celice srpaste oblike

Table 2. Reference Values for Common Hematology Determinations in Adult Dogs and Cats*†

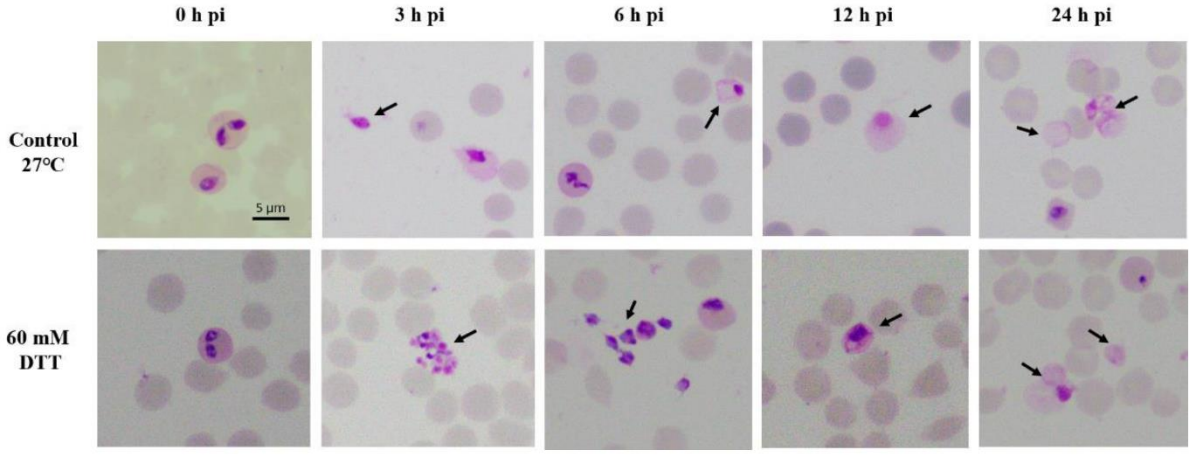
Parameter	Canine	Feline
WBC count (/μL)	4,000–15,500	3,000–14,800
RBC count ($\times 10^6$/μL)	4.8–9.3	5.92–9.93
Hemoglobin (g/dL)	12.1–20.3	9.3–15.9
Hematocrit (%)	36–60	29–48

anemija zaradi parazitizma...

Infection type	Mean value ± confidence interval		
	RBC count ($\times 10^6/\mu l$)	Hb concn (g/dl)	HCT value (%)
Noninfected	7.29 ± 0.29	11.6 ± 0.31	32.0 ± 0.91
T. orientalis	5.90 ± 0.35[a]	11.2 ± 0.49	30.1 ± 1.39
B. ovata	7.24 ± 0.63	11.4 ± 0.44	31.7 ± 1.45
T. orientalis and *B. ovata*	5.64 ± 0.56[a]	10.3 ± 0.88[a]	27.8 ± 2.33[a]

[a] Statistically significant reduction in the mean value obtained compared to that for the noninfected animals ($P < 0.01$).

- *Babesia ovata, Theileria orientalis*



VAJA (EX. 11, Acitivity 1)

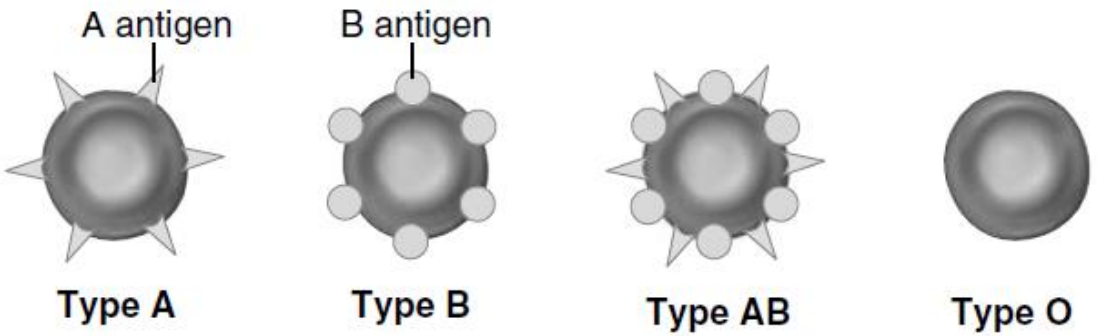
- **Sample 1:** healthy male, living in Boston
- **Sample 2:** healthy female, living in Boston
- **Sample 3:** healthy male, living in Denver
- **Sample 4:** healthy female, living in Denver
- **Sample 5:** male with aplastic anemia
- **Sample 6:** female with iron-deficiency anemia

- BOSTON – pri morju
- DENVER – ok. 1600 m n.v.

DOLOČANJE KRVNE SKUPINE

- vse celice v telesu – obdane z membrano z genetsko določenimi glikoproteini – ANTIGENI
  - na eritrocitih (RBC) – antigeni (aglutinogeni), ki določajo krvno skupino ljudi

- ljudje – ABO sistem krvnih skupin
  - določa prisotnost anitgenov - tip A, tip B antigeni



(b) Blood type surface antigens

antigeni genetsko določeni – vsak 2 kopiji (alela) genov za protein

Allele I^A produces antigen **A**

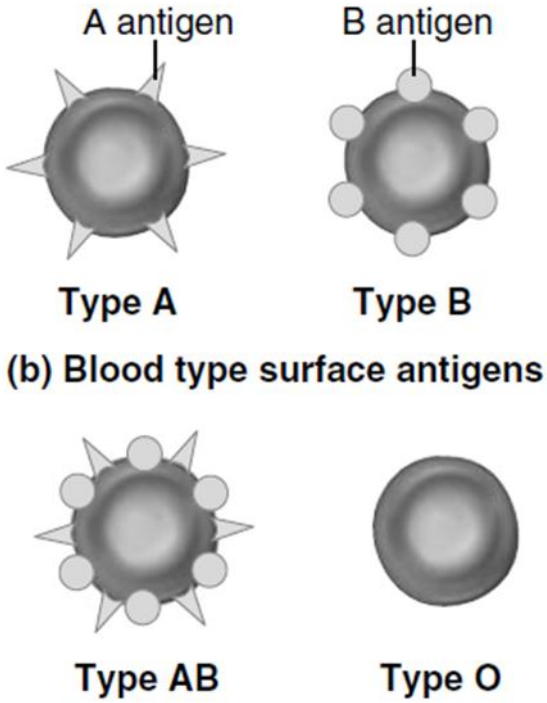
Allele I^B produces antigen **B**

Allele **i** produces **no** antigen.

dominantna alela

recesiven alel

Genotypes	Phenotypes (blood types)
$I^A I^A$	A
$I^A I^B$	AB
$I^A i$	A
$I^B I^B$	B
$I^B i$	B
ii	O

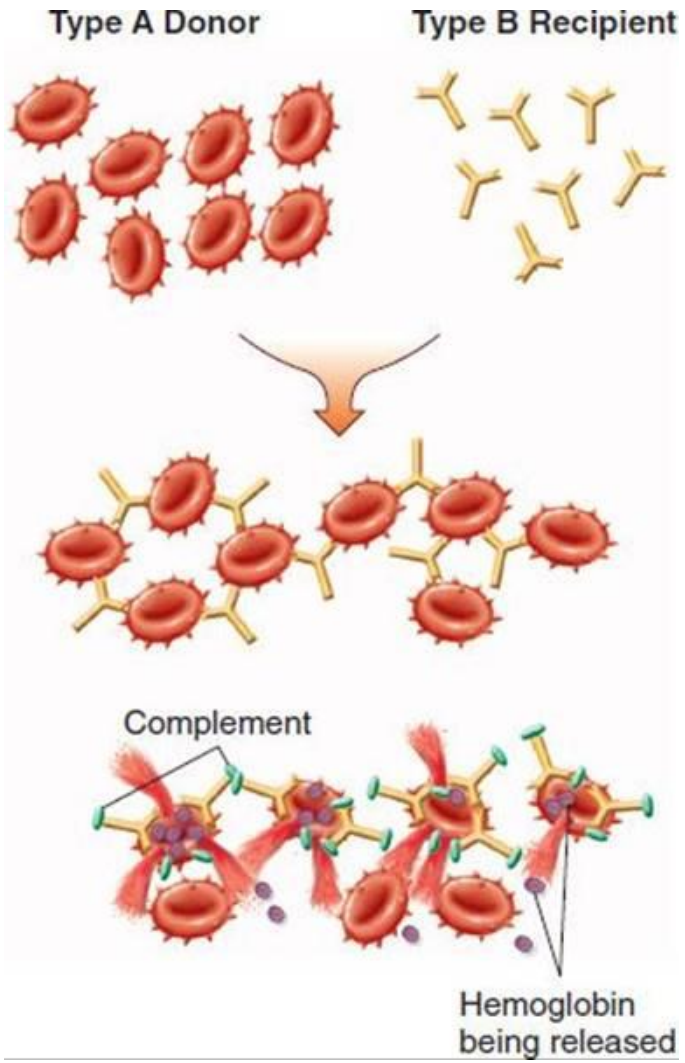


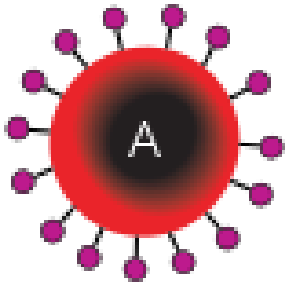
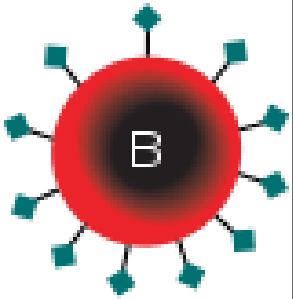
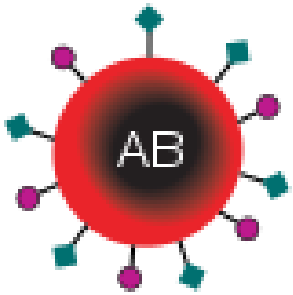
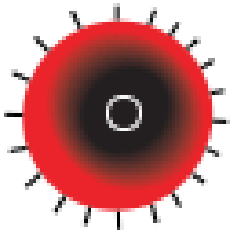
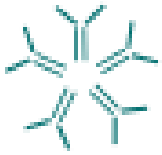
PROTITELESA (aglutinini), ang. antibody

- tvorijo se v krvni plazmi
- reagirajo z antigeni (ki jih ni na rd. krvničkah)
- protitelesa proti “tujemu antigenu”

- **transfuzija** – če protitelesa reagirajo z antigeni donorja – rdeče krvničke se skrčijo (agglutinated), razgradijo → potencialno življenjsko nevarna reakcija transfuzije

- ABO in Rh antigeni povzročijo potencialno smrtne reakcije pri transfuziji



	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

UNIV. DONOR???

UNIV. PREJEMNIK???

RH SISTEM DOLOČANJA KRVNE SKUPINE

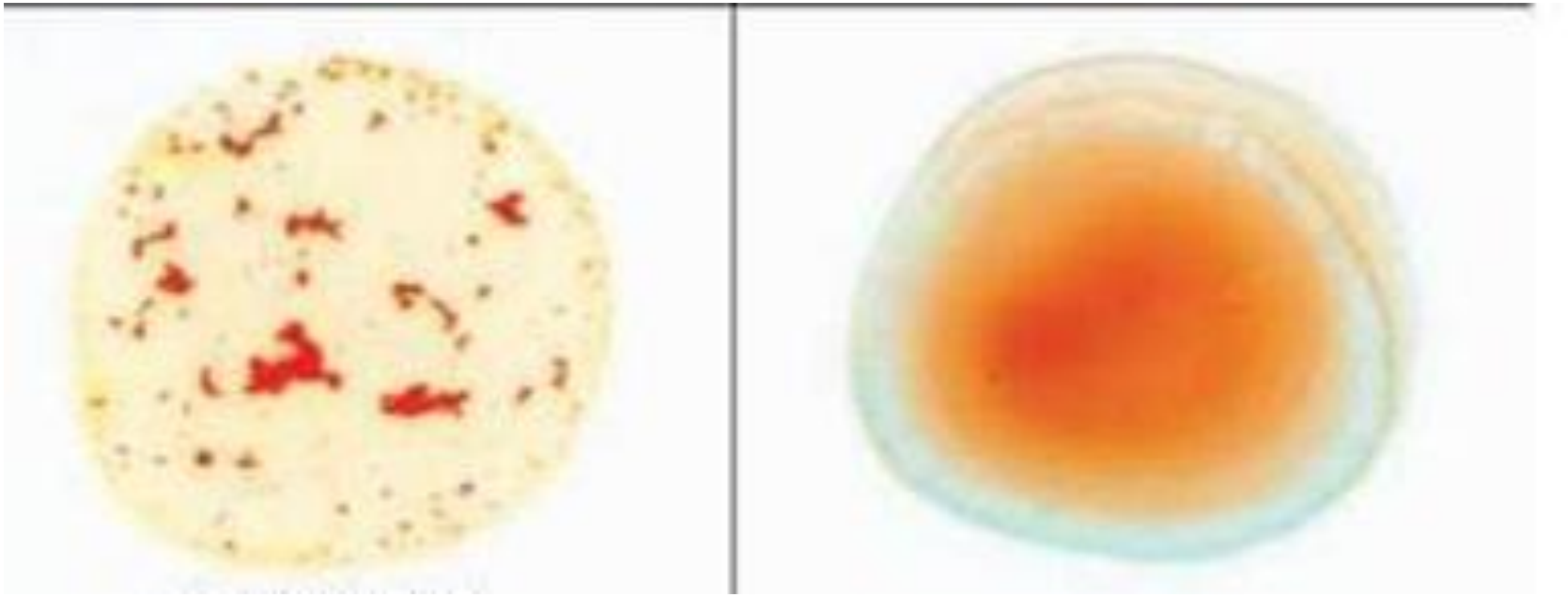
- več skupin Rh antigenov, Rh oznaka krvne sk. – Rh (D) antigen
- Rh+ RBC imajo Rh antigen
- Rh- nimajo
  - protitelesa proti RH antigenu tvorijo ob izpostavitvi le-temu

Rh- - recesivno, Rh+ dominantno

ABO +RH NSTA EDINA SISTEMA!!

	A -	A +	B -	B +
Red blood cells				
Antigens present	A antigen	A antigen Rh antigen	B antigen	B antigen Rh antigen

DOLOČANJE KRVNE SKUPINE:

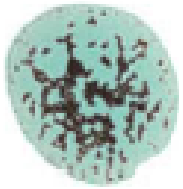


- kapljice krvi pomešamo z antiserumom s protitelesi proti A, B ali Rh antigenom

- aglutinacija – prisotnost aglutinogena (antigena) na
- npr. krvna sk. A+ - aglutinacija z antiserumom s protitelesi A

Blood sample	Agglutination with Anti-A Serum	Agglutination with Anti-B Serum	Agglutination with Anti-Rh Serum	Blood Type
1	positive	negative	positive	
2	negative	positive	positive	
3	positive	positive	negative	
4	negative	negative	negative	
5	positive	positive	positive	
6	negative	positive	negative	

Sample 1



Anti-A

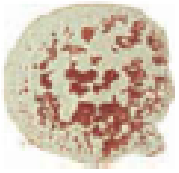
Anti-B

Anti-Rh

Sample 2

Anti-A

Anti-B



Anti-Rh

KRVNE SKUPINE PRI ŽIVALIH...

- **psi** – več kot 20 krvnih sk, nekaj pomembnejših, 1 - 8 DEA (dog erythrocyte antigen)
- **mačke** – A, B ali AB
- **konji** – 30 krvnih skupin, 8 pogostejših, A, C, D, K, P, Q, U, + T
- **ovce** – 7 krvnih skupin

kompatibilnost odvisna od prisotnosti protiteles v krvni plazmi!

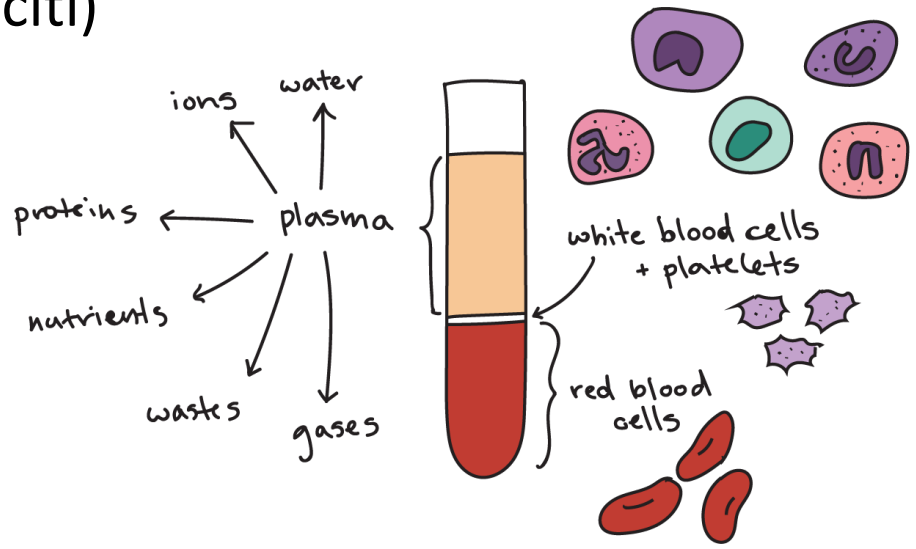
RAČUNALNIŠKE VAJE

- Exercise 11: Blood analysis
  - Activity 1: Hematocrit determination
  - Activity 4: Blood typing

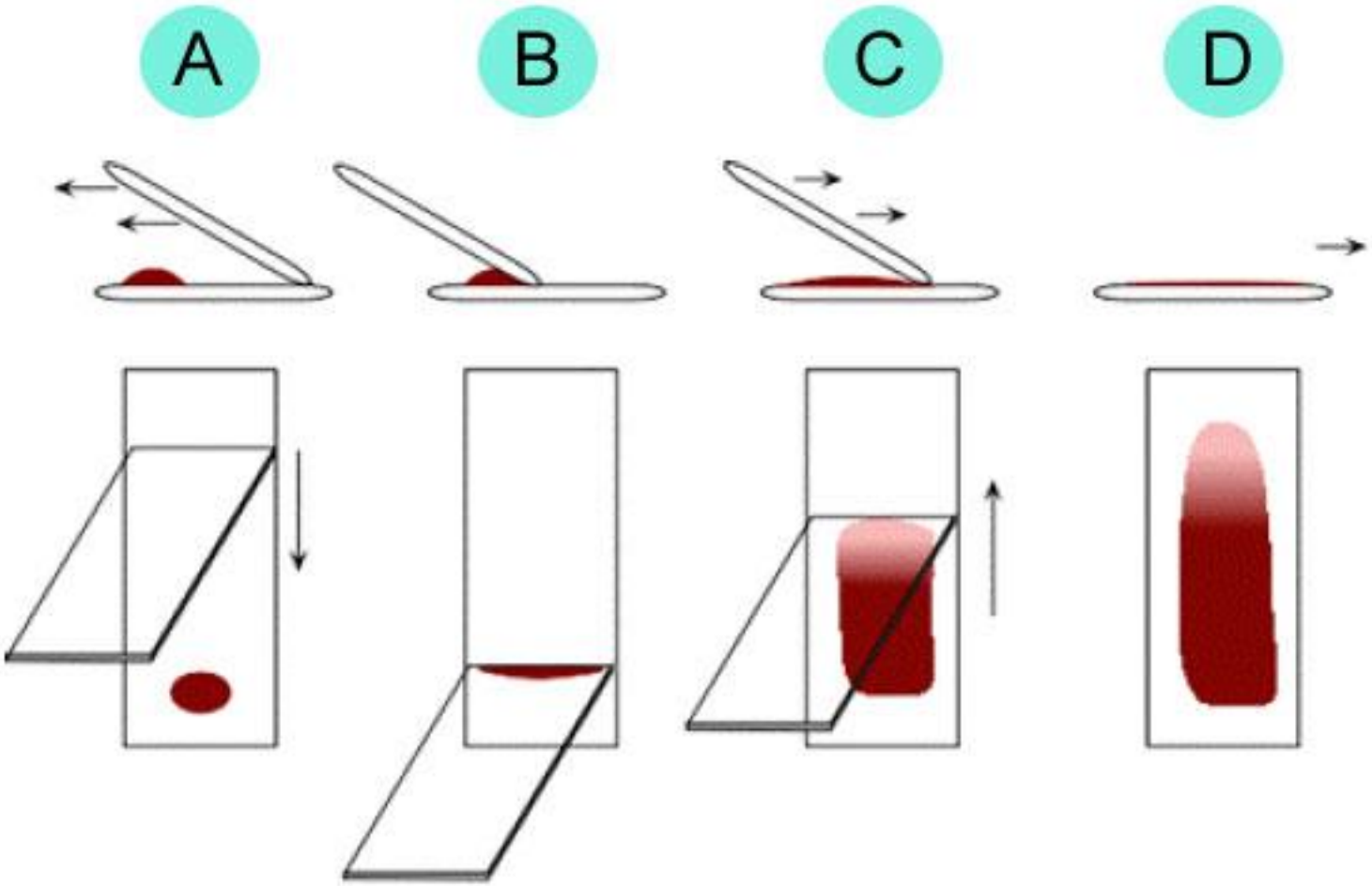
KRVNE CELICE – DOLOČANJE V VZORCU KRVI

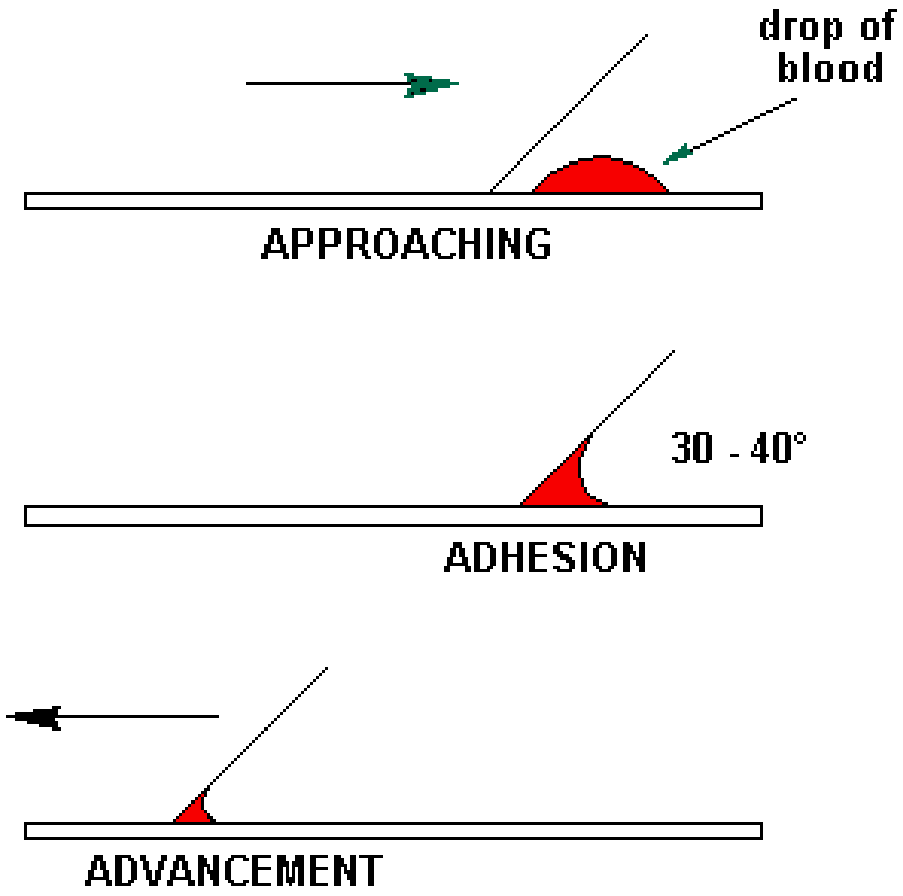
SESTAVA KRVI

- PLAZMA
- KRVNE CELICE:
  - rdeče krvničke (eritrociti) -RBC
  - bele krvničke (levkociti)
  - krvne ploščice (trombociti)

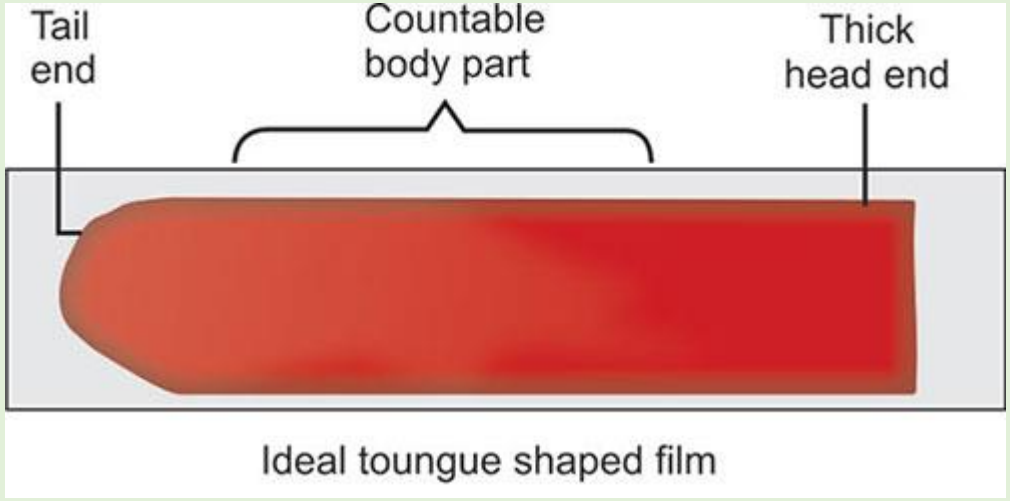


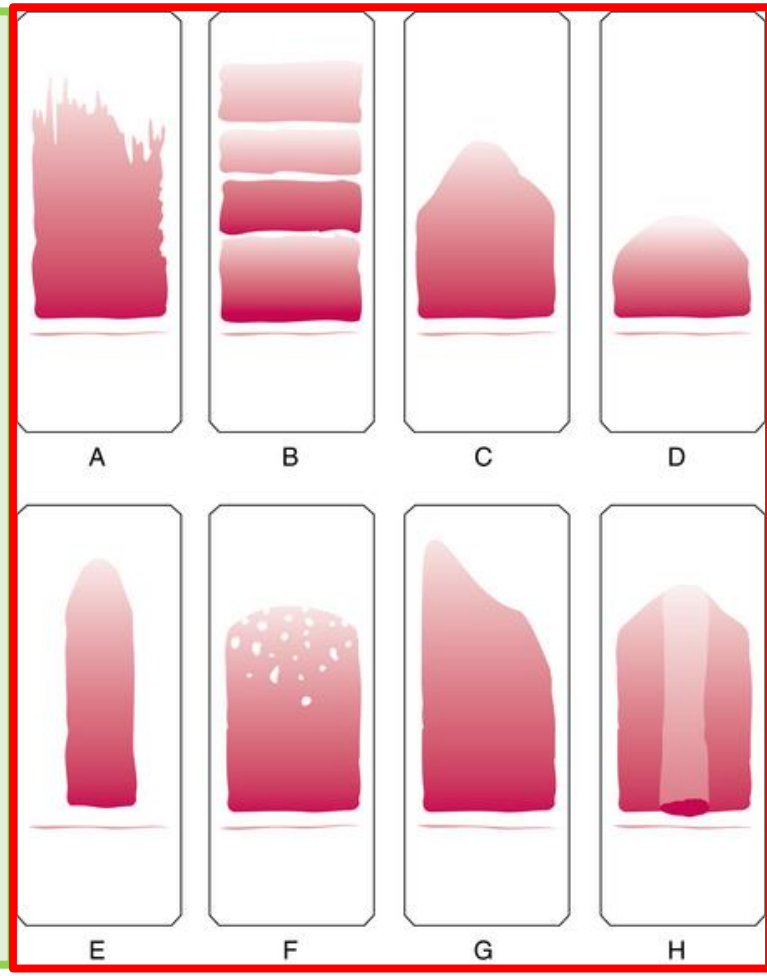
PRIPRAVA MIKROSKOPSKIH PREPARATOV




drop of
blood
APPROACHING
30 - 40°
ADHESION
ADVANCEMENT

- tanek, enakomeren, ravni stranski robovi
- pokriva naj približno 2/4 površine v sredini objektnega stekla, ne sme segati do roba objektnega stekla
- na koncu mora biti zaobljen






Baso
Diff-Quik Fix
Diff-Quik Stain
Baso
Diff-Quik I
Baso
Diff-Quik II

POSTOPEK BARVANJA

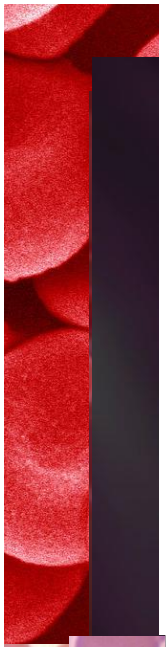
- **3 tekočine:**
- Fiksativ (metanol)
- Stain solution 1 (Eosin G in phosphate buffer) - rdeča
- Stain solution 2 (Thiazine dye in phosphate buffer) - modra

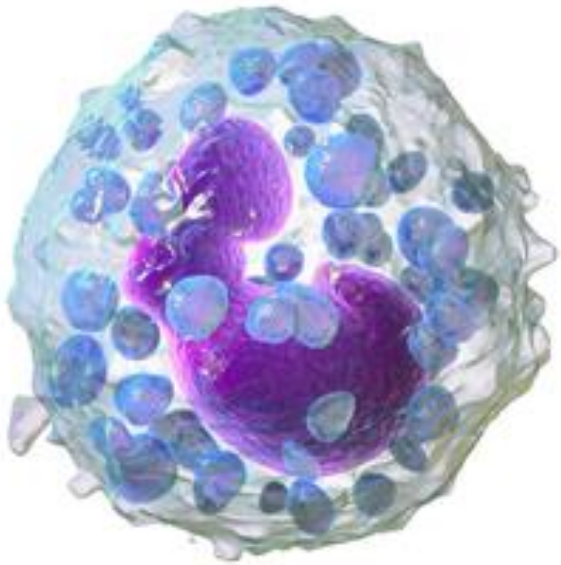
Metoda barvanja

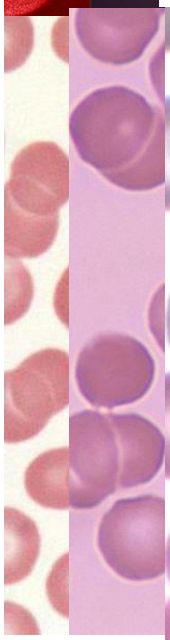
- posušite preparate
- potopite preparat 5x po 1s v fiksativ → odcedite na brisački
- potopite v barvo I – 5x počasi → odcedite na brisački
- potopite v barvo II – 3x počasi → odcedite na bisački
- potopite v vodo
  - dokler ne teče več modra barva
- posušite

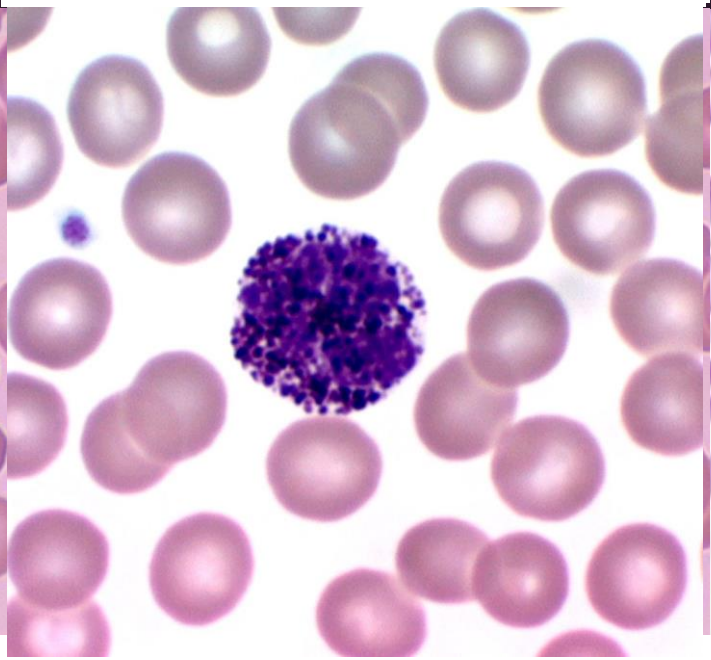
KRVNE CELICE

- ERITROCITI
- LEVKOCITI
  - NEZRNATI
    - monociti (ledvičasto jedro)
    - limfociti (okroglo veliko jedro)
  - ZRNATI = GRANULOCITI
    - nevtrofilci (2-5 režnjev jedra)
    - eozinofilci (2 režnja, številne granule)
    - bazofilci (2-3 režnji, temne granule)
- TROMBOCITI









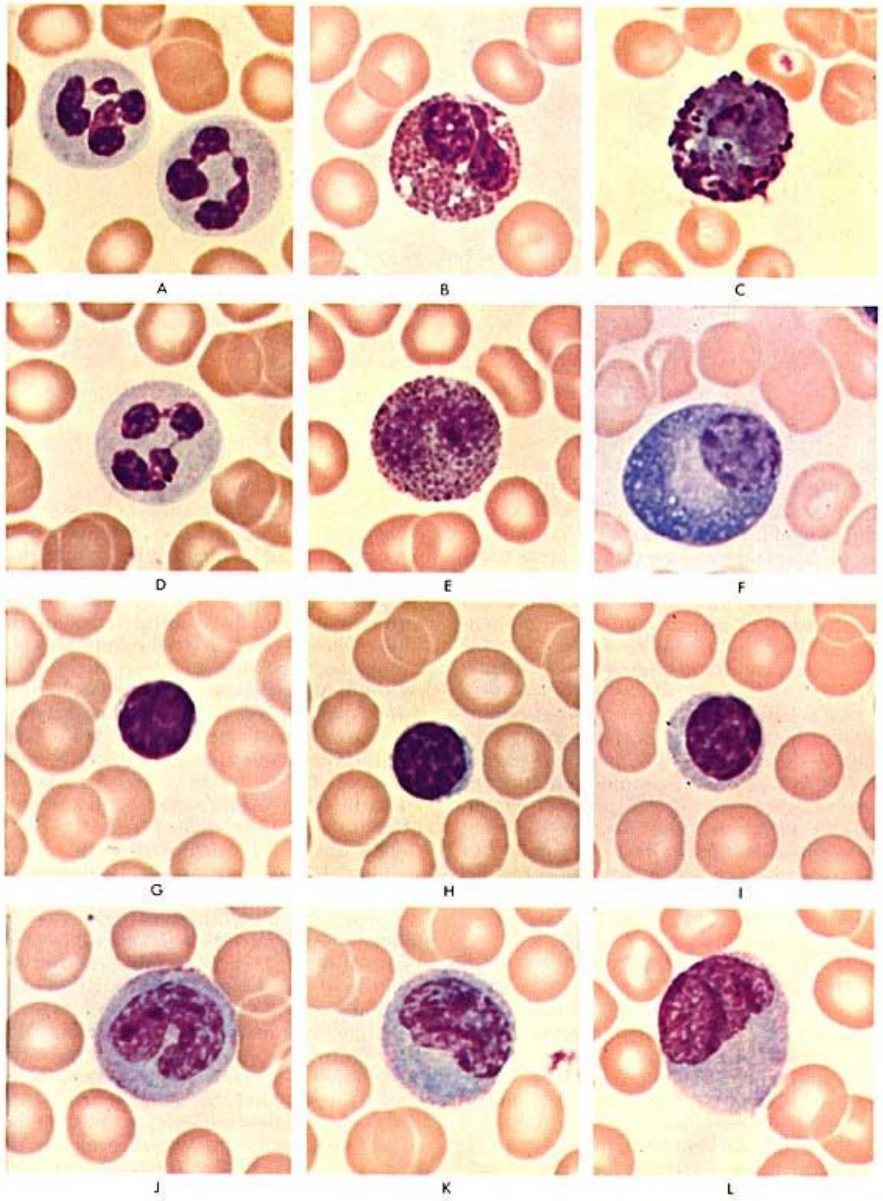
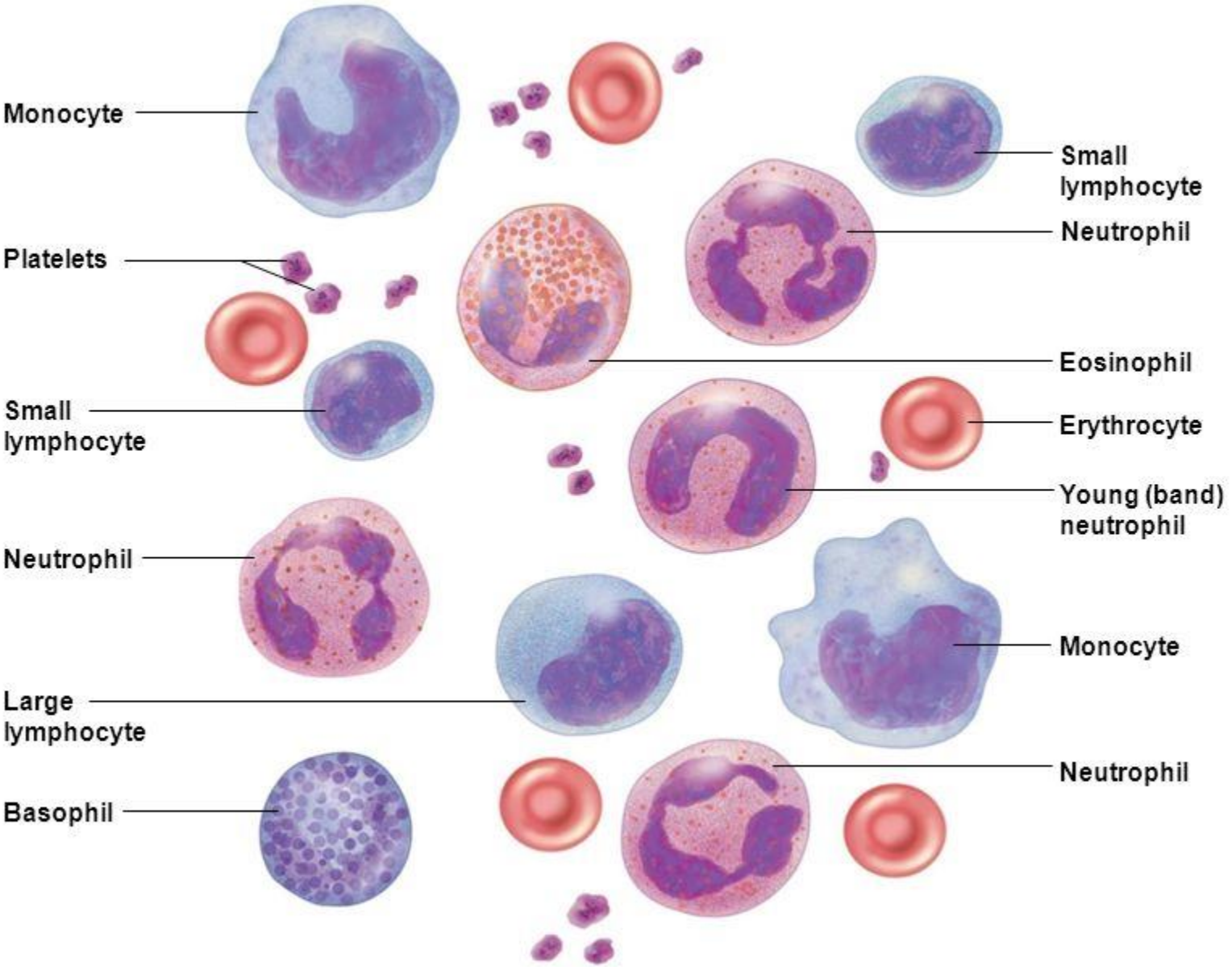
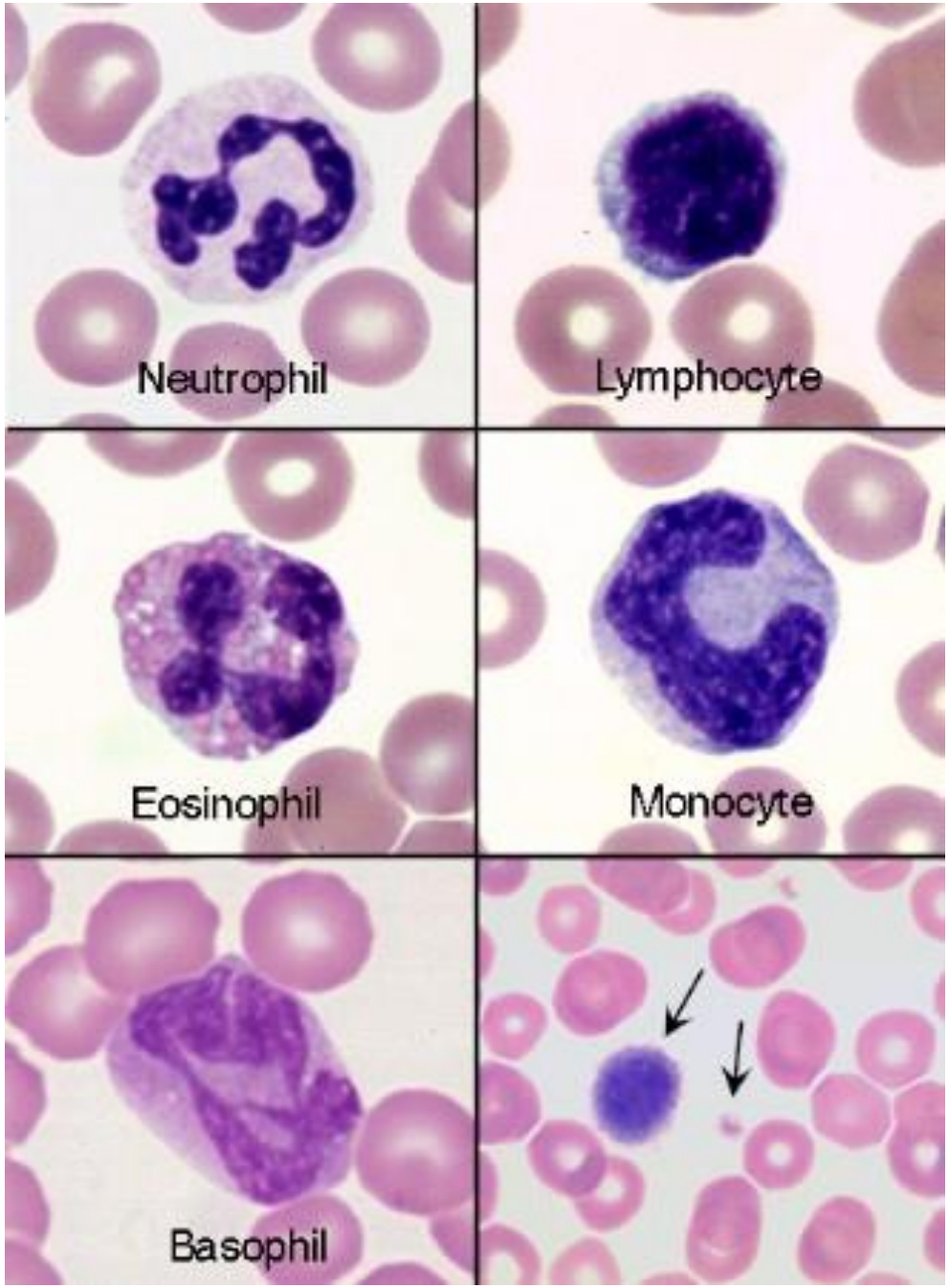


Figure 4–7. Human blood cells form a smear after Wright's stain. *A* and *D*, Neutrophilic leukocytes. *B* and *E*, Eosinophilic leukocytes. *C*, Basophilic leukocyte. *F*, Plasma cell; this is not a normal constituent of the peripheral blood but is included here for comparison with the nongranular leukocytes. *G* and *H*, Small lymphocytes. *I*, Medium lymphocytes. *J*, *K*, and *L*, Monocytes.

- A,D – nevtrofilci
- B, E – eozinofilci
- C – bazofilci
- J, K, L - monociti

Fig. 18.1




Neutrophil
Lymphocyte
Eosinophil
Monocyte
Basophil

NALOGA – ŠTETJE LEVKOCITOV

- Preštejte 50 levkocitov in zapišite število:

- NEZRNATI
  - monociti
  - limfociti
- ZRNATI
  - eozinofilci
  - nevtrofilci
  - bazofilci

NALOGA – ŠTETJE LEVKOCITOV

- Preštejte 50 levkocitov in zapišite število:

- NEZRNATI
  - monociti – 5,3 %
  - limfociti – 30 %
- ZRNATI
  - eozinofilci – 2,3 %
  - nevtrofilci – 62 %
  - bazofilci – 0,4 %