Laboratory Approach to Haematological Disorders

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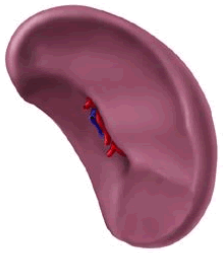
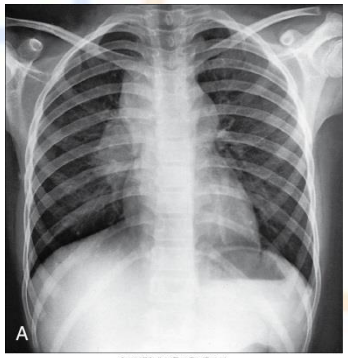
Learning Objectives

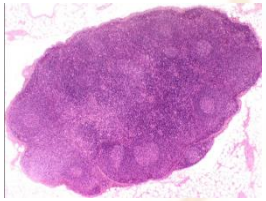
- Understand the scope of haematological disorders
- Describe laboratory tests useful for investigation
- Rationalise the use of various tests in patient management

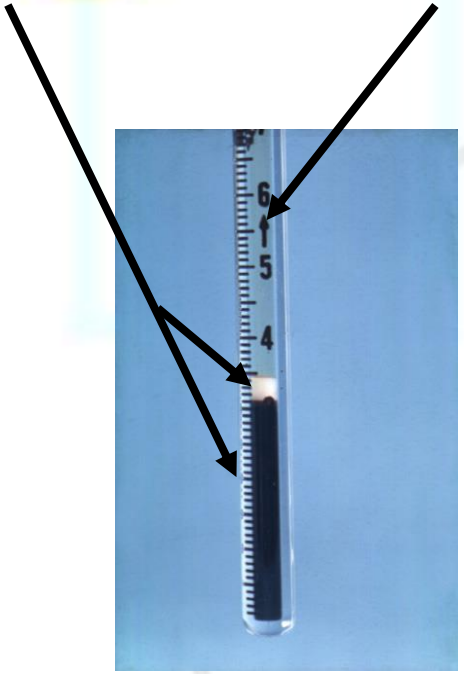
Elements of the Haematological System

- Organs
  - Bone marrow
  - Thymus
  - Spleen
  - Lymph nodes
- Cells
  - White blood cells
  - Red blood cells
  - Platelets
- Plasma
  - Coagulation factors
  - Anticoagulants









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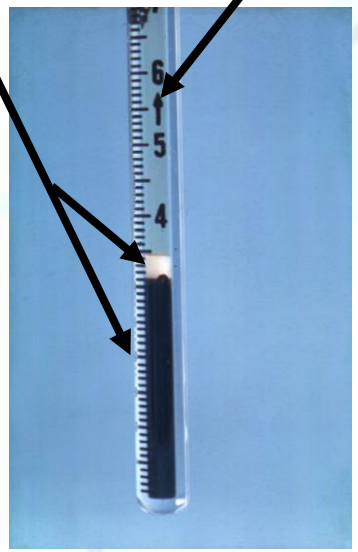

Nature of Haematological Disorders

- Hereditary vs acquired
- Benign/reactive vs malignant
- Primary vs secondary (to systemic diseases)

Laboratory Investigations for Haematological Diseases

- Aim of clinical laboratory investigation
  - Establish diagnosis, predict prognosis, guide treatment, help monitoring

Collect Date :	05/04/03
Collect Time :	--:--
Receive Date :	05/04/03
Receive Time :	18:00
Request No. :	HN021608
Urgency :	URGENT
Complete Blood Count	
WBC	8.7
RBC	2.96 L
HGB	8.3 L
HCT	0.248 L
MCV	83.8
MCH	27.9
MCHC	33.3
RDW	13.5
PLT	218
MPV	9.8
Film Review	N

Basic Principles of Clinical Laboratory Investigation

- Simple fast screening to complex confirmatory tests
- Cheap to expensive tests
- Non-invasive to invasive tests

Blood Cell & Bone Marrow Investigations

Complete Blood Counting - The Simplest Haematology Test

- Simple, fast, cheap, non-invasive and excellent screening

- Indicate cell count abnormalities and average red cell characteristics

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- Non-specific as to the underlying diagnosis

Automated Blood Counting

What is the Cause of Anaemia?

- Get the most information from simple screening tests - Complete Blood Counting
  - Red cell indices
  - White cells and platelets
  - Reticulocyte count
  - Peripheral blood smear

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CBP Report

Collect Date :	05/04/03
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Clinical Classification of Anaemia

- By red cell size (Mean Cell Volume)

Microcytic

Iron deficiency

Thalassaemia

Normocytic

Haemolysis

Anaemia of chronic disease

Renal failure

Macrocytic

Megaloblastic anaemia

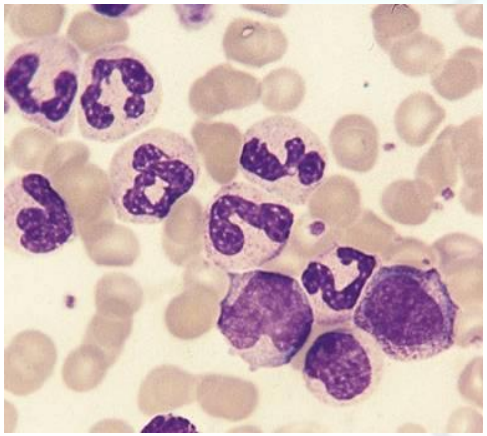
Aplasic anaemia

Myelodysplasia

Liver disease

Peripheral Blood Smear Examination - A Useful Supplement to CBC

- Confirm blood counts, assess morphology
- Provisional diagnosis
- Guide further special investigation



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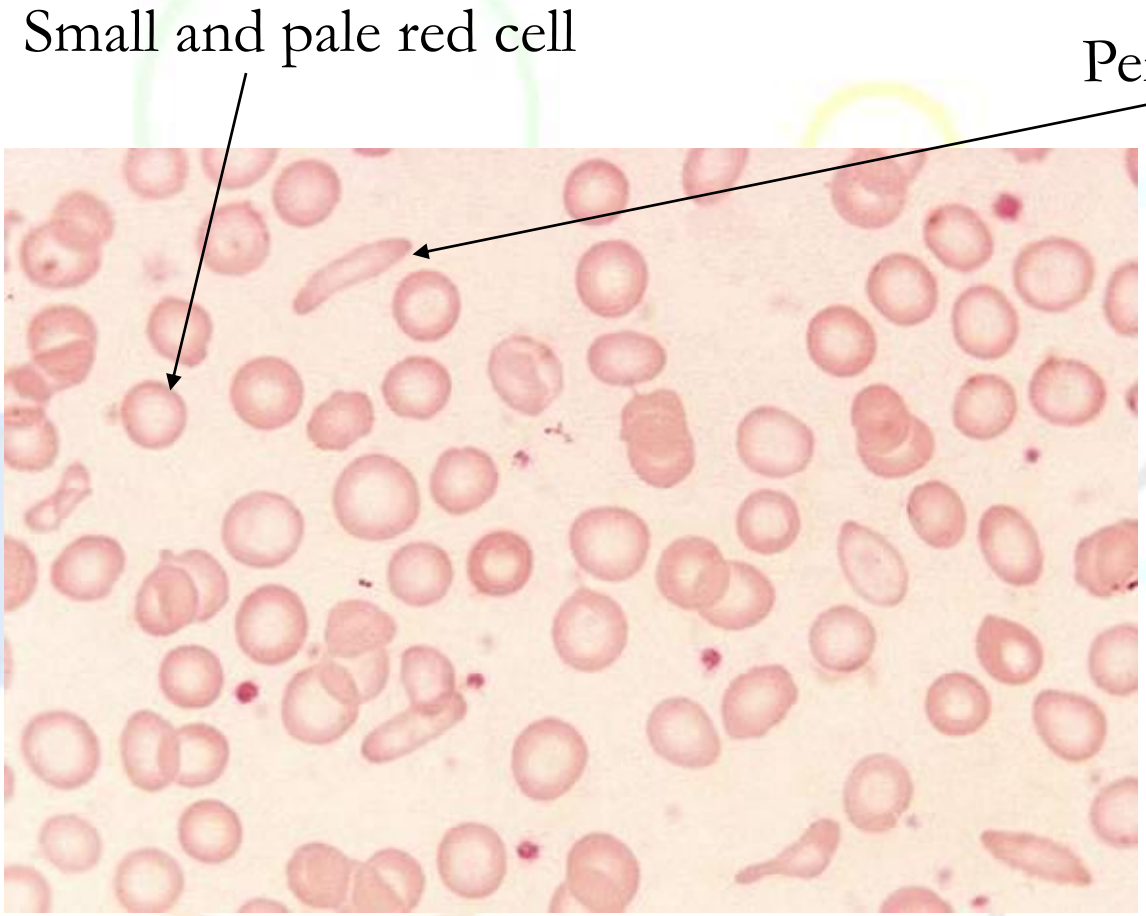
Blood Smear in Iron Deficiency Anaemia

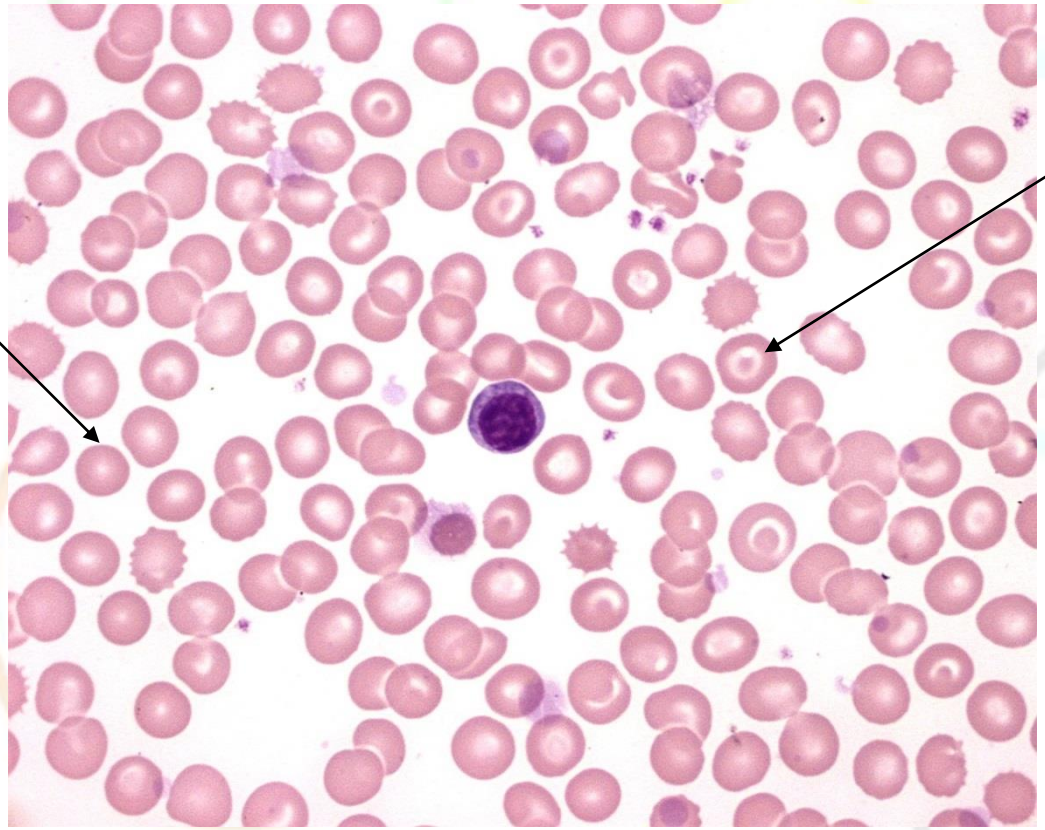


Confirmation of Iron Deficiency

Biochemical findings

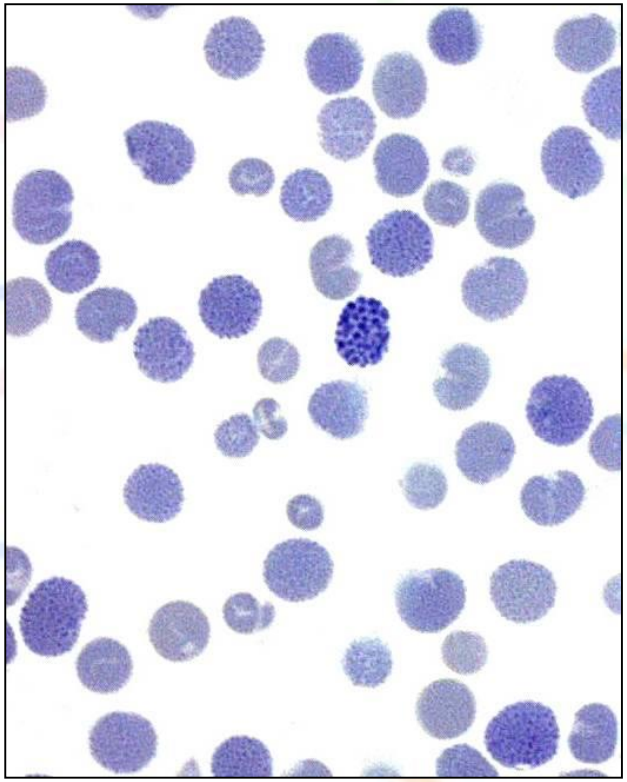
- low serum iron (usable iron)
- high serum transferrin (compensatory response aiming to increase transport)
- low transferrin saturation
- low serum ferritin (body iron storage)

Blood Smear in Thalassaemia Trait



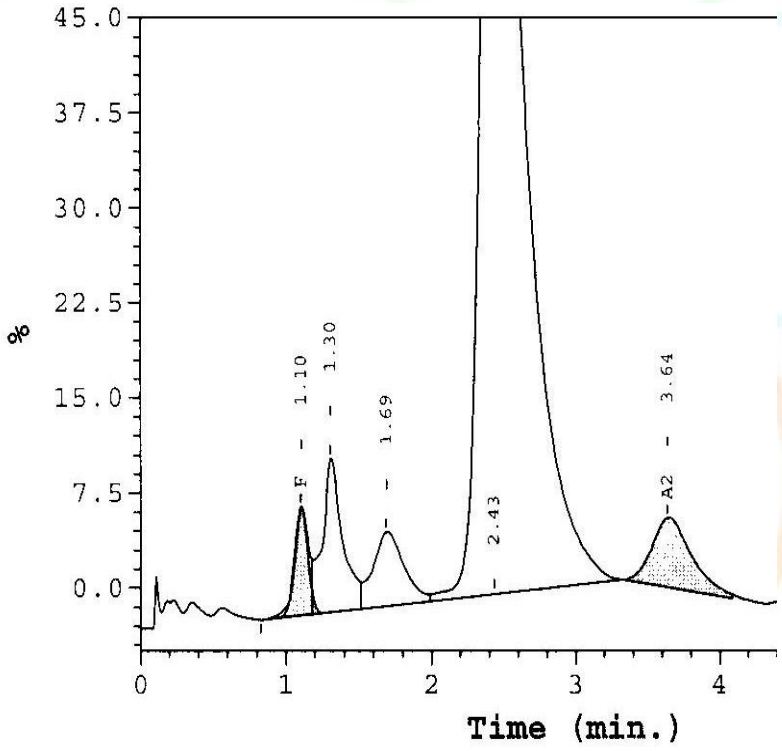
α-Thalassaemia Diagnosis

Detection of HbH (β_4) Inclusion Bodies

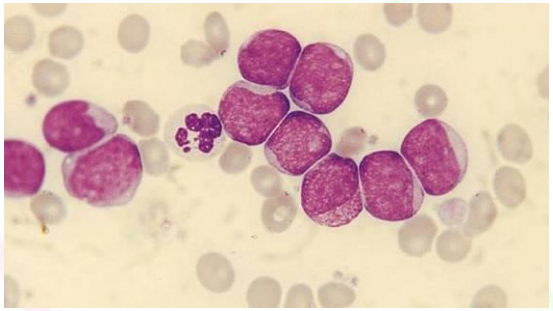


β-Thalassaemia Diagnosis

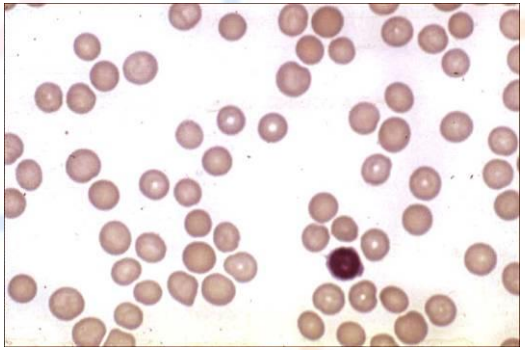
High Performance Liquid Chromatography to Measure Hb A_2



Some peripheral blood smear examination results suggest a bone marrow disease



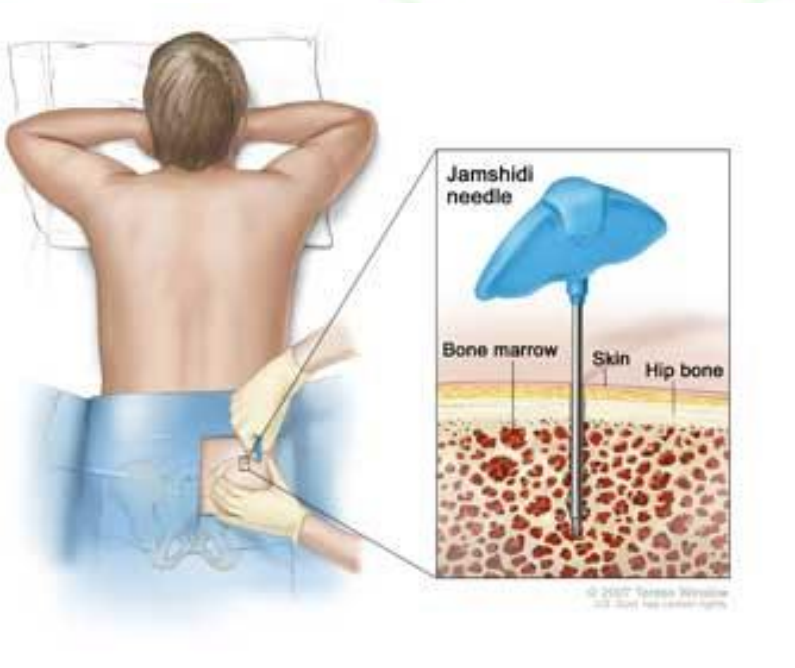
circulating blasts

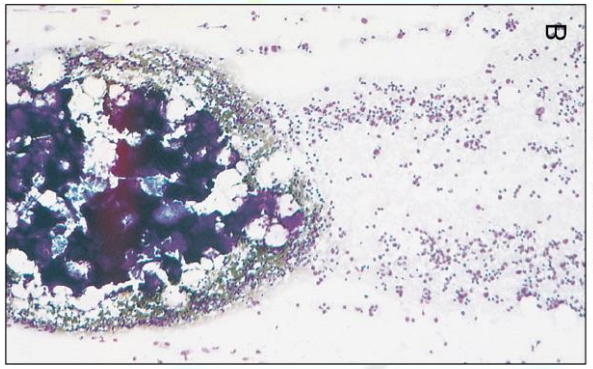


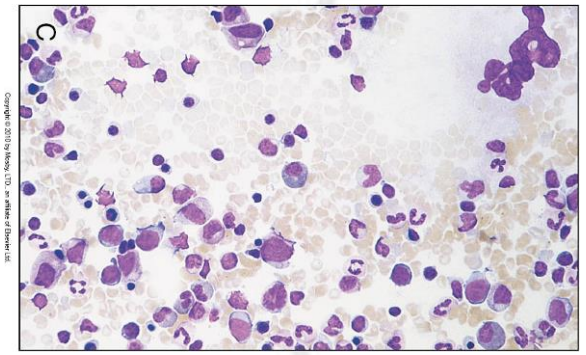
unexplained cytopenia

Bone Marrow Examination

- Invasive but safe, requires expertise in assessment





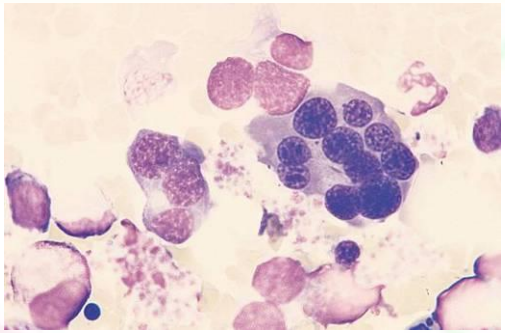


Bone Marrow Examination

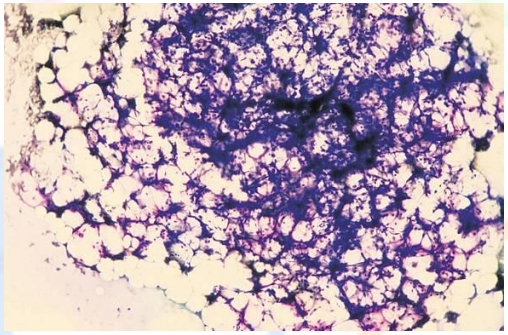
- Determine whether the marrow is the primary site of abnormality
  - Inadequate vs ineffective haemopoiesis in cytopenia

  - Presence of abnormal cells - blasts, infiltrates, dysplasia

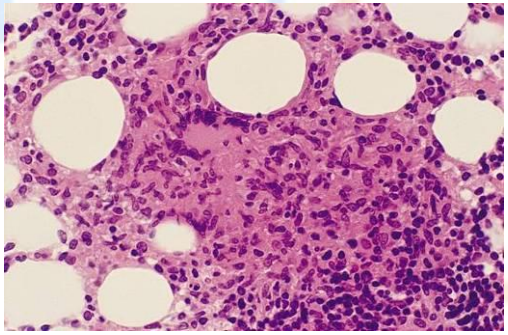
Bone Marrow Examination



congenital dyserythropoietic anaemia

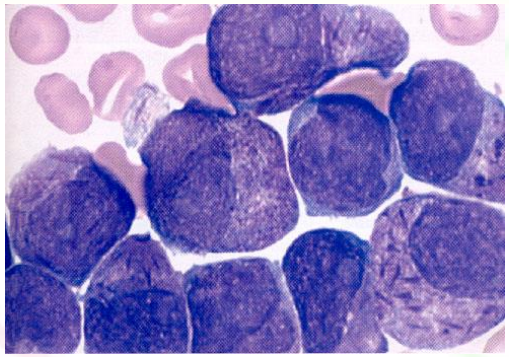


aplastic anaemia

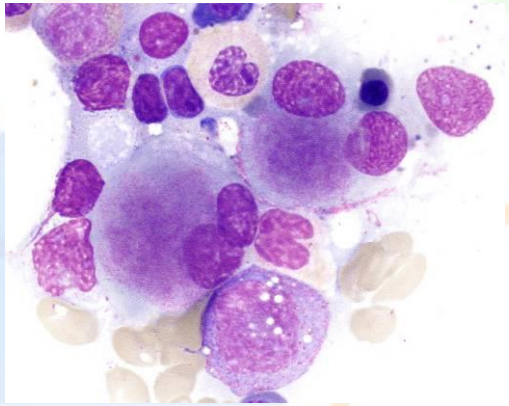


miliary tuberculosis

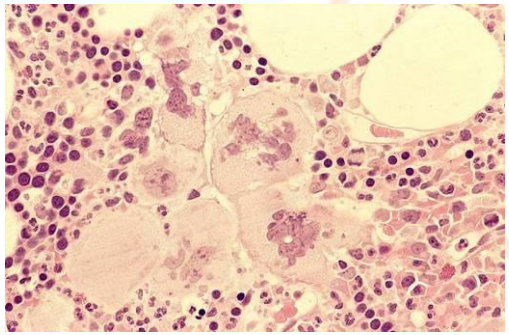
Bone Marrow Examination



acute promyelocytic leukaemia



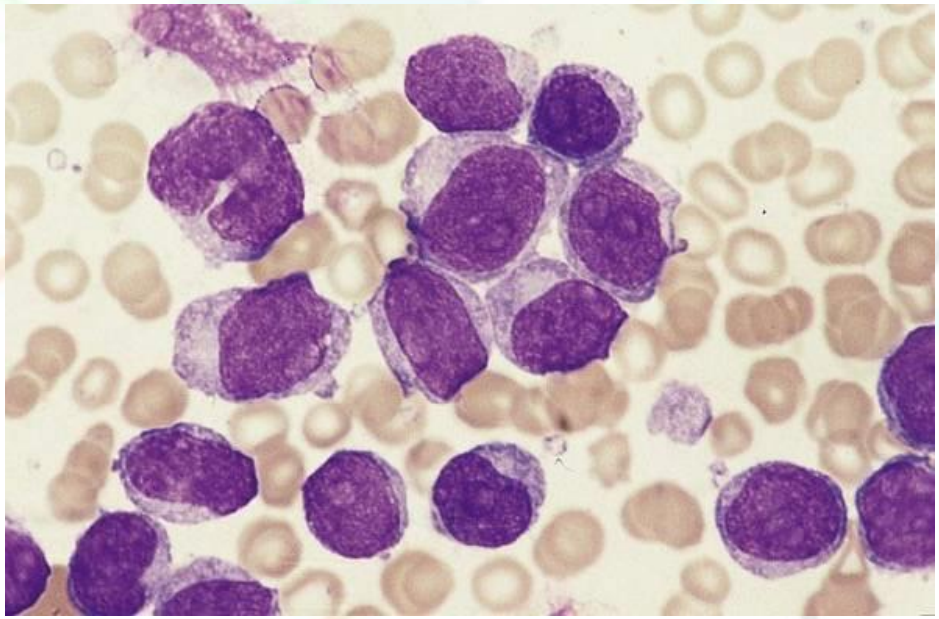
myelodysplastic syndrome



primary myelofibrosis

Bone Marrow Examination

- Provide optimal material for further special investigations in malignant cases

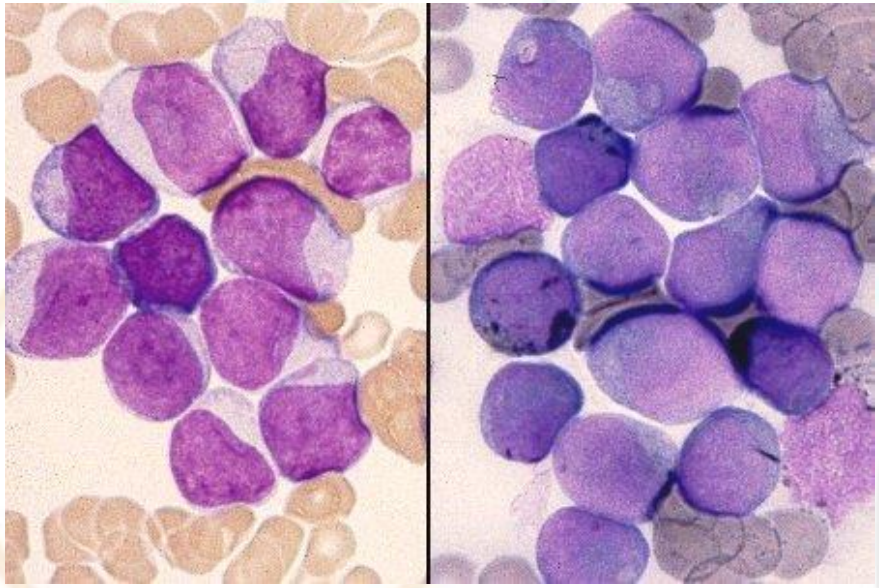


Special Investigations for Malignant Haematological Diseases

- To classify malignant disease for choice of specific treatment
  - Different myeloid types, B, T, natural killer cell
- To predict prognosis for counselling and risk-adapted therapy
- To monitor residual disease after treatment
  - Minimal residual disease (MRD) not detectable by morphology

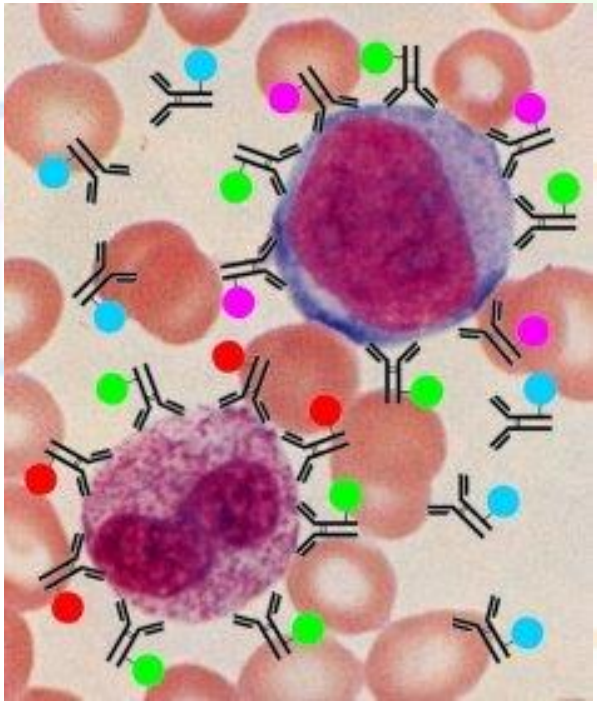
Special Investigations for Malignant Haematological Diseases

- Cytochemistry
  - Classification of acute leukaemia
  - Demonstrate enzymatic function of blasts



Special Investigations for Malignant Haematological Diseases

- Immunophenotyping
  - Classification of leukaemias
  - Using antibodies to detect lineage-associated antigens in abnormal cells



Haemopoietic precursors: CD34, Tdt

B-lineage: CD19, CD20, CD22, CD79a

T-lineage: CD3, CD4, CD5, CD8, CD7, TCR

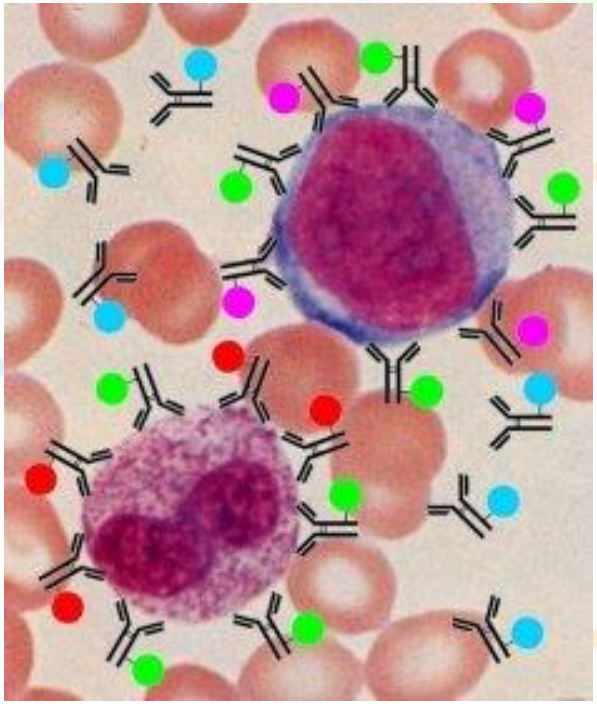
Myeloid: CD13, CD33, MPO

Megakaryocytic: CD41, CD42b, CD61

Erythroid: glycophorin A

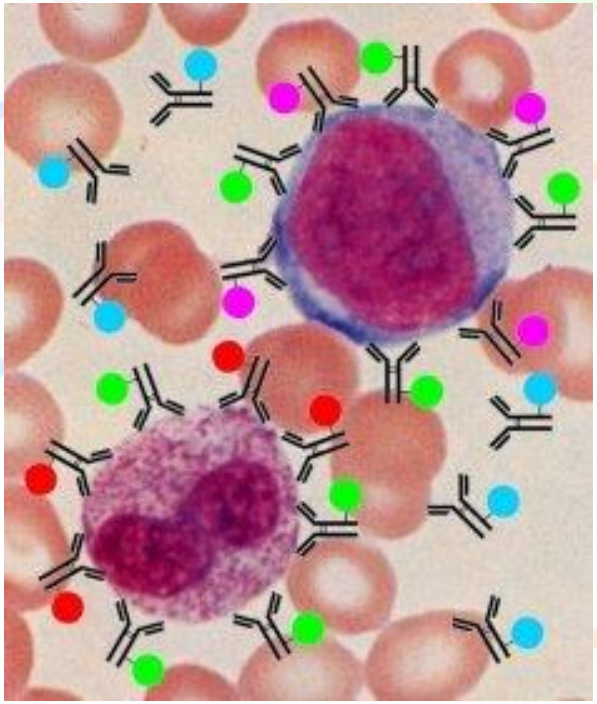
Special Investigations for Malignant Haematological Diseases

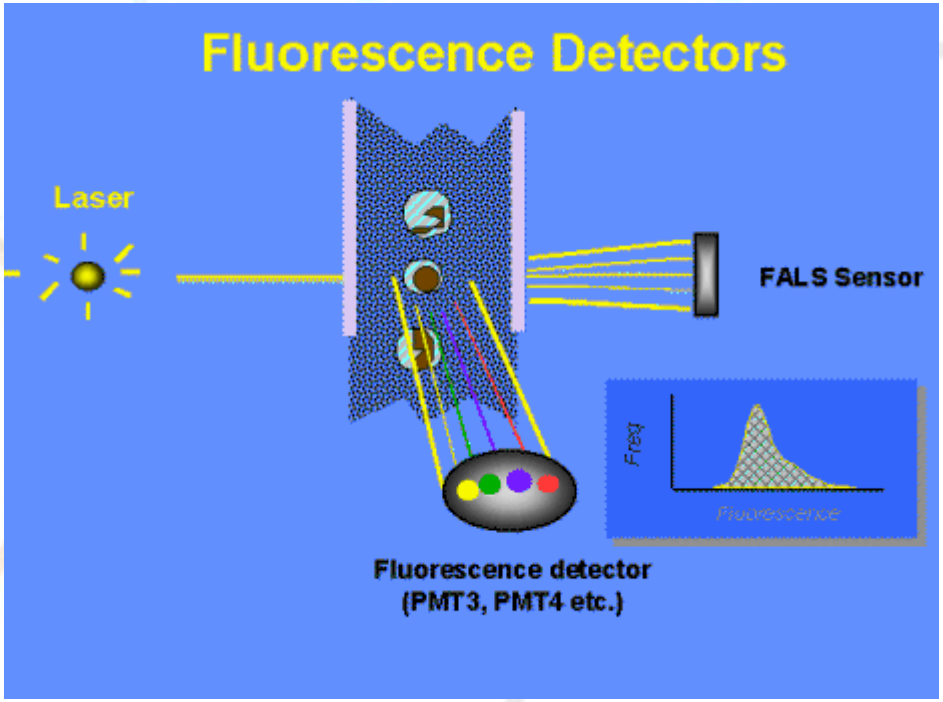
- Immunophenotyping
  - Flow cytometer - automated machine for fast and detailed immunophenotyping



Special Investigations for Malignant Haematological Diseases

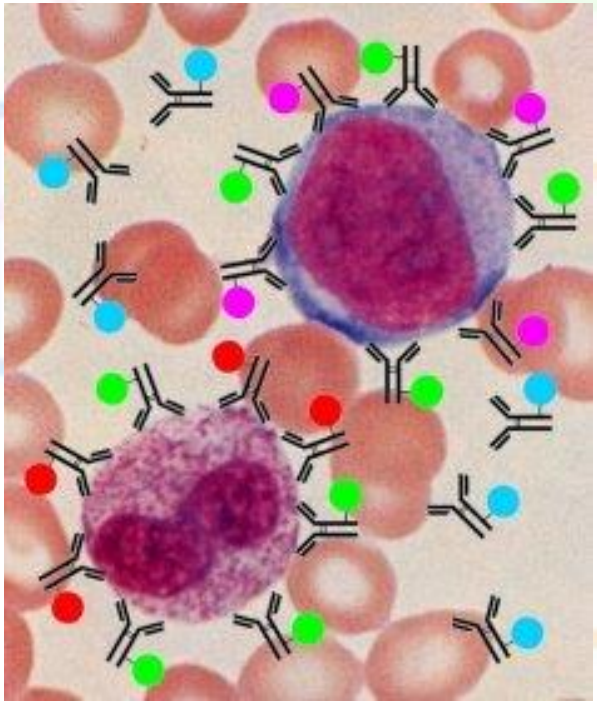
- Immunophenotyping
  - Laser light excites fluorochrome on antibodies bound to abnormal cells

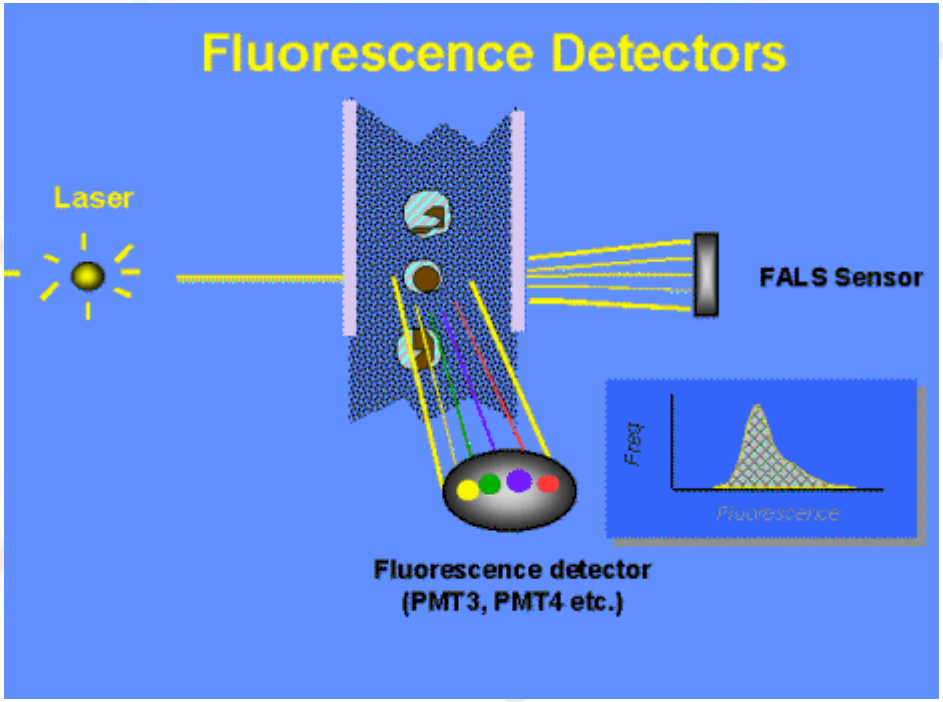




Special Investigations for Malignant Haematological Diseases

- Immunophenotyping
  - MRD monitoring if leukaemic cells have a specific immunophenotype





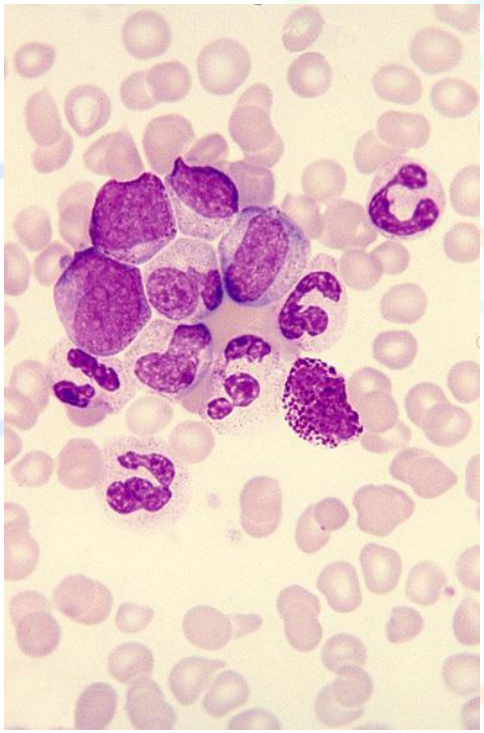
Genetic Testing for Haematological Diseases

- As confirmation for inherited haematological diseases with known genetic defects (e.g. thalassaemia)

- Malignant haematological diseases are also genetic diseases
  - Acquired mutations

Genetic Testing for Malignant Haematological Diseases

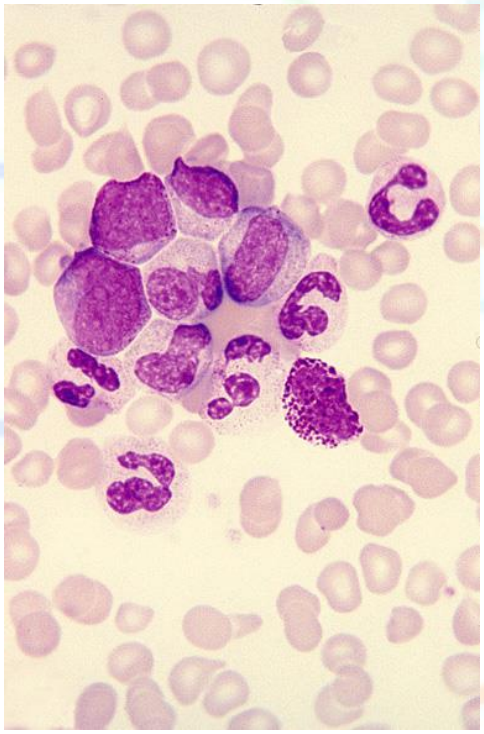
- Cytogenetic studies
  - Diagnosis and classification of leukaemias
  - Culture malignant cells and examine their chromosomes

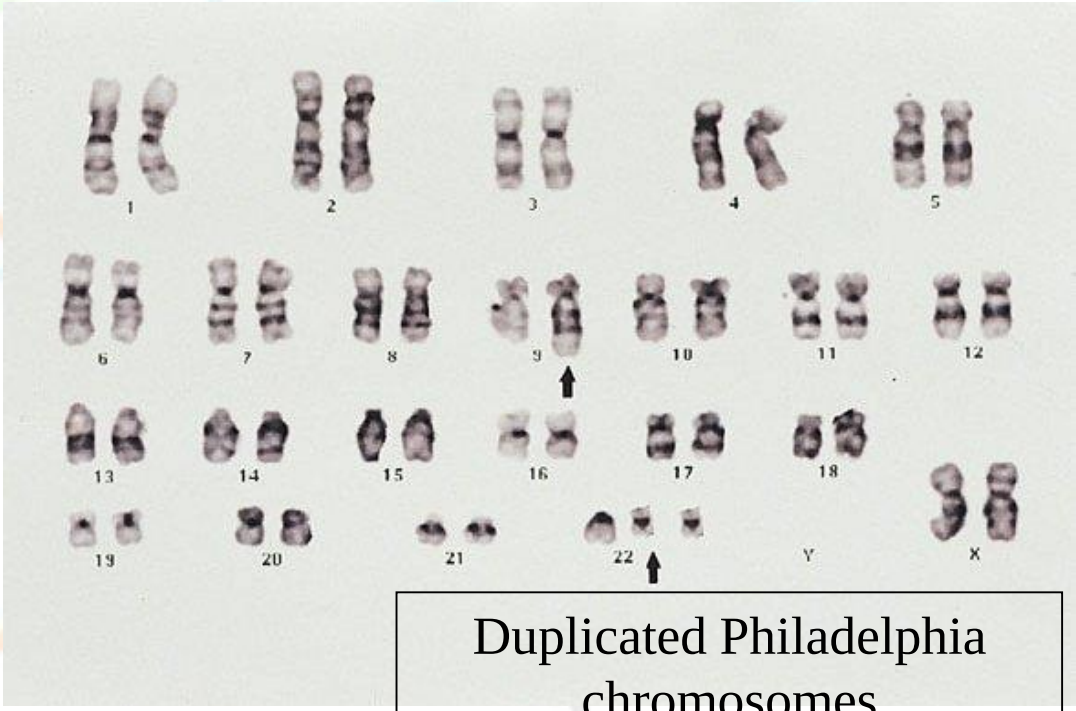


Philadelphia chromosome

Genetic Testing for Malignant Haematological Diseases

- Cytogenetic studies
  - Prediction of prognosis
  - Culture malignant cells and examine their chromosomes

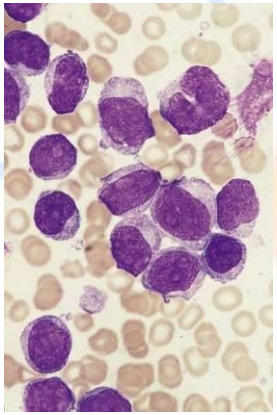


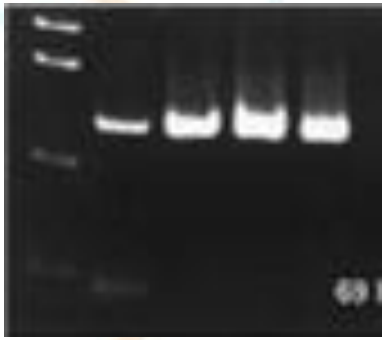


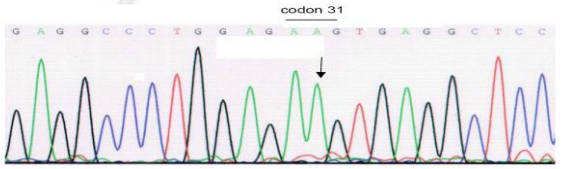
Duplicated Philadelphia chromosomes

Genetic Testing for Malignant Haematological Diseases

- Molecular genetic tests
  - Classification, prediction of prognosis and MRD monitoring
  - Use sensitive molecular techniques to determine lineage of abnormal cells, gene mutation and gene fusion

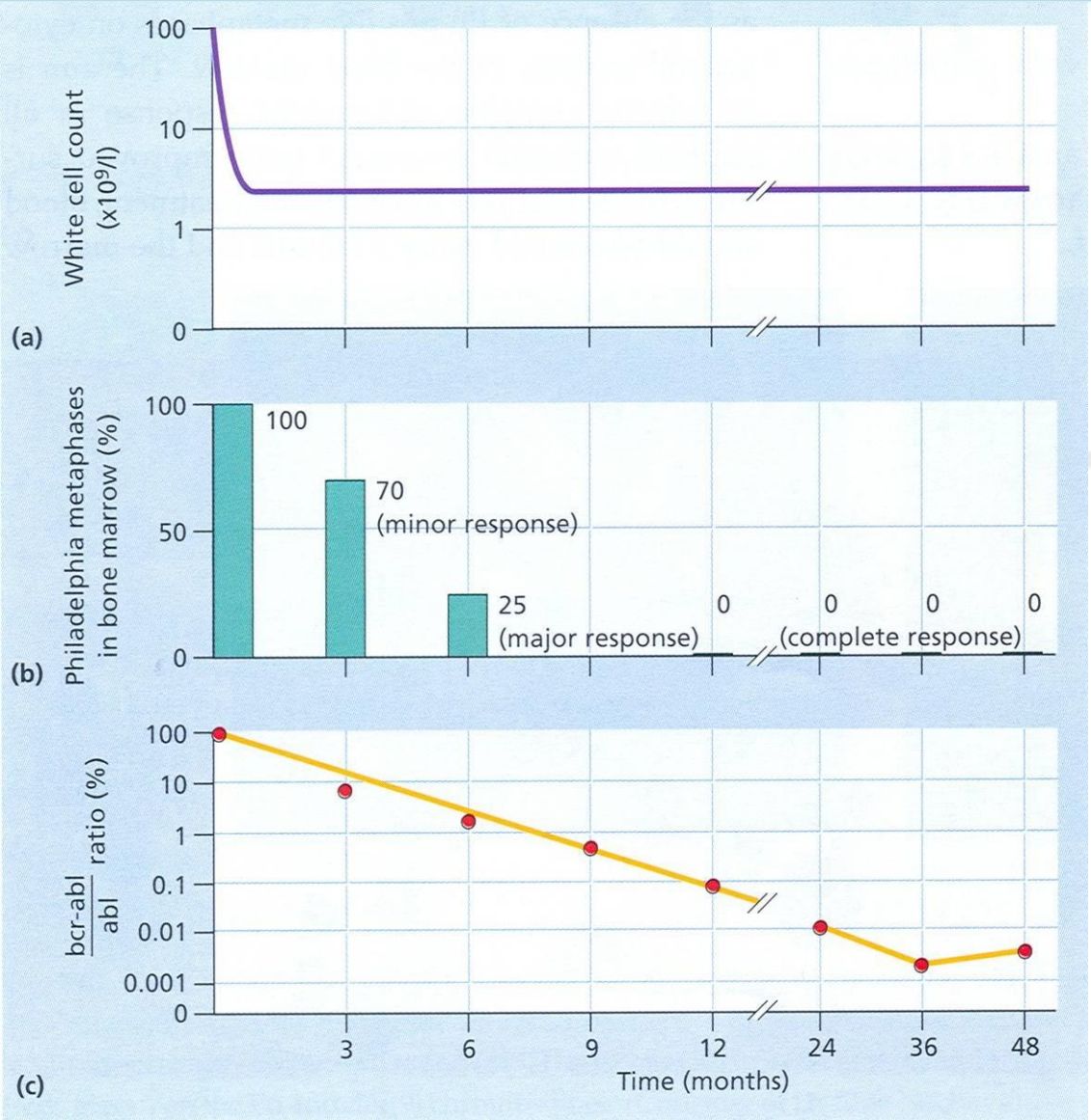






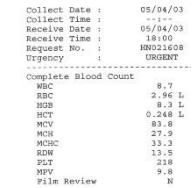
Genetic Testing for Malignant Haematological Diseases

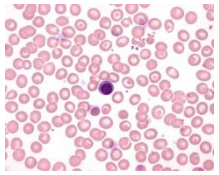
- Molecular genetic tests for MRD monitoring



Clinical Suspicion

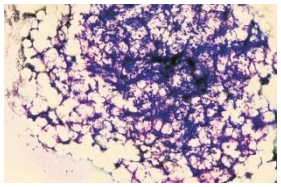
Blood Counts & Smear Morphology





Blood Tests

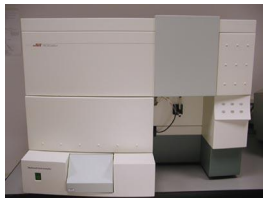
Bone Marrow Morphology



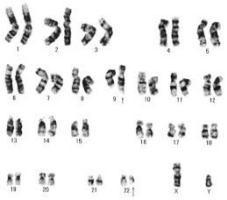
Non-malignant Dx

? malignant

Cytochemistry / Immunophenotyping



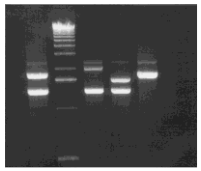
Cytogenetics / Molecular Tests



Malignant Dx

treatment

Immunophenotyping / Molecular Tests



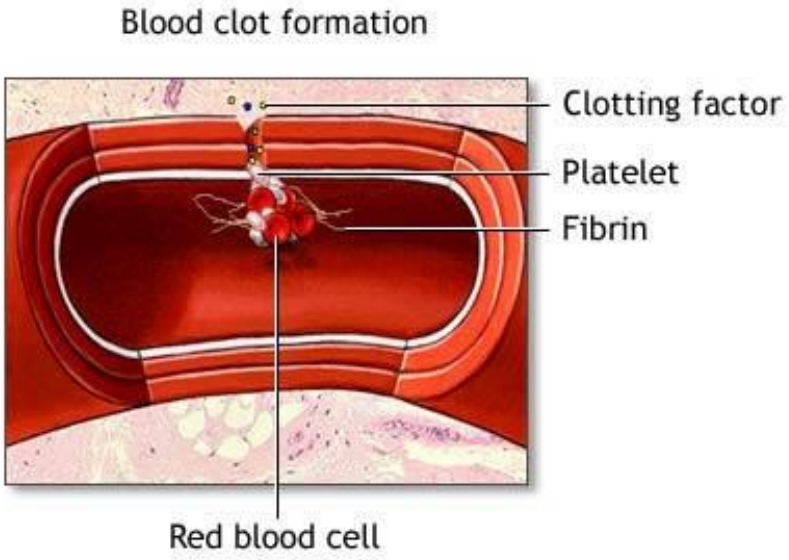
MRD Detection

Bleeding & Thrombosis Investigations

You Have a Patient with Bleeding

Components of Haemostasis

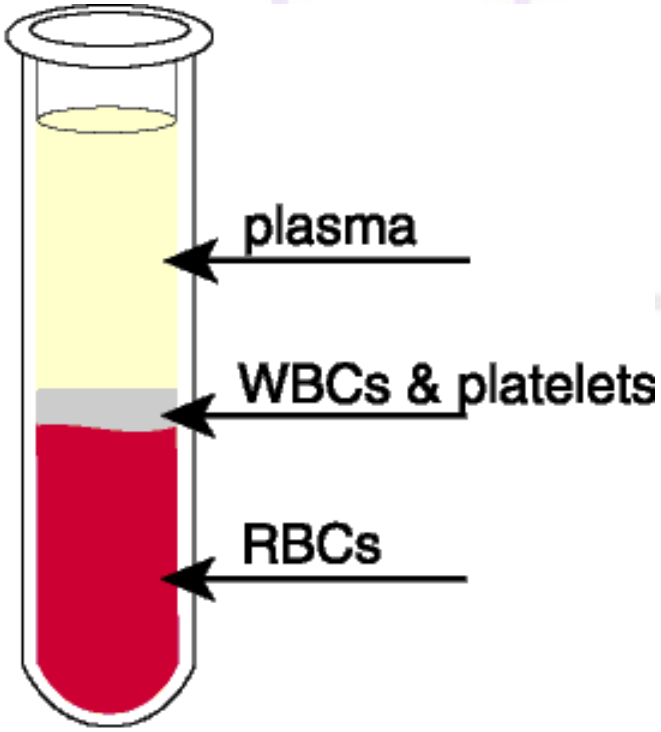
- Blood vessel
- Platelets
- Coagulation factors
- von Willebrand factor (VWF)
- Natural anticoagulants (protein C, protein S, antithrombin)



Coagulation Factors, VWF & PC, PS AT Are in Patient's Plasma

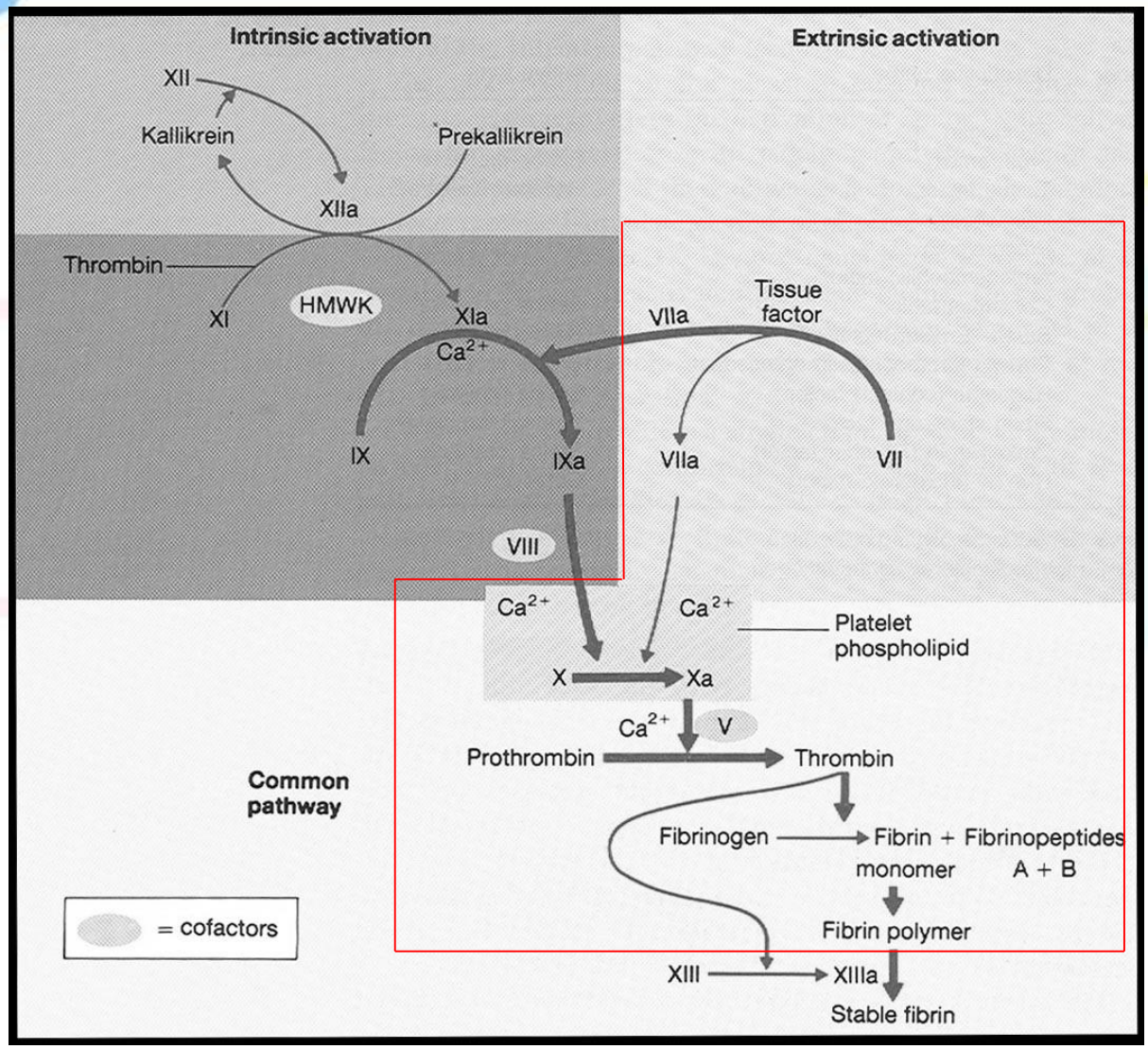
citrate bottle

anticoagulated blood



Coagulometers

Prothrombin Time (PT)



Abnormal PT

factor VII

factor X

factor V

prothrombin

fibrinogen

Prothrombin Time

anticoagulated patient plasma

+

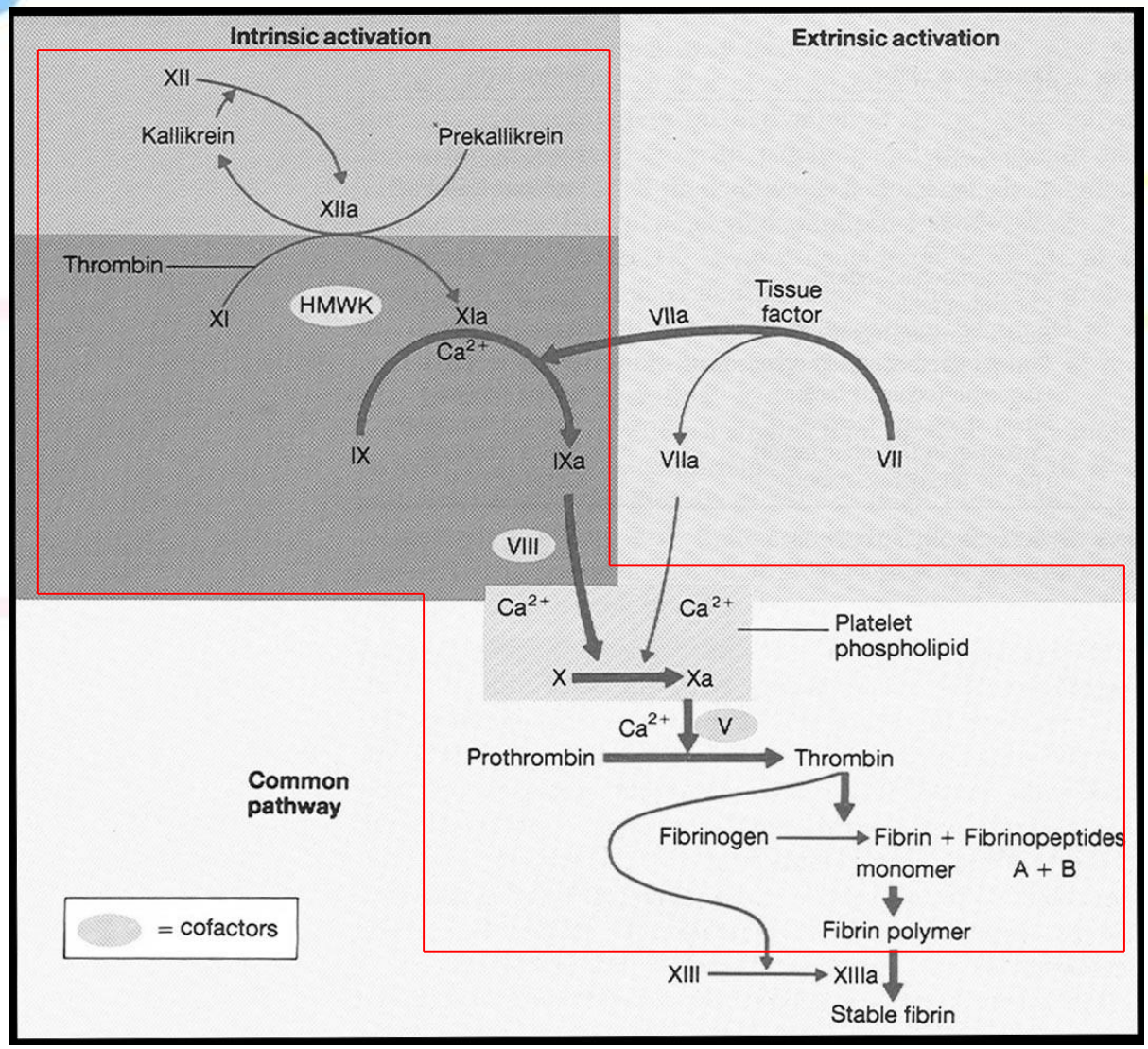
thromboplastin (tissue factor & phospholipid)

+

calcium

= PT (sec)

Activated Partial Thromboplastin Time (APTT)



Abnormal APTT

factor XII

factor XI

factor IX

factor VIII

factor X

factor V

prothrombin

fibrinogen

Activated Partial Thromboplastin Time

anticoagulated patient plasma

+

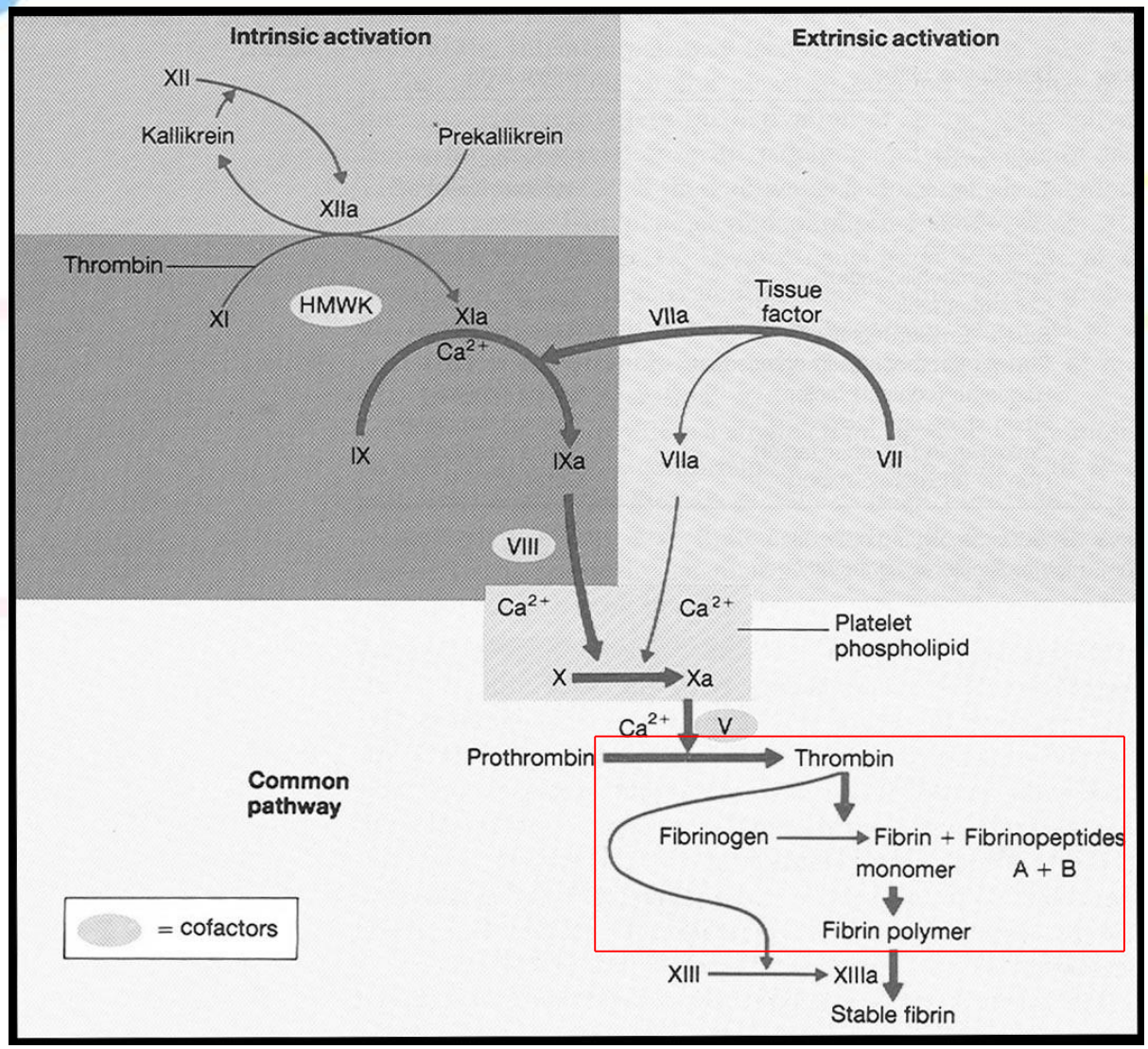
contact activator

+

phospholipid & calcium

= APTT (sec)

Thrombin Time (TT)



Abnormal TT

fibrinogen

Thrombin Time

anticoagulated patient plasma

+

thrombin

+

= TT (sec)

Other Tests in Blood & Plasma for Bleeding and Thrombotic Diseases

- Depending on the clinical presentation and screening test results
  - Specific coagulation factor assay
  - Coagulation inhibitor testing
  - VWF testing
  - PC, PS and AT assay
  - Platelet function test

Conclusions

- Laboratory investigations provide disease diagnosis, prognosis and monitoring
- Rational use of special haematology tests allow cost-effective use of resources for patient management

Photo Credits and References

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