

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Hematological Investigations

الاستقصاءات الدموية

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Routine Hematology

الاستقصاءات الدموية

Anticoagulant of choice: EDT

مضادات التخثر و خياراها

- Complete Blood Counts (CBCs) تعداد الدم الكامل
- Manual WBC Differentials صيغة البيض اليدوية
- Peripheral blood smear
- Erythrocyte Sedimentation Rates (ESRs) سرعة التثفل
- Reticulocyte Counts تعداد الشبكيات

تعداد الدم الكامل CBC

• **تعداد الدم الكامل Complete blood count**

- مع أو بدون صيغة With or without differential
- يتم سحب الدم من الأوردة المحيطية بأنبوب خاص يحتوي مادة خاصة مضادة للتخثر تدعى (EDTA)
- ترفض العينة Unacceptable specimen المتخثرة أو المسحوبة لأكثر من 48 ساعة
- Methodology of testing: طريقة الاختبار
 - Whole blood analyzer محلل الدم الكامل
- How often is the test available for hospitalized patients?
 - 7 days/week (24/7)

Complete Blood Counts (CBCs)

ما هي مكونات تعداد الدم? What is measured?

- RBC count
 - Hematocrit (packed cell volume) Hemoglobin
 - Mean corpuscular volume (MCV)
 - Mean cell hemoglobin MCH
 - Mean corpuscular hemoglobin concentration (MCHC)
 - Red blood cell distribution width (RDW)
 - White blood cell data
 - ✓ Total white blood cell (leukocyte) count (WBC)
 - ✓ A white blood cell count differential may also be ordered
 - Platelet Count (PLT)
-
- Under normal conditions the production, release, and survival of blood cells is a highly regulated process. Quantitative and/or qualitative hematologic abnormalities may result when there is an **imbalance between cell production, release, and/or survival.**

Measurement (units)	Men	Women
Hemoglobin (gm/dL)	13.6-17.5	12.0-16.0
Hematocrit (%)	40-52	37-47
Red cell count ($10^6/\mu\text{L}$)	4.5-6	4-5.5
Reticulocyte count (%)	0.4-2	
Mean cell volume (μm^3) MCV	78-96	
Mean corpuscular hemoglobin (pg) MCH	28-32	
Mean corpuscular hemoglobin concentration (gm/dL) - MCHC	32-36	
RBC distribution width RDW	11.5-14.5	

Red blood cell indices

المناسب الكريوية

Mean cell volume (MCV): حجم الكرية الحمراء الوسطي

$$(Ht \times 10) / (\text{red cell count} \times 10.000000)$$

$$86(\pm 10) \text{ fl}$$

Mean cell hemoglobin (MCH): خضاب الكرية الوسطي

$$(Hb \times 10) / (\text{red cell count} \times 10.000000)$$

$$30(\pm 3) \text{ pg}$$

Mean cell Hb. concentration (MCHC): تركيز خضاب الكرية
الوسطي

$$(Hb \times 10) / (Ht) \text{ or } MCH/MCV$$

$$33(\pm 2) \text{ g\%}$$

Red cell distribution width (RDW) : توزع تفاوت حجم الكريات
الاحمر

$$RDW = \text{Standard deviation of RBC size} \div MCV \quad 11.5 - 14.5$$

فائدة استخدام حجم الكرية الوسطي

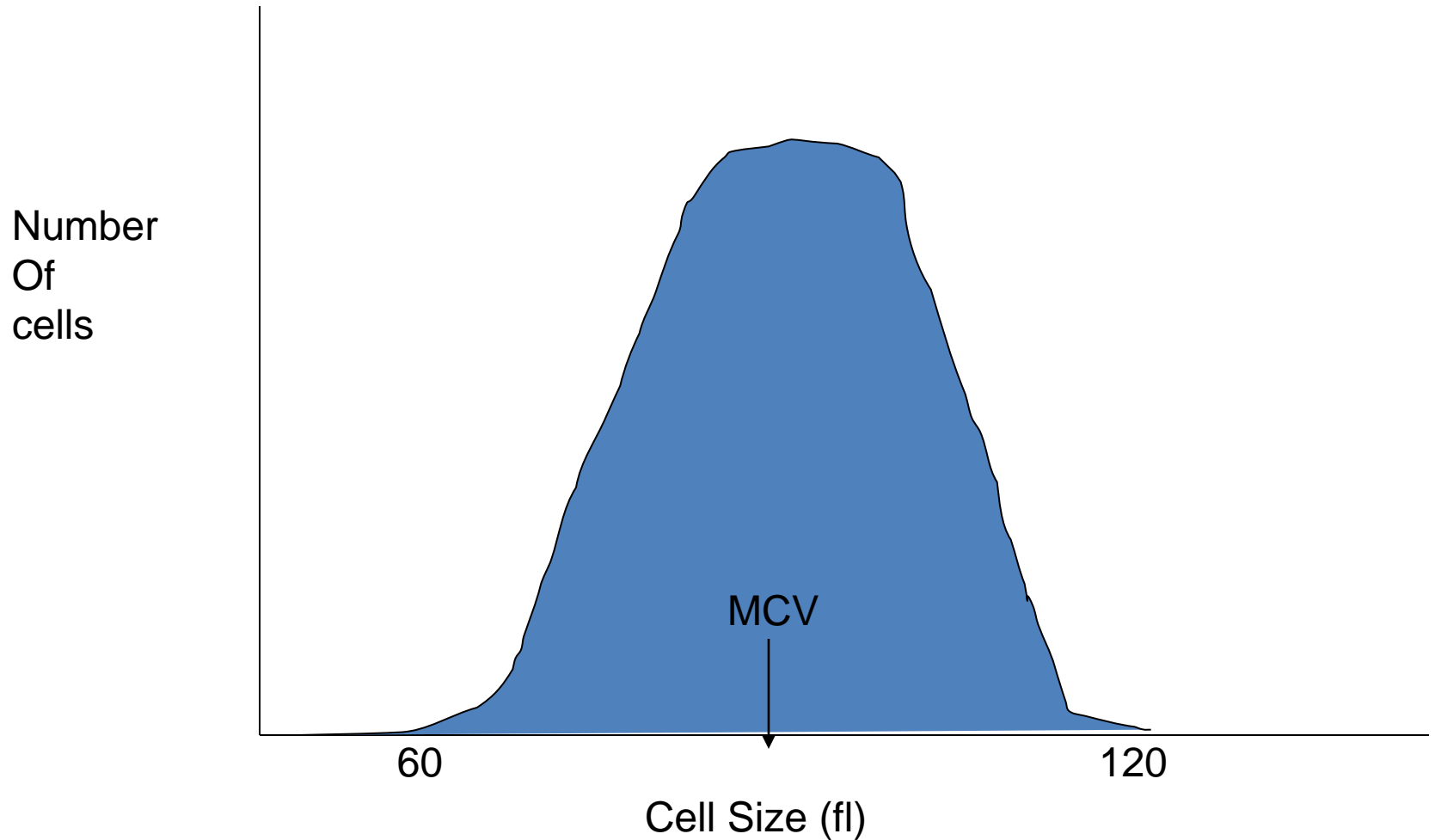
- أهميته في تصنيف فاكات الدم

Normal MCV= normocytic – فقر الدم سوي المناسب
anemia

Decreased MCV= microcytic – فقر الدم صغير المناسب
anemia

Increased MCV= macrocytic – فقر الدم كبير المناسب
anemia

Red Cell Distribution Histogram



Red Blood Cell Distribution Width

منسب تفاوت حجوم الكريات الحمر

- RDW is an indication of the variation in the RBC size (referred to anisocytosis)
- It is derived from the red blood cell histogram and represents the coefficient of variation of the curve
- **In general, an elevated RDW** (indicating more variation in the size of RBCs) has been associated with anemias with various deficiencies, such as iron, B12, or folate
- **Minor Thalassemia** is a microcytic anemia that characteristically has a normal RDW

White Blood Cell Count

تعداد الكريات البيض

- A count of the **total** WBC, or leukocyte, count in 1mm^3 of peripheral blood
- A decrease in the number of WBCs =
 - Leukopenia قلة البيض
- An increase in the number of WBCs =
 - Leukocytosis كثرة البيض

WBC Differential صيغة الكريات البيض

- When a differential is ordered, the percentage of each type of leukocyte present in a specimen is measured.
- Name the types of leukocytes
 - Neutrophils (includes bands) العدلات
 - Lymphocytes اللمفيات
 - Monocytes الوحيدات
 - Eosinophils الحمضات
 - Basophils الأسسات
- WBC differentials are either performed manually or by an automated instrument

إجراء الصيغة اليدوية Manual Differentials

- “Manual” WBC differentials are performed by trained medical technologists who count and categorize typically 100 white blood cells via microscopic examination of a Romanowsky-stained peripheral blood smear
 - In addition to the differential count, evaluation of the smear provides the opportunity to morphologically evaluate all components of the peripheral blood, including red blood cells, white blood cells and platelets
 - The manual differential allows for the detection of disorders that might otherwise be lost in a totally automated system
 - This applies to < 20% of specimens

Automated Differentials إجراء الصيغة الآلية

- The clinical laboratory may perform an “automated differential”
 - Via instruments with the capability of performing differential leukocyte counts
 - Usually based on the determination of different leukocyte cellular characteristics that permit separation into subtypes by using flow-cytometric techniques

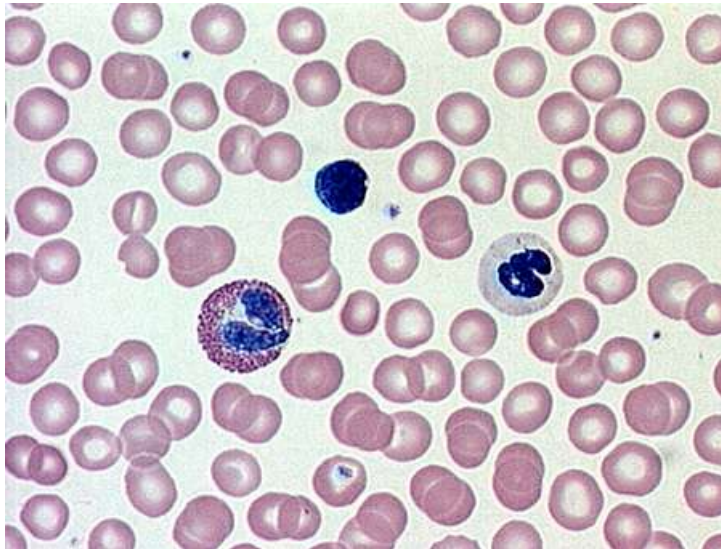
فقر الدم و اللطاخة الدموية – Anaemia Workup

Peripheral Smear Study

- Are all RBC of the same size ?
- Are all RBC of the same normal discoid shape ?
- How is the colour (Hb content) saturation ?
- Are all the RBC of same colour/ multi coloured ?
- Are there any RBC inclusions ?
- Are intra RBC there any hemo-parasites ?
- Are leucocytes normal in number and D.C ?
- Is platelet distribution adequate ?

Red Blood Cells

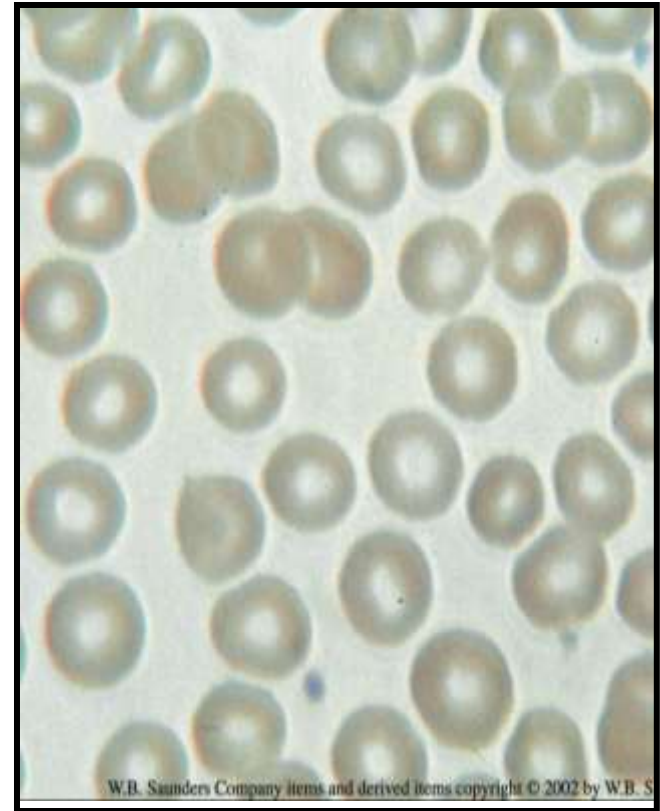
- Peripheral blood smear: اللطاخة الدموية المحيطية
 - Normal blood film



Erythrocytes

الكريات الحمر

- Normal range 4.2-5.5 million per mm^3 in adults.
- Biconcave shape.
- Diameter 7 microns.
- Cells for transport of O_2 and CO_2 .
- Life span 120 days.

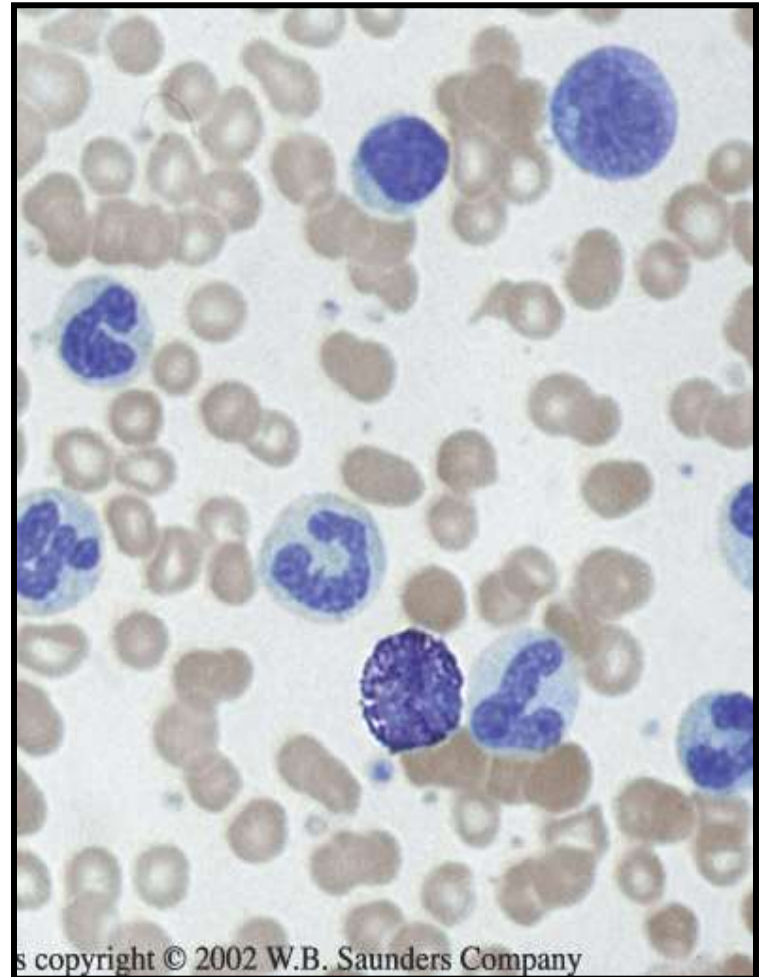


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Leukocytes

الكريات البيض

- Normal range 4 - 11 thousand per mm^3 in adults.
- Five types.
- Size 8-20 microns.
- Involved in fighting infection, combatting allergic reactions, and immune responses.

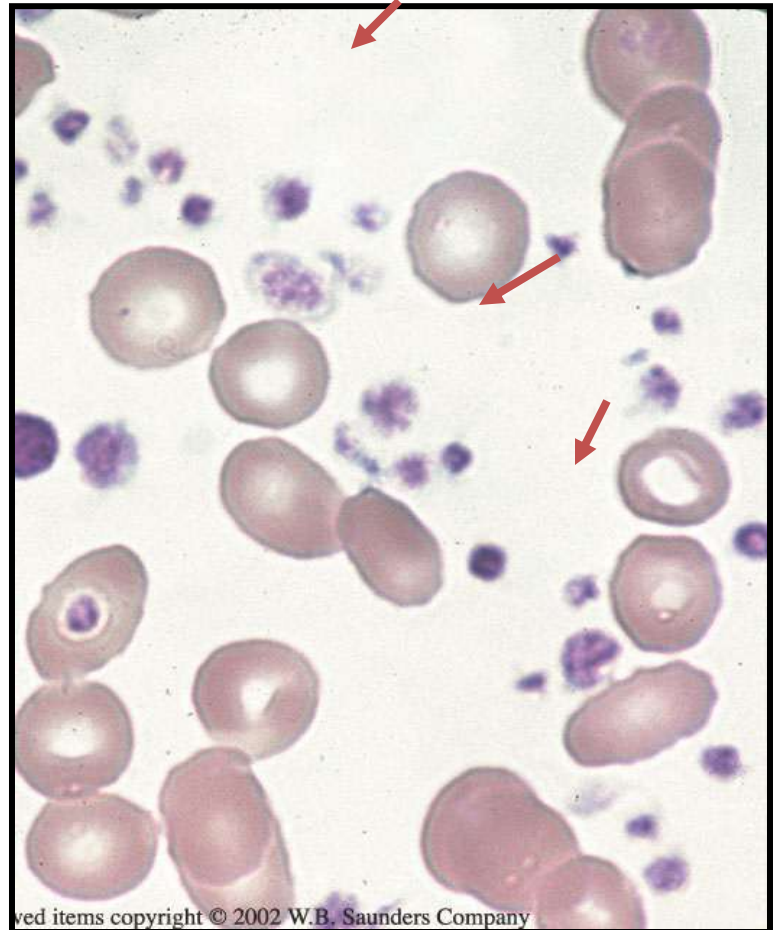


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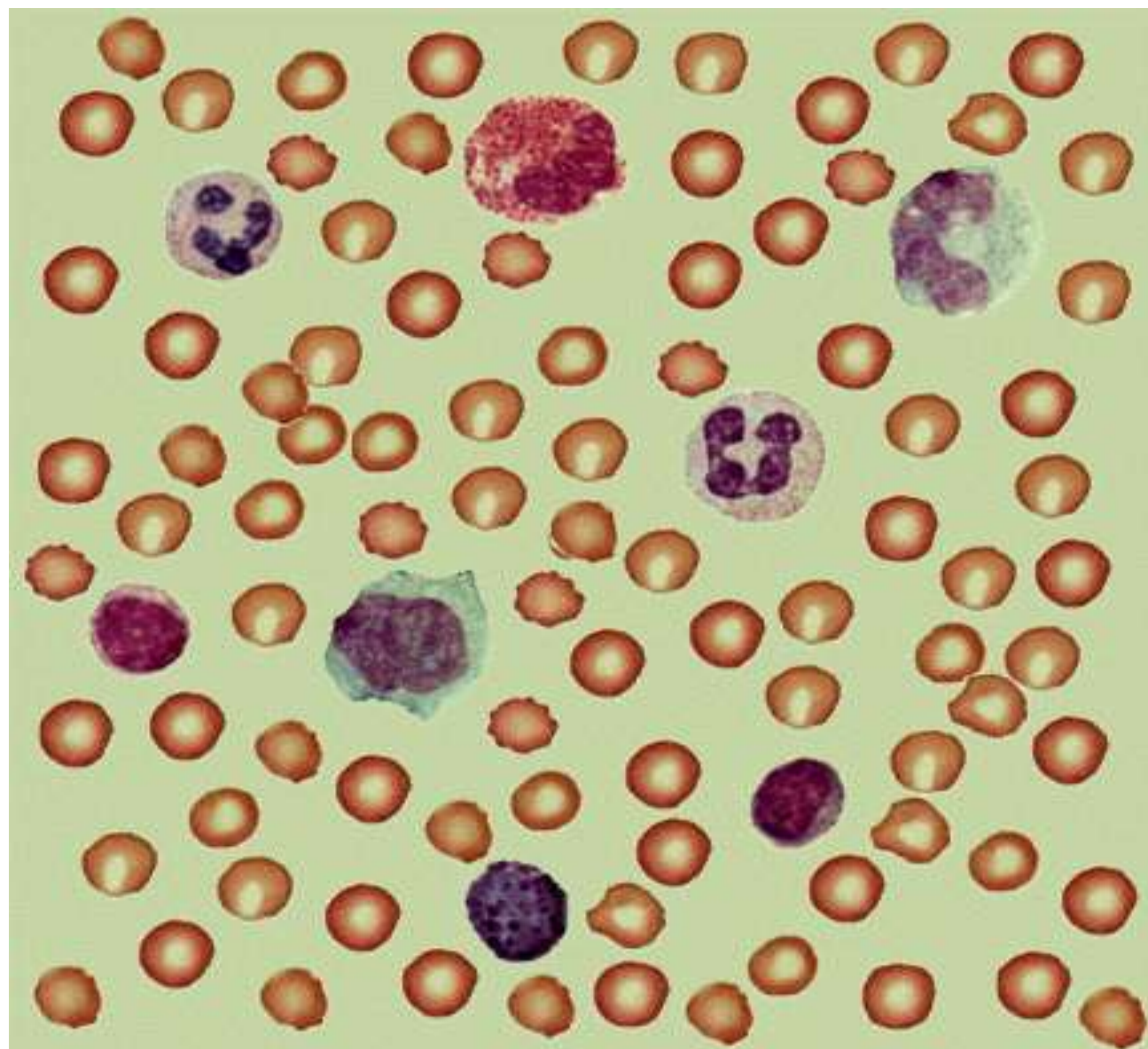
Thrombocytes

الصفائح

- Smallest cells in the blood.
- Normal range 150,000-400,000.
- Active role in coagulation and hemostasis.



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اللطاخة المحيطة Blood film

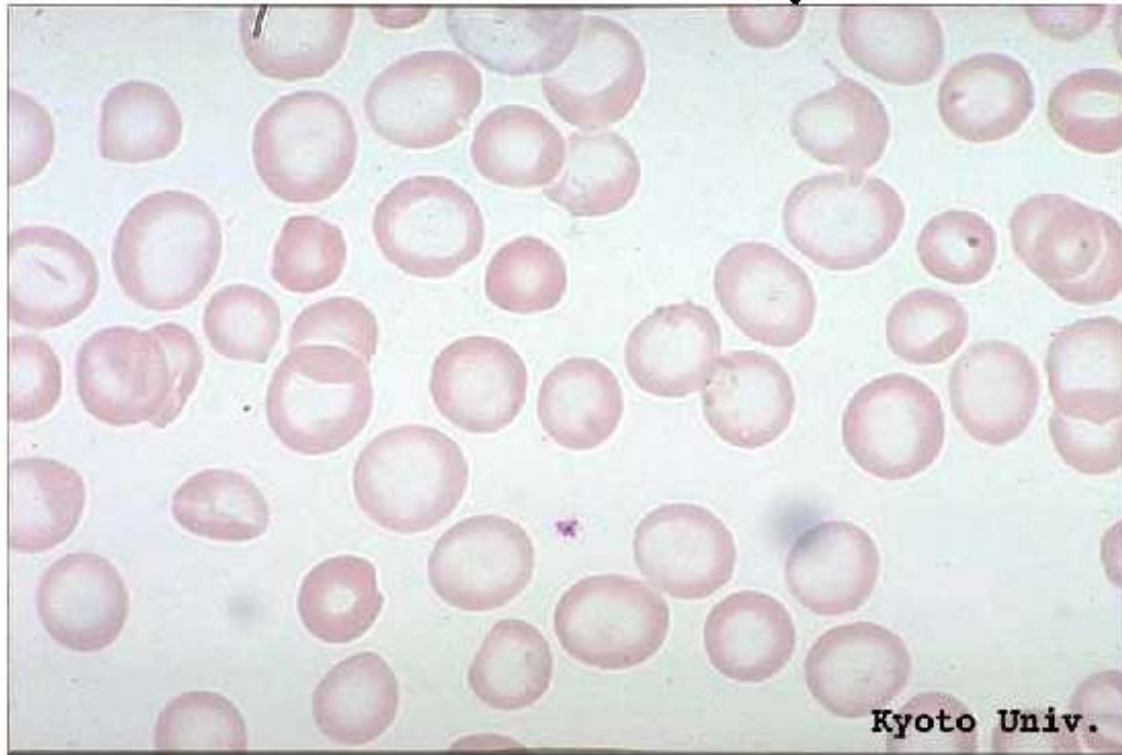
1-تفاوت حجم الكريات الحمر (MCV) Anisocytosis: و هو
يشير لاختلاف حجوم الكريات الحمر
تصنف الكريات الحمر:

- خلايا سوية الحجم Normocytic
- خلايا كبيرة الحجم Macrocytic
- خلايا صغيرة الحجم Microcytic

Blood film

- 2- الصباغ: بالمجهر يتوزع خضاب الكرية الحمراء في محيطها (بسبب شكلها مقعر الوجهين) ويكون مركزها شاحبا ولايتجاوز هذا الشحوب المركزي ثلث قطر الكرية وهنا ندعوها سوية الصباغ (Normochromic)
- وعندما يزداد الشحوب المركزي تدعى ناقصة الصباغ (Hypochromic) وتشاهد بفقر الدم بعوز الحديد مثلاً.
- في بعض الحالات تأخذ الكرية الحمراء شكل حلقة رقيقة محيطية بسبب نقص الصباغ الشديد (الخلية الخاتمية Ring cell)
- اما اذا زال المركز الشاحب فندعوها زائدة الصباغ (Hyperchromic)

Hypochromic/Microcytic Anemia Iron Deficiency



اللطخة الدموية Blood film

- 3- اختلاف الشكل Poikilocytosis
يشير لاختلاف الشكل

ABNORMALITIES IN THE SHAPE OF ERYTHROCYTES



SPHEROCYTE



**OVALOCYTE
(ELLIPTOCYTE)**



**LEPTOCYTE
(PLATICYTE)**



ACANTHOCYTE



**MEGALOCYTE
(MACROOVALOCYTE)**



STOMATOCYTE



SCHIZOCYTE



POIKILOCYTE



DACRYOCYTE



**ROULEAUX
FORMATION**















Red cell abnormalities	Causes	Red cell abnormalities	Causes
	Normal		Microspherocyte Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
	Macrocyte Liver disease, alcoholism, Oval in megaloblastic anaemia		Fragments DIC, microangiopathy, HUS, TTP, burns, cardiac valves
	Target cell Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy		Elliptocyte Hereditary elliptocytosis
	Stomatocyte Liver disease, alcoholism		Tear drop poikilocyte Myelofibrosis, extramedullary haemopoiesis
	Pencil cell Iron deficiency		Basket cell Oxidant damage—e.g. G6PD deficiency, unstable haemoglobin
	Echinocyte Liver disease, post-splenectomy		Sickle cell Sickle cell anaemia
	Acanthocyte Liver disease, abetalipoproteinaemia, renal failure		Microcyte Iron deficiency, haemoglobinopathy

Figure 2. Note the hypersegmented neutrophil (7-8 lobes)

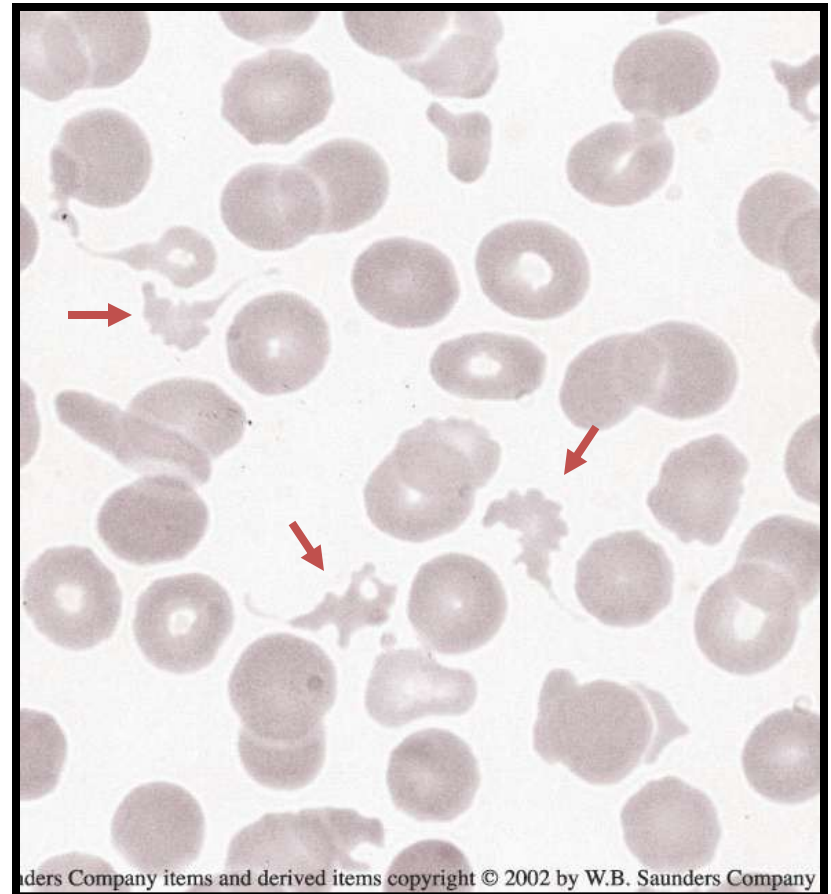


Schrier, S. ASH Image Bank 2001;2001:100231

Sickle Cells



Schistocytes(Fragments)



داء الكريات البيضوية الإهليلجية Eliptocytosis



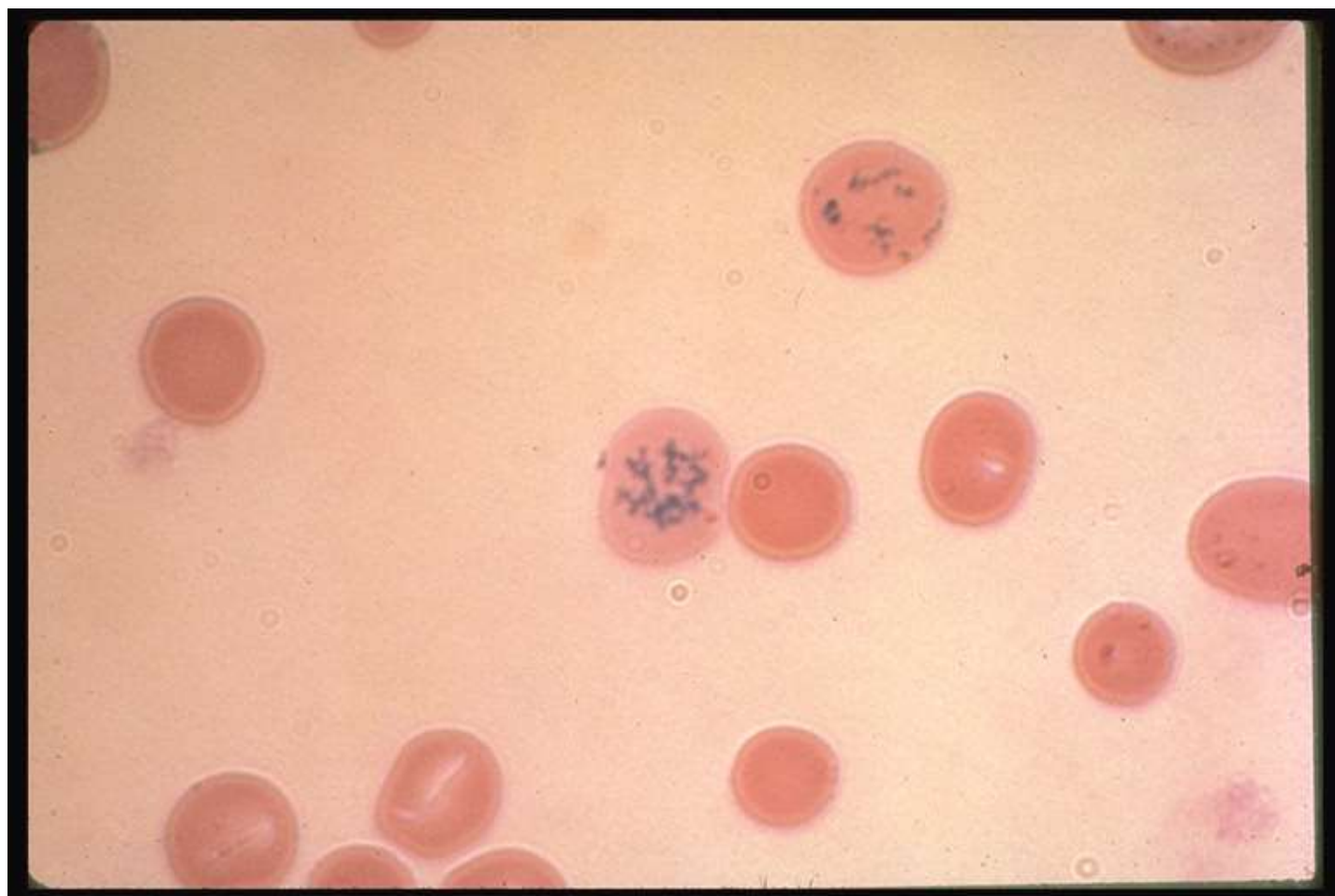
Blood film

4-polychromsia تعدد الصباغ أو الحؤول اللوني

(RETICULOCYTOSIS) Reticulocytes appear as polychromatic cells on stained slides. They are immature red cells newly released from the marrow sinusoids and takes about a day or two to mature in the peripheral circulation in those with intact spleen.

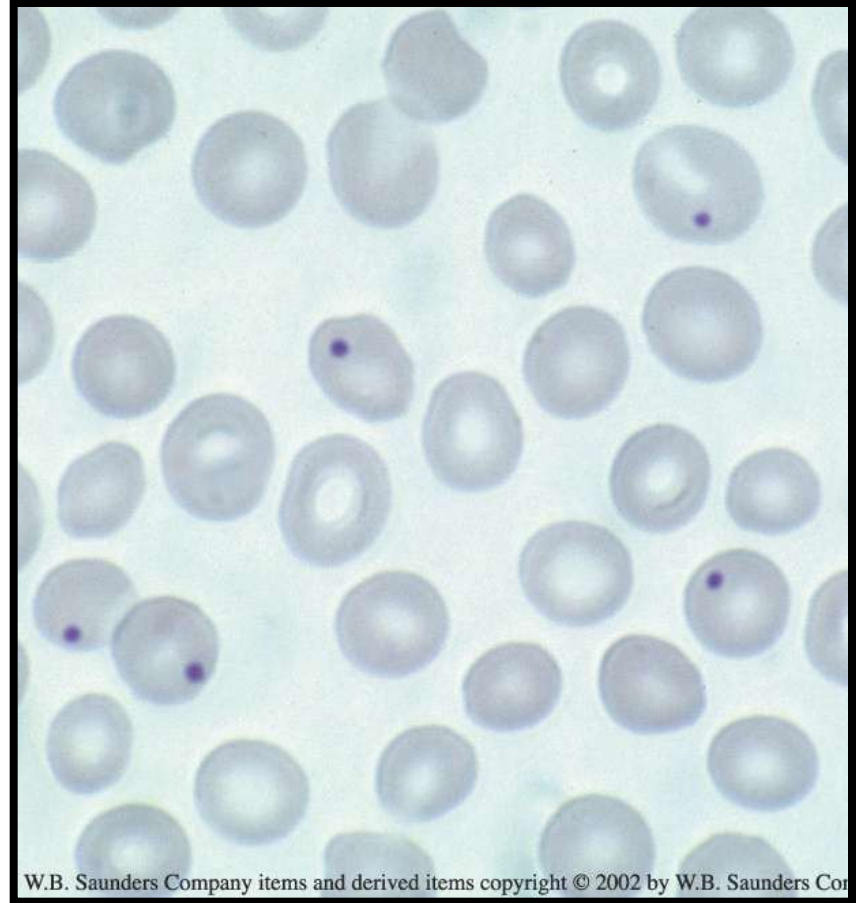
(الخلية الشبكية) Reticulocyte

- An immature red blood cell that has a fine reticulum formed of the remains of ribosomal RNA— form in marrow where they mature for 3 days and then spend 1 day in circulation (before maturing to RBC)
- Given avg life span of RBC of 100-120 days, 1% of RBCs are destroyed each day
- Retics form 1% of circulating RBCs qd(0.4-2%)
 - With epo, can increase to 250,000 retics/uL blood qd (given nl marrow and replete iron, folate, B12, hemolysis, bleeding)
 - Decreased in Vit B12 and folic acid deficiency, aplastic anemia, pure red cell aplasia



5- Howell-Jolly Bodies

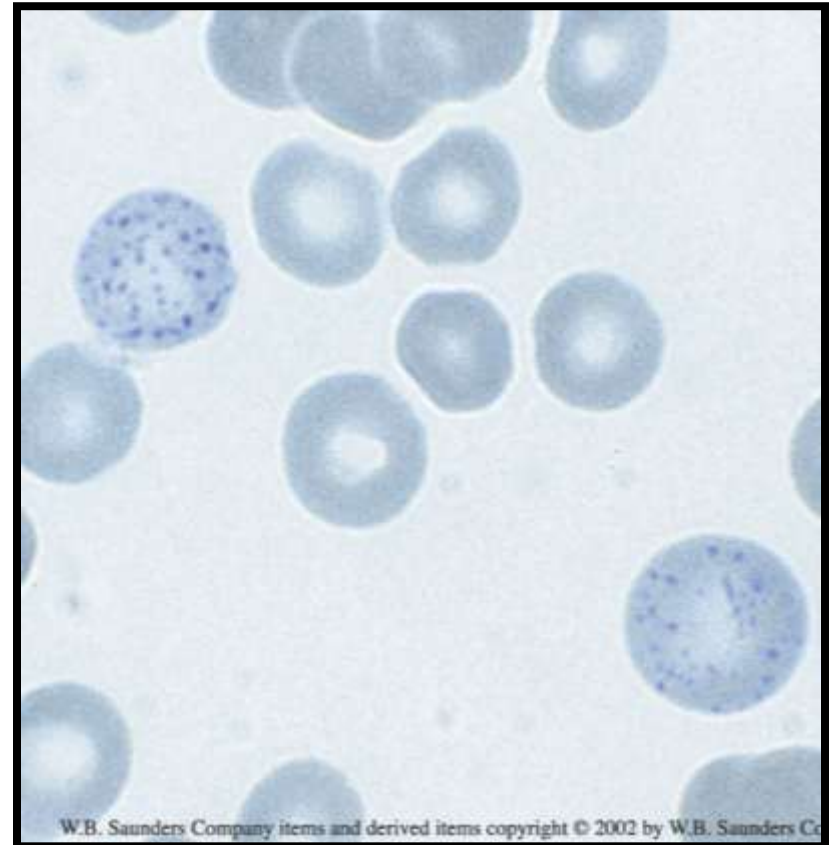
- Round, purple inclusions in RBCs.
- Composed of DNA.
- Commonly seen in patients with hypofunctioning spleens.
- Splenectomy.
- Vit B₁₂ deficiency
- Thalassemia, sickle cell disease



6-Basophilic Stippling

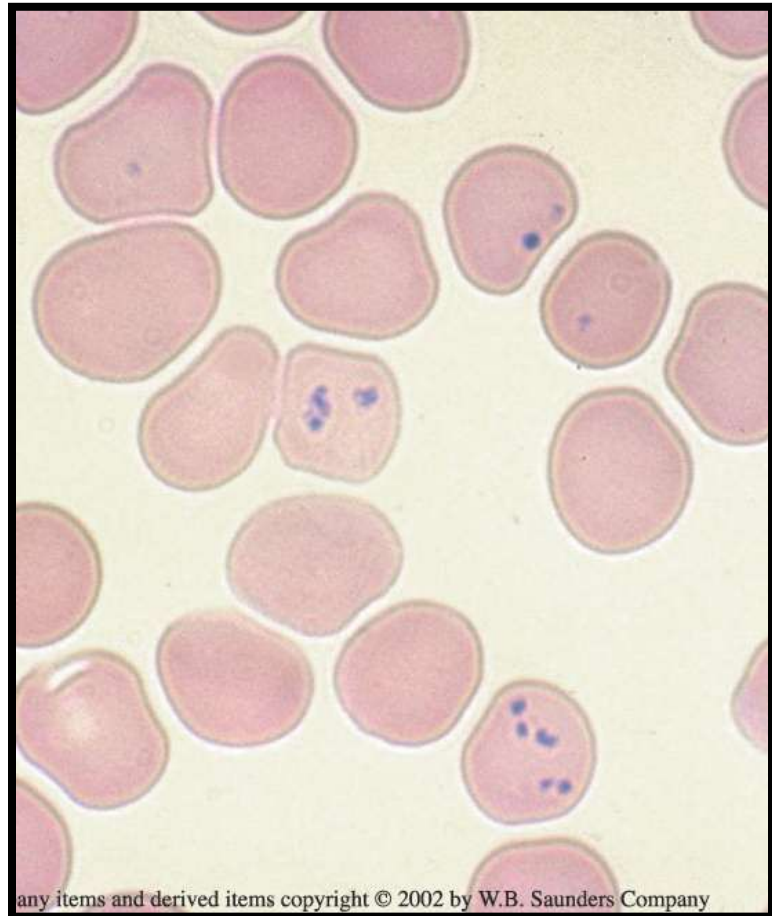
الخلايا المرقطة

- Numerous, small purple inclusions in RBCs. Indicate
- Aggregates of ribosomal RNA.
- Most commonly seen in lead poisoning, Sideroblastic anemia, thalassemia, MDS



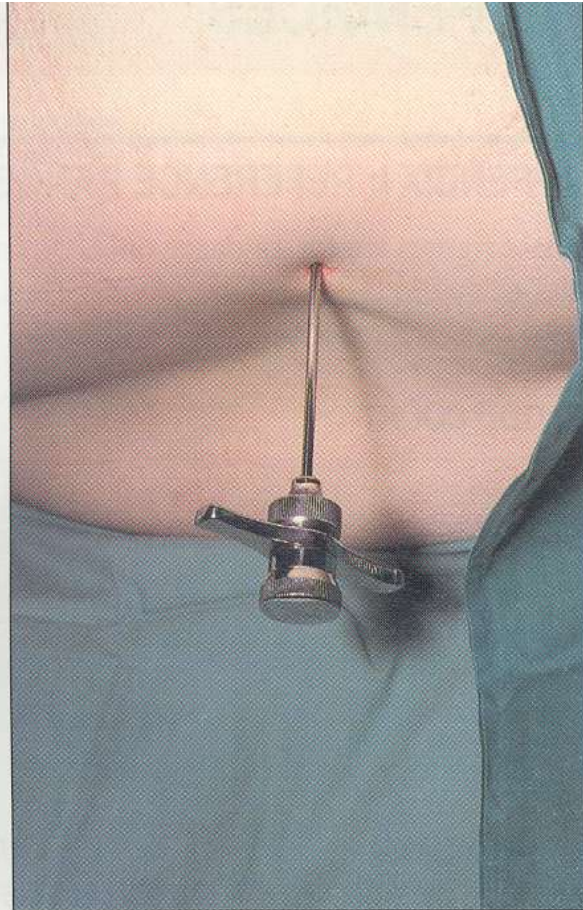
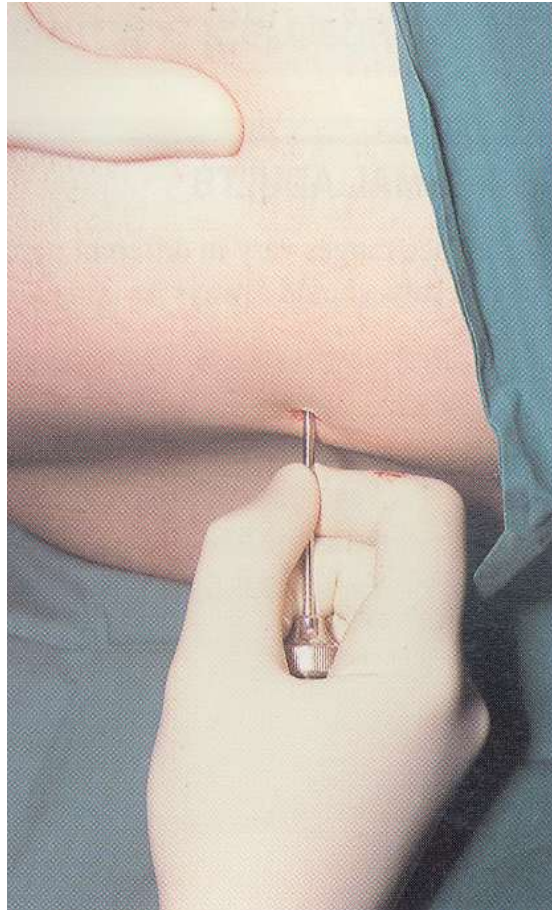
7- Pappenheimer Bodies

- Clusters of dark blue granules, irregular in size and shape.
- Composed of iron and ribosomal RNA.
- Seen in sideroblastic and hemolytic anemias.



Techniques for obtaining bone marrow

- **The technique** should be explained to the patient and consent obtained **Aspiration** Site - usually iliac crest
- Give local anaesthetic injection
- Use special bone marrow needle (e.g. Salah)
- Aspirate marrow
- Make smear with glass slide
- Stain with:
 - Romanowsky technique
 - Perls' reaction (acid ferrocyanide) for iron.
- **Trephine** Indications include:
 - 'Dry tap' obtained with aspiration
 - Better assessment of cellularity, e.g. aplastic anaemia
 - Better assessment of presence of infiltration or fibrosis.
- **Technique** Site - usually posterior iliac crest
- Give local anaesthetic injection
- Use special needle (e.g. Jamshidi - longer and wider than for aspiration)
- Obtain core of bone
- Fix in formalin; decalcify - this takes a few days
- Stain with:
 - Haematoxylin and eosin
 - Reticulin stain.



Indication of bone marrow aspiration

استطرابات بزل النقي

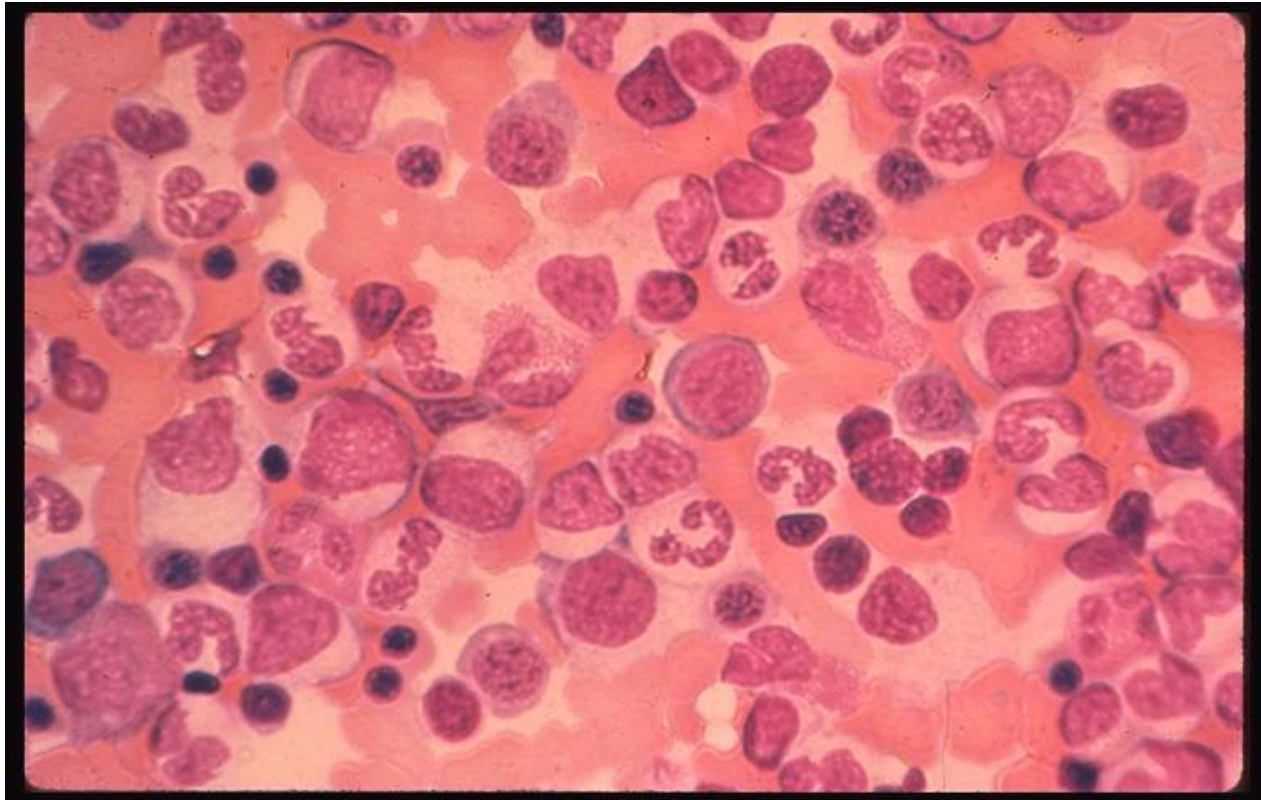
- some types of anemia بعض فاقت الدم
- neutropenia قلة العدلات
- Leukemia الابيضاض
- suspected megakaryocyte abnormalities
- Multiple myeloma الورم النقوي المتعد
- some infections بعض الأخماج
- Hypersplenism فرط الطحالية

فحص نقي العظم

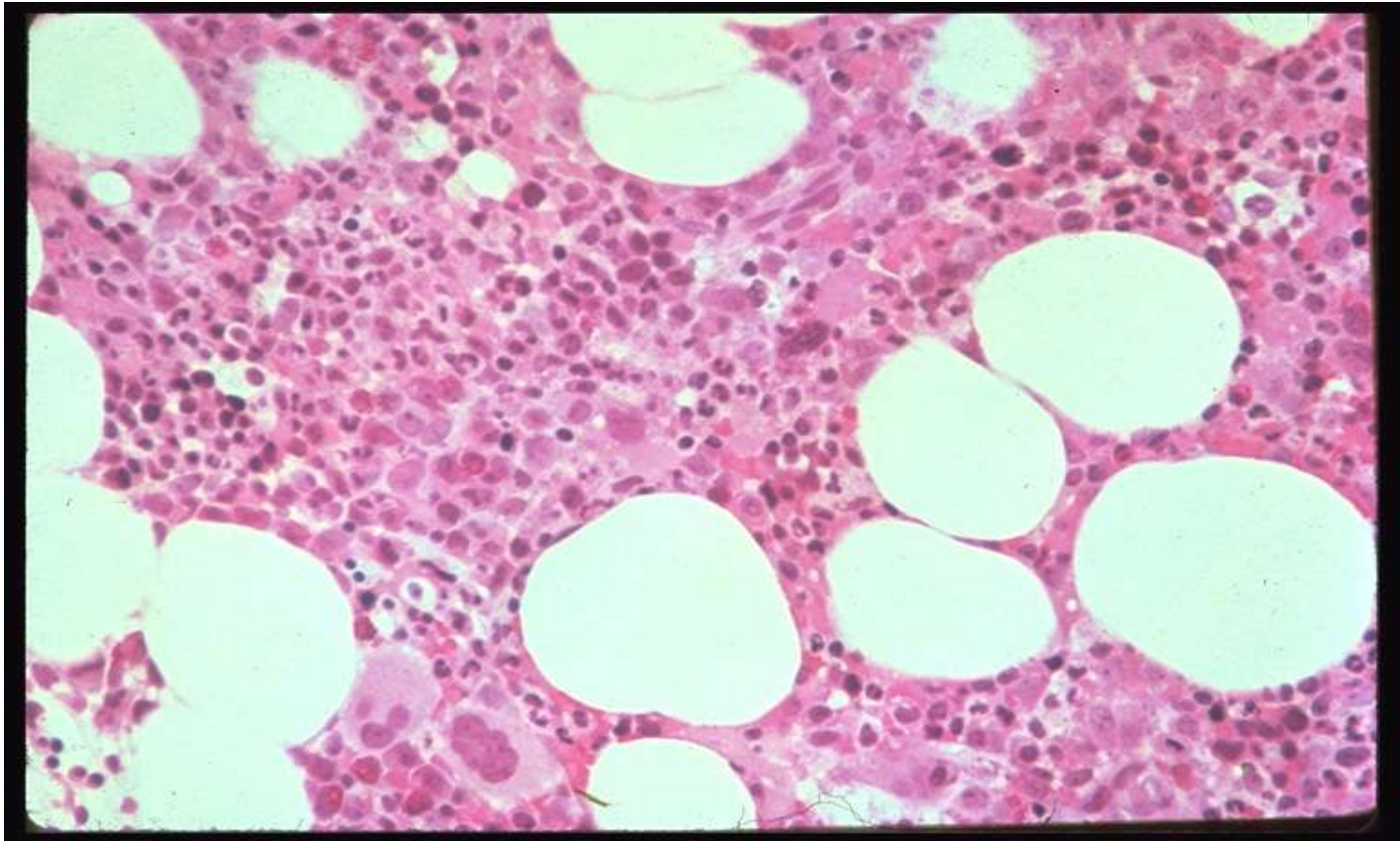
Bone Marrow Examination

Aspirate	Trephine Biopsy
cellularity M:E ratio morphology of cells megakaryocyte numbers presence of foreign cells iron stores presence of bacteria chromosomal abnormalities special staining features	archetecture red marrow / fat ratio abnormal cells abnormal structure

Normal Bone Marrow Aspirate

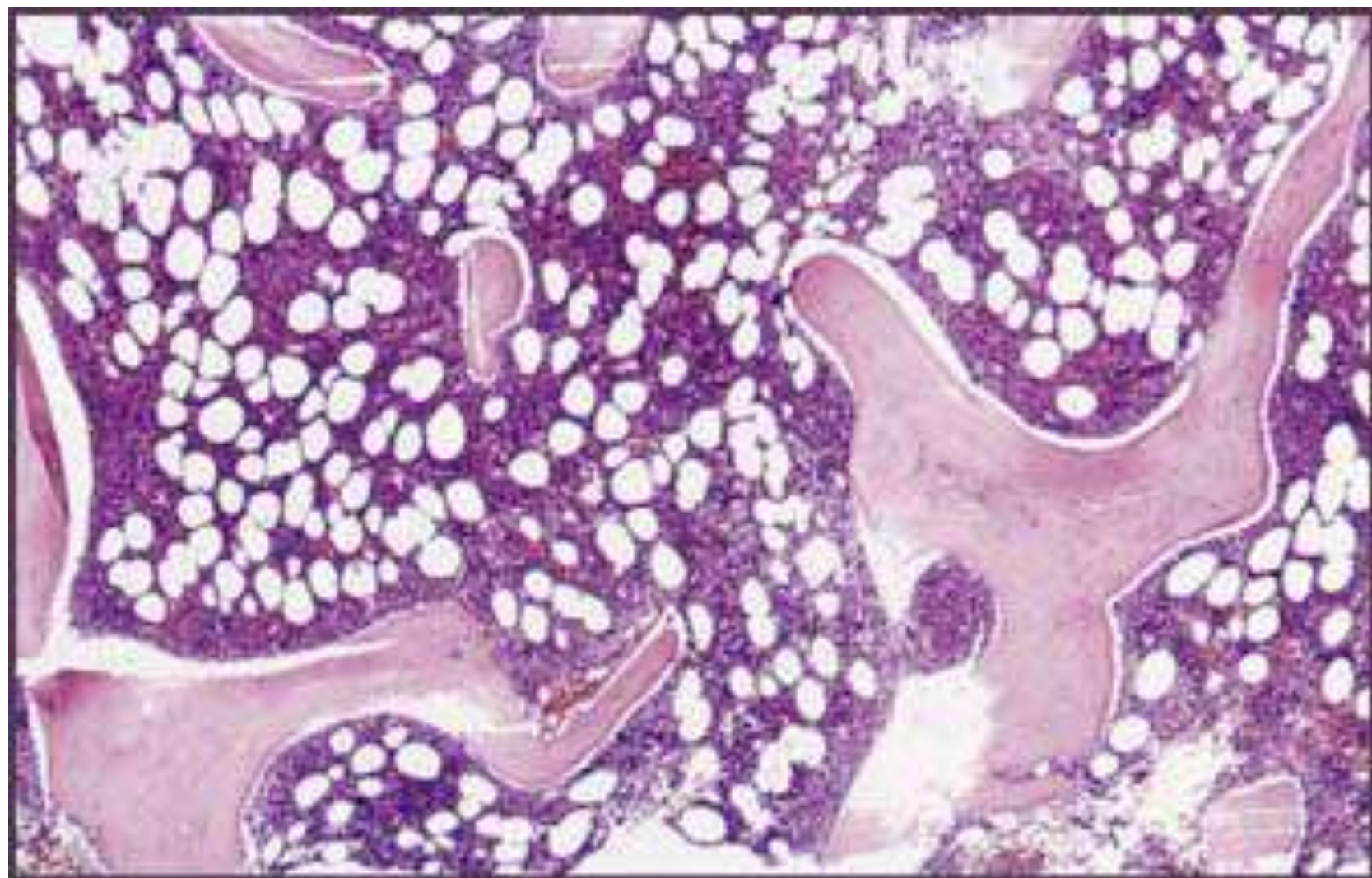


Normal Bone Marrow Biopsy



Indication of bone marrow biopsy

- pancytopenia نقص الكريات الشامل
- dry tap البزل اللف (infiltration , fibrosis ,aplastic anemia)
- lymphoma اللمفوما
- Some infection(TB)
- Storage disease أدواء الخزن
- Unknown fever الحمى مجهولة السبب
- Myeloproliferative disorders اضطرابات النقي التكاثرية



Erythrocyte sedimentation rate (ESR) **سرعة التثفل**

Anticoagulated whole blood (4 parts whole blood: 1 part Na Citrate) is placed in a narrow-bore glass tube. Erythrocytes are allowed to sediment at room temperature.

- سرعة التثفل ESR تقيس مسافة ترسب الكريات الحمر في ساعة

- بروتينات البلازما هي التي تحدد سرعة التثفل, ترتفع طور الالتهاب الحاد

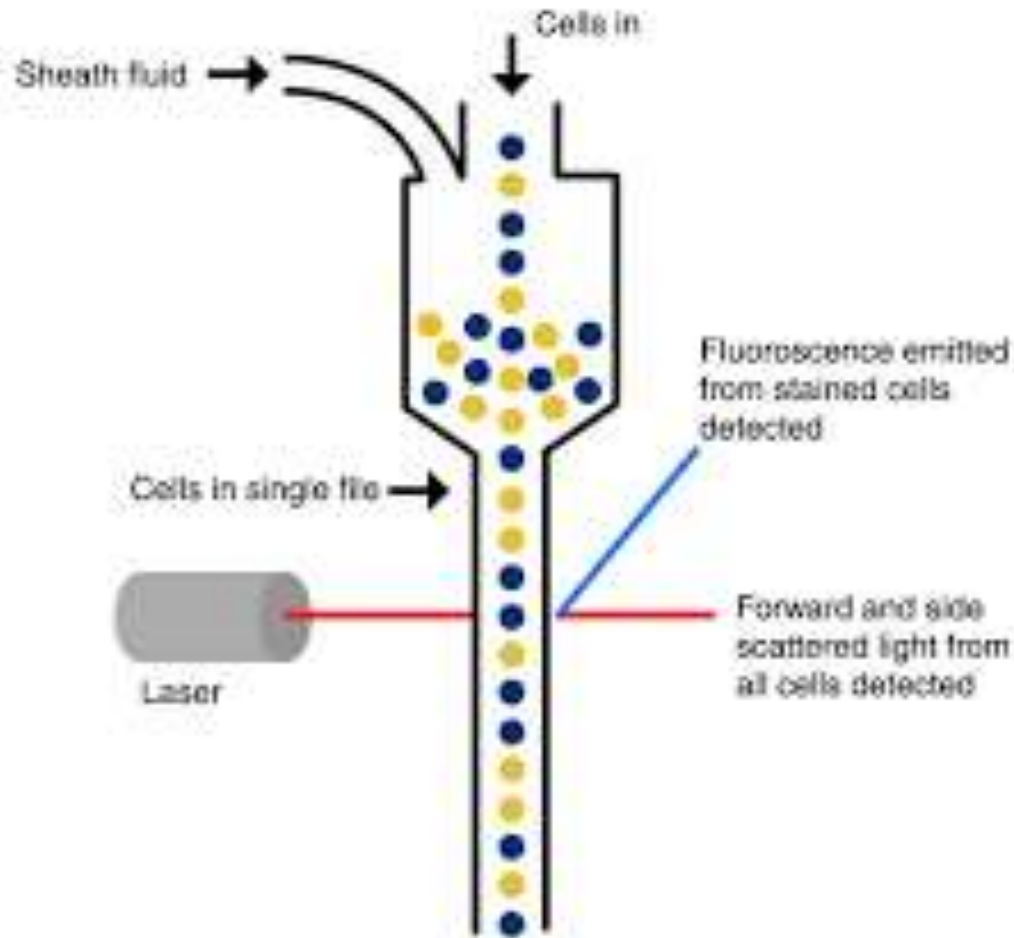
. Used to follow rheumatoid arthritis, SLE, vasculitis and many inflammatory conditions, infection , malignancy.

- **VERY LOW SPECIFICITY** **تنخفض بشدة في**
- **Decreased in:** -Polycythemia Vera **كثرة الكريات الحقيقية**
-Sickle cell disease **فقر الدم المنجلي**

فحوصات أخرى

- ✓ **Immunochemistry:** التلوين المناعي
 - Histoimmunochemistry : التلوين النسيجي المناعي
 - lymphoma
 - Flowcytometry : مقياس الخلايا بالتدفق
 - Leukemia
- ✓ **Karyotype:** النمط النووي
- ✓ Leukemia
- ✓ **PCR: Polymerase chain Reaction** التفاعل التسلسلي التضخيمي
- ✓ Leukemia

التنميط المناعي بمقياس الخلايا بالتدفق



التنميط المناعي

