



FIRST FACULTY OF MEDICINE
CHARLES UNIVERSITY IN PRAGUE

GENERAL UNIVERSITY
HOSPITAL IN PRAGUE

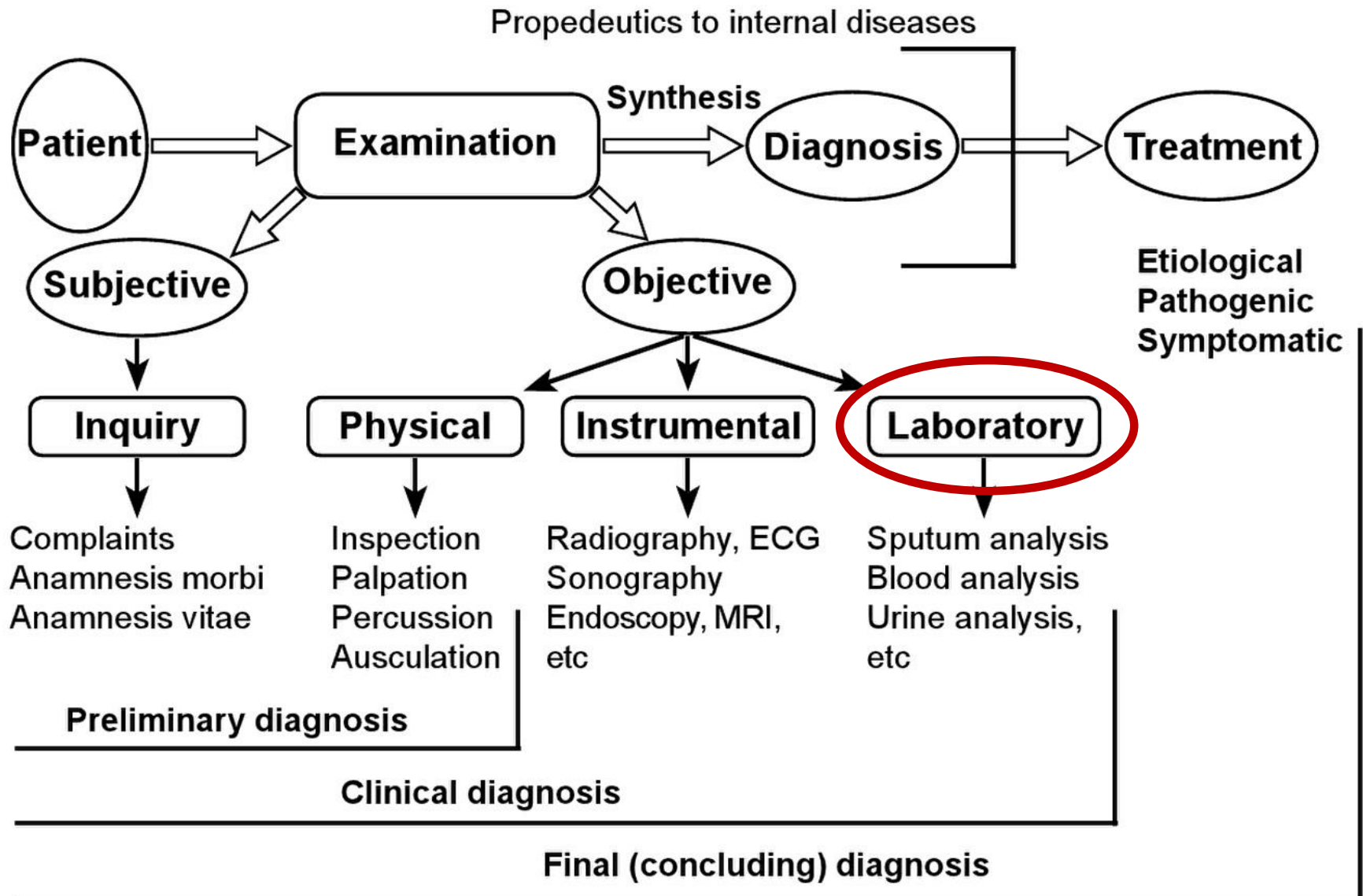


Laboratory Hematology

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and Central Hematology Laboratory

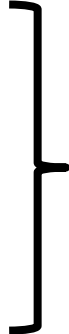
Algorithm of clinical examination



Overview

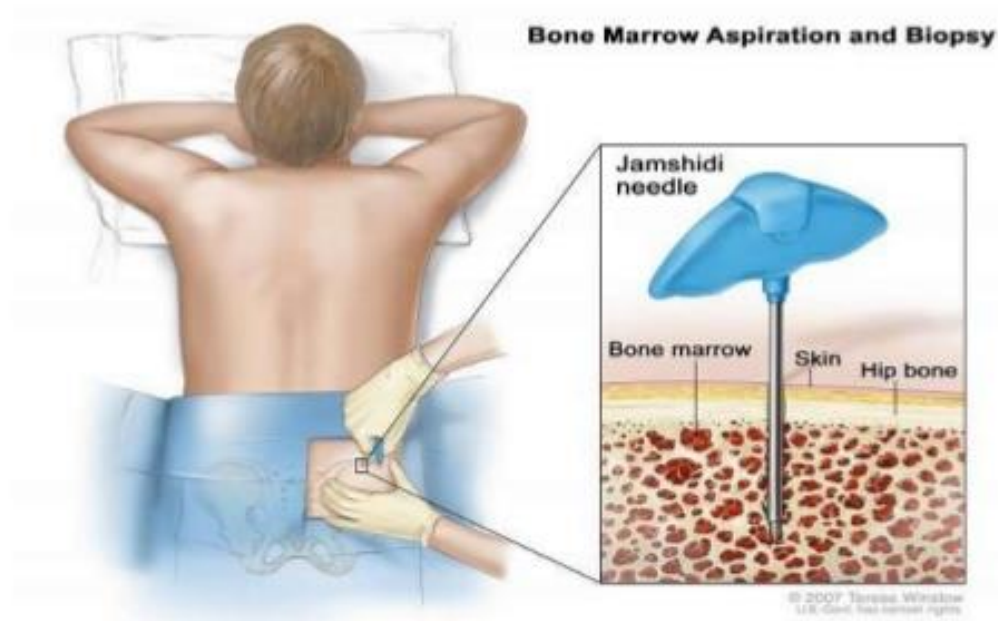
- Materials
- Full blood count + differential
- Coagulation tests
- Flow cytometry (immunophenotyping)

Materials

- Full blood (EDTA): FBC
 - Plasma (citrate): coagulation
 - Serum: biochemistry
 - Bone marrow
 - Cerebrospinal fluid
 - Lymph node
 - Other
- 
- Peripheral blood

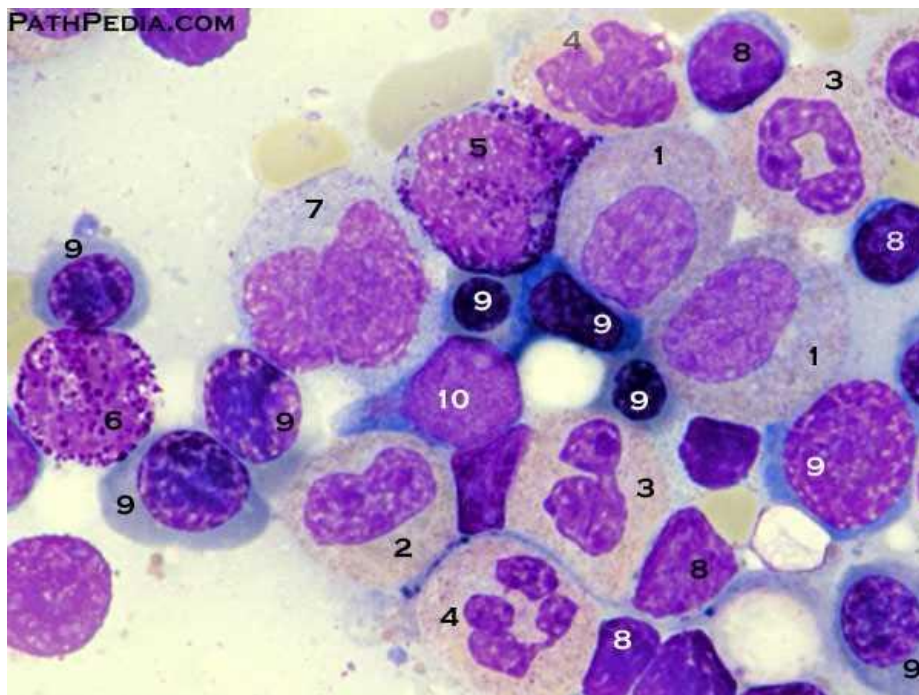
Bone marrow examination

- Aspiration: sternum
- Biopsy (trephine biopsy): iliac crest



Bone marrow examination

- Aspirate: cytology (smear)



- Trephine biopsy: histology



Overview

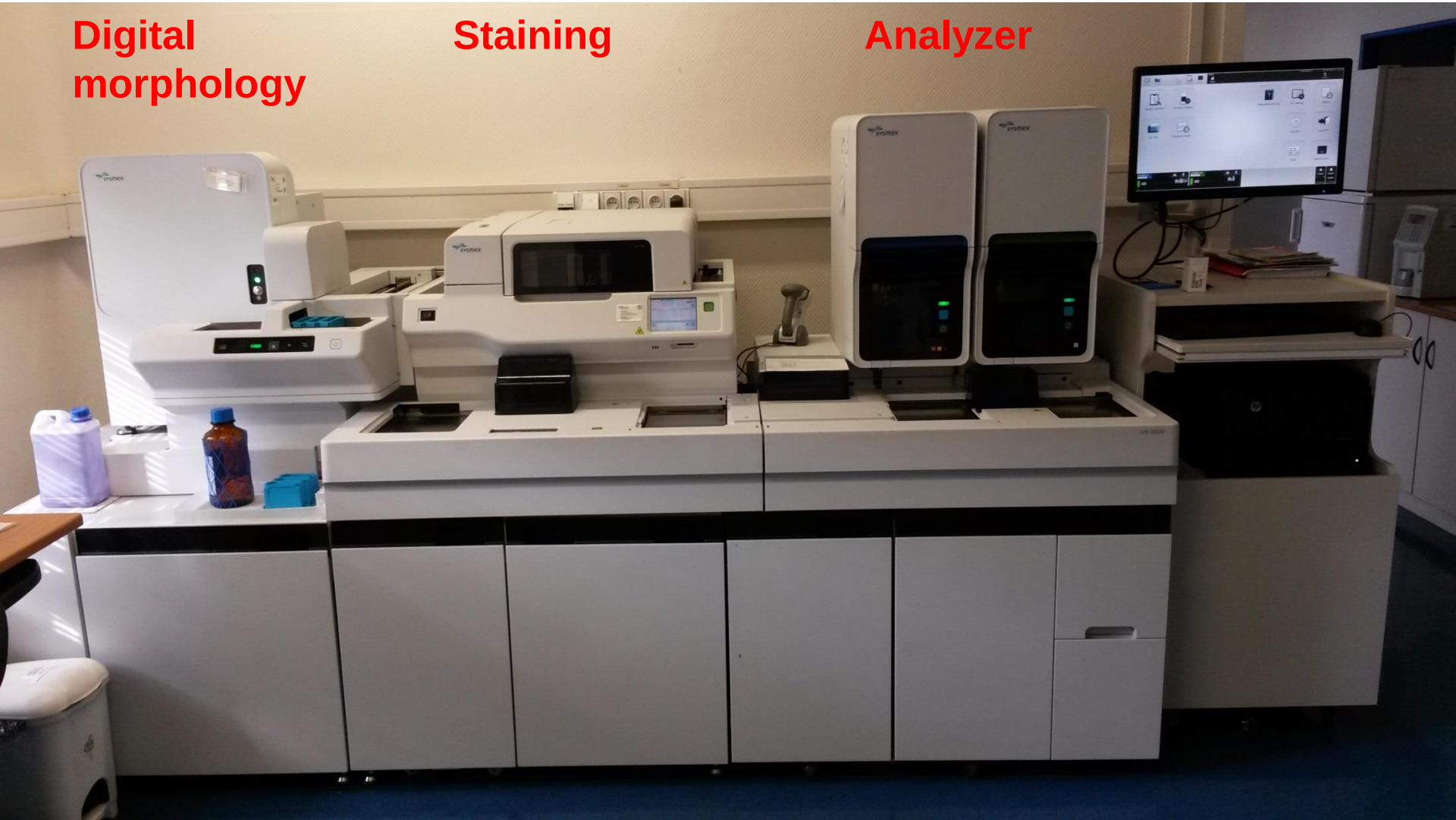
- Materials
- **Full blood count + differential**
- Coagulation tests
- Flow cytometry (immunophenotyping)

Hematology Analyzer Sysmex XN

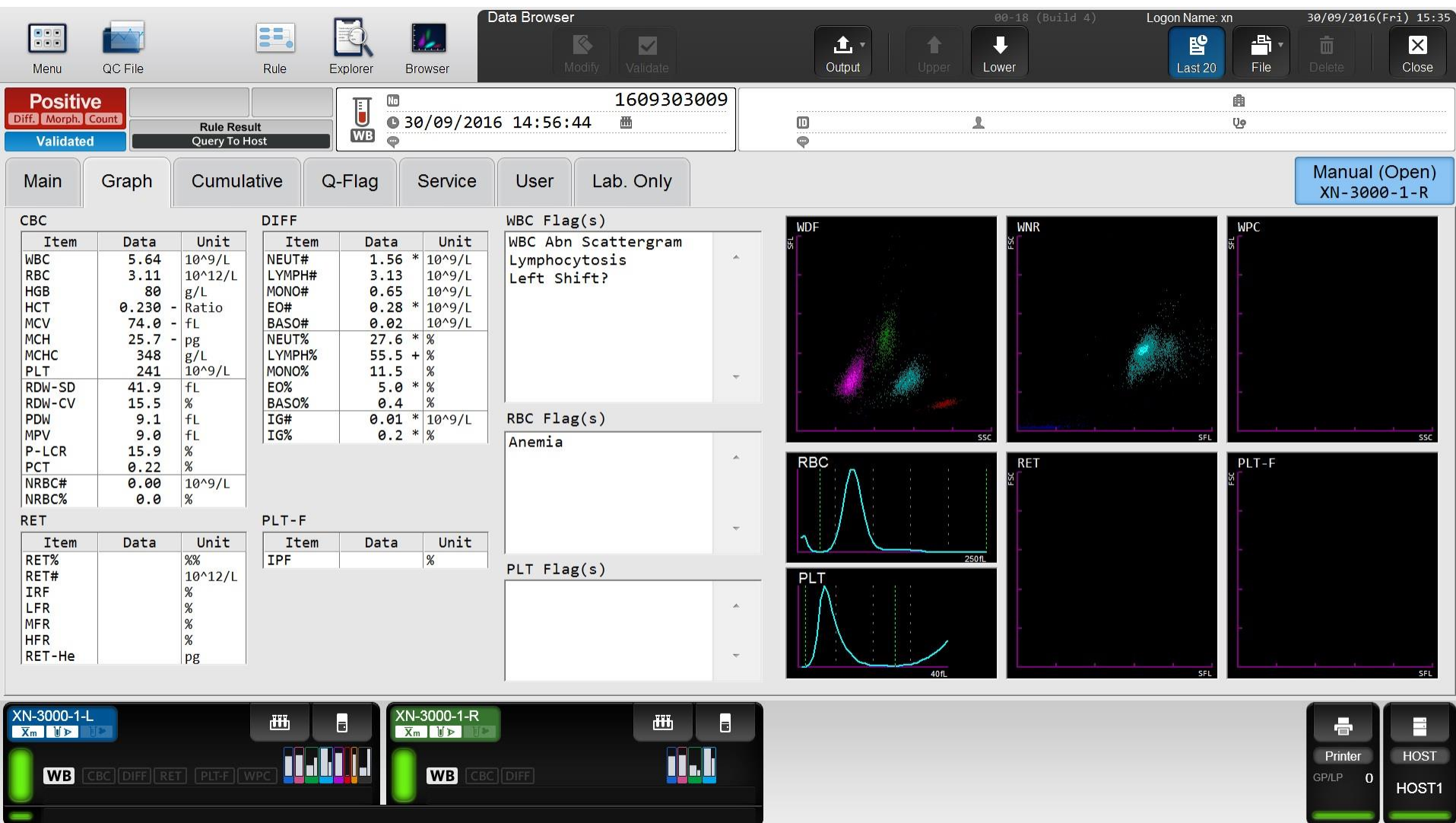
Digital
morphology

Staining

Analyzer

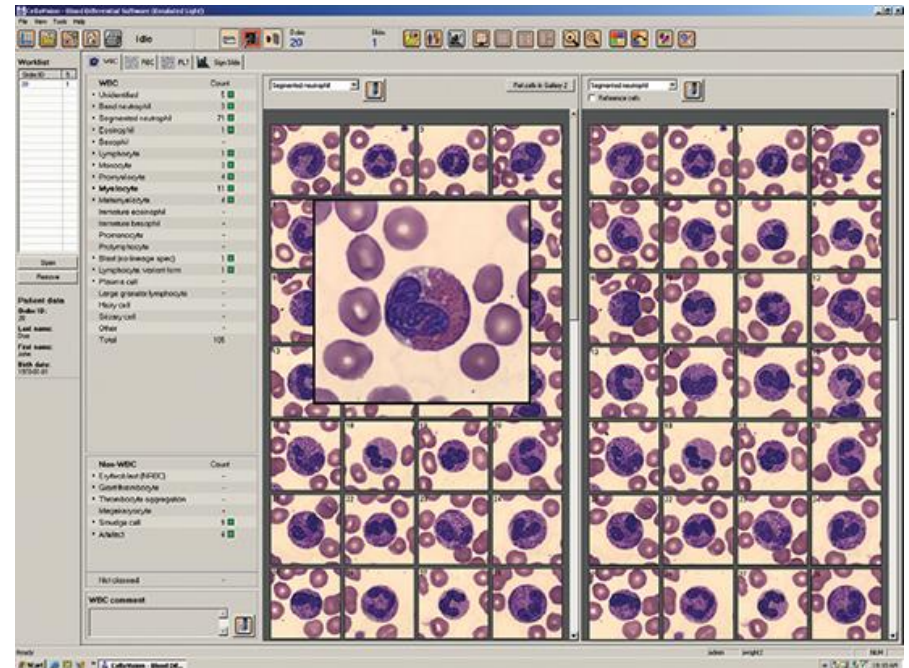
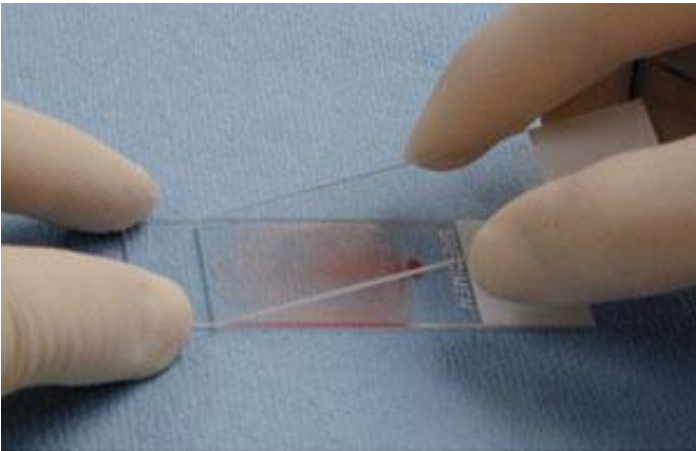


Full blood count + differential



Differential of leukocytes

- Hematology analyzer
- Microscopic differential (= blood smear)
 - Manual
 - Automatic (digital morphology)



FBC + Diff + Rtc

- **Full blood count**
 - Leukocytes: WBC
 - Erythrocytes: RBC, HGB, HCT, MCV, MCH, MCHC, RDW
 - Platelets: PLT, MPV, PCT, PDW; *IPF*
 - *NRBC: normoblasts*
- **FBC + Differential of leukocytes**
 - Neutrophils, lymphocytes, monocytes, eosinophils, basophils, *IG*
 - Manual (microscopic) differential
- **Reticulocytes**
 - absolute number and percentage

FBC – New parameters

- **IPF** – Immature Platelet Fraction
- **NRBC** – Nucleated Red Blood Cell
- **IG** – Immature Granulocytes

ITP

- **IPF** – Immature Platelet Fraction
– ↑ ITP, HIT, DIC...
- 34-year-old woman
- GP > dermatology > internal dpt
- Petechia

		Krevní obraz-perifer	
10 ⁹ /l	4,00..10,00	Leukocyty WBC	4,54
10 ¹² /l	3,80..5,20	Erytrocyty RBC	4,59
g/l	120..160	Hemoglobin HGB	134
l	0,350..0,470	Hematokrit HCT	0,380
fl	82,0..98,0	Stř.obj.ery MCV	82,8
pg	28,0..34,0	Stř.mn.hem.v ery MCH	29,2
g/l	320..360	Stř.konc.hem. v ery MCH	353
%	10,0..15,2	Distr.křiv.ery RDW	12,3
10 ⁹ /l	150..400	Trombocyty PLT	2
%	0,8..6,3	Mladé frakce trombocytů IPF	34,4
fl	7,8..11,0	Stř.obj.trombo MPV	<u>Txt+His</u>
%	0,120..0,350	Tromb.hematokrit PCT	<u>Txt+His</u>
fl	9,0..17,0	Distr.křiv.tr. PDW	<u>Txt+His</u>
-		Shluky PLT	
		Dif.stroj. relativní	
%	45,0..70,0	Neutrofily NE	49,8
%	20,0..45,0	Lymfocyty LY	46,5
%	2,0..12,0	Monocyty MO	3,3
%	0,0..5,0	Eozinofily EO	0,0
%	0,0..2,0	Bazofily BA	0,4
		Dif.stroj. absolutní	
10 ⁹ /l	2,00..7,00	Neutrofily abs. NE	2,26
10 ⁹ /l	0,80..4,00	Lymfocyty abs. LY	2,11
10 ⁹ /l	0,08..1,20	Monocyty abs. MO	0,15
10 ⁹ /l	0,00..0,50	Eozinofily abs. EO	0,00
10 ⁹ /l	0,00..0,20	Bazofily abs. BA	0,02
		Diferenciál manuální	
		<+>	
		Ostatní hematologie-	
%	0,0..0,6	Nezralé granulocyty	0,2
10 ⁹ /l	0,00..0,09	Nezralé granulocyty abs.	0,01
-	0,00..0,00	Normoblasty / 100 L	0,00
10 ⁹ /l	0,00..0,00	Normoblasty abs. strojov	0,00
%	5..25	RTC stroj.	10
10 ¹² /l	0,025..0,100	RTC abs. stroj.	0,044
pg	28,0..38,0	Koncentrace hem. v RET	29,2

NRBC (Nucleated Red Blood Cell)

- Erythroblasts = normoblasts = immature red blood cells
- NRBC → reticulocyte → erythrocyte
- Normal value = 0
 - ↑ hemolytic anemia (thalassemia, sickle cell disease, AIHA)
 - ↑ hematological malignancies (leucoerythroblastic blood film)

IG (Immature Granulocytes)

- Promyelocytes, myelocytes, metamyelocytes
- Normal range:
 - IG # $0,00 - 0,09 \times 10^9 / l$
 - IG % $0,00 - 0,60 \%$
- $\uparrow$ = ,deeper' left shift, infection; CML



Peripheral blood film

- White cells
 - Manual differential
 - Morphology, abnormalities (toxic granulation etc.)
 - Presence of abnormal cells (blasts etc.)
- Red cells
 - Size, shape, inclusions
- Platelets
 - Number, size, shape

Terminology

- **Left shift**
 - Increase in the number of immature leukocytes in the peripheral blood
- **Leukemoid reaction**
 - Physiological response to infection
 - Marked ,left shift' (WBC $> 50 \times 10^9/L$)
- **Hiatus leucaemicus**
 - Lack of intermediate forms of leukocytes
 - Presence of blasts and mature granulocytes

Normal ranges

- **WBC:** 4-10 x 10⁹/l
- **Hgb:** men 135-175 g/l, women 120-160 g/l
 - Significant anemia: < 80 g/l
- **PLT:** 150-400 x 10⁹/l
 - Spontaneous bleeding: < 20 x 10⁹/l

Overview

- Materials
- Full blood count + differential
- **Coagulation tests**
- Flow cytometry (immunophenotyping)

Coagulation tests

- APTT (activated partial thromboplastin time)
- Prothrombin time (PT, Quick, INR)
- Thrombin time (TT)
- Fibrinogen
- Antithrombin
- D-dimer

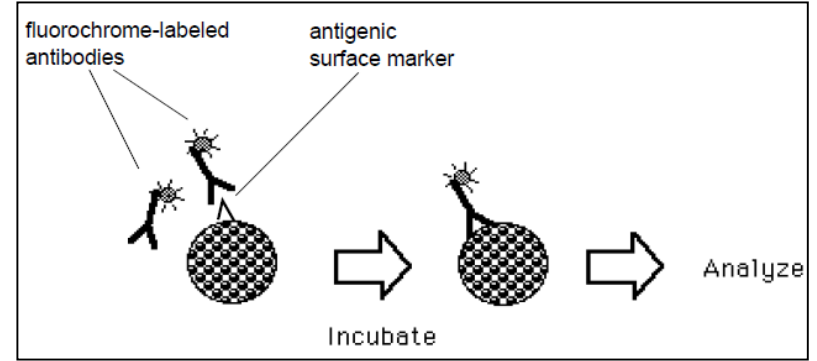
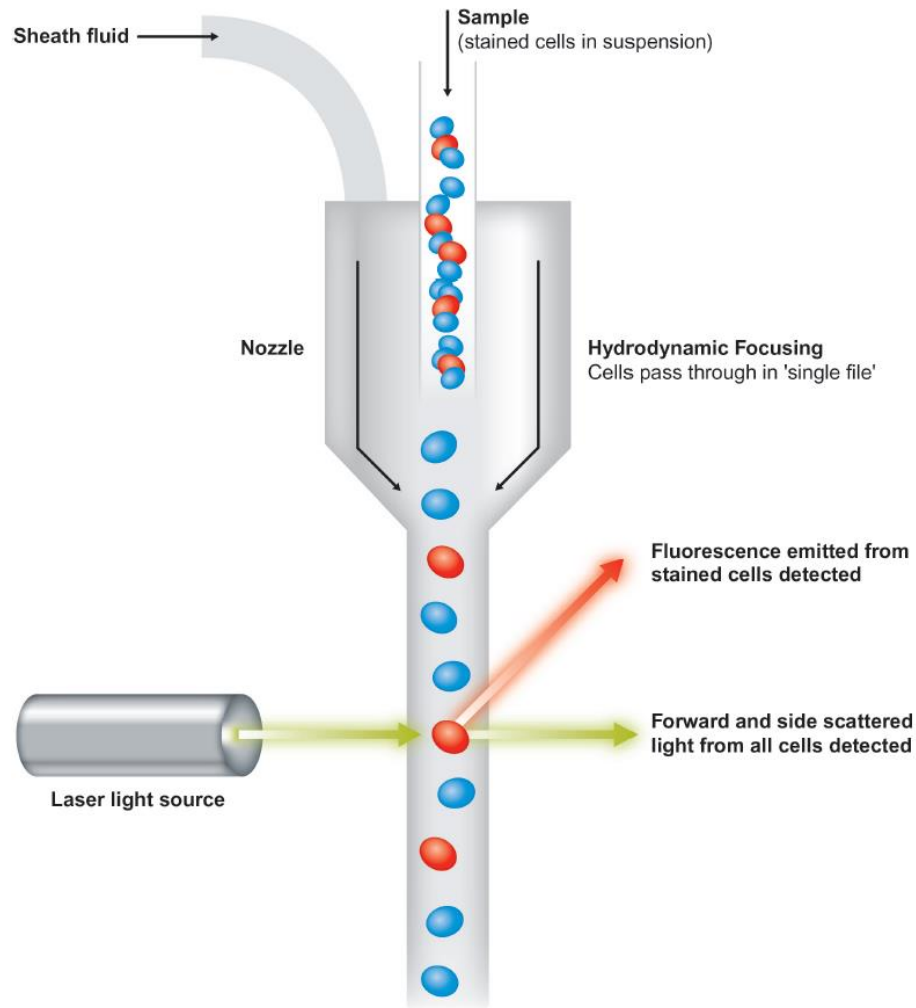
Coagulation tests

- APTT: $\uparrow$ *heparin treatment*
- Prothrombin time (PT, Quick, INR): $\uparrow$ *warfarin*
- Thrombin time (TT): $\uparrow$ *heparin*
- Fibrinogen: $\uparrow$ *inflammation*
- Antithrombin: $\downarrow$ *thrombophilia*
- D-dimer: $\uparrow$ *DVT*

Overview

- Materials
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- Coagulation tests
- **Flow cytometry (immunophenotyping)**

Immunophenotyping



Immunophenotyping

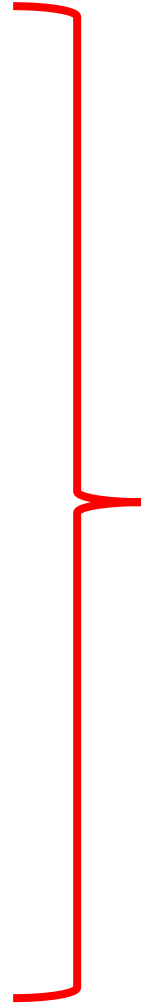
- Test used to identify cells using fluorochrome conjugated antibodies
- CD – „cluster of differentiation“
- Samples
 - PB, BM, lymph nodes, CSF, etc.
- Diagnosis
- MRD monitoring

Immunophenotyping

- Hemato-Oncology
 - **Lymphoproliferative disease (lymphocytosis)**
 - Acute leukemia
 - Multiple myeloma
 - Myelodysplastic syndrome
 - PNH (paroxysmal nocturnal hemoglobinuria)
- Hematology
 - HIT (heparin induced thrombocytopenia)
 - Fetal erythrocytes

Screening test

- CD20+CD4
- CD45
- CD8+IgL
- CD56+IgK
- CD5
- CD19
- CD3
- CD38



- **B cells (CD19+)**
 - clonality
 - development
 - CD5+
- **T cells (CD3+)**
 - CD4, CD8
 - CD5, CD38
- **NK cells (CD3-CD56+)**