Romanowsky stains
Why they remain valuable

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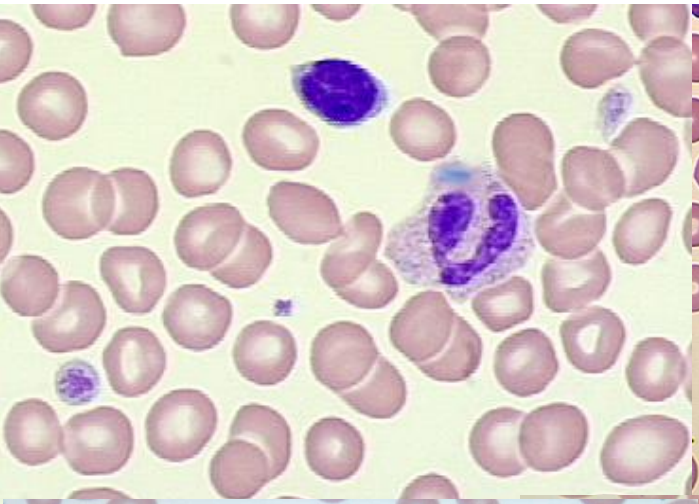
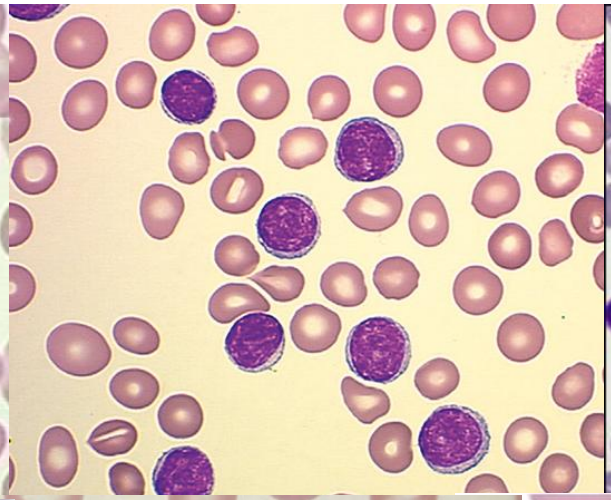
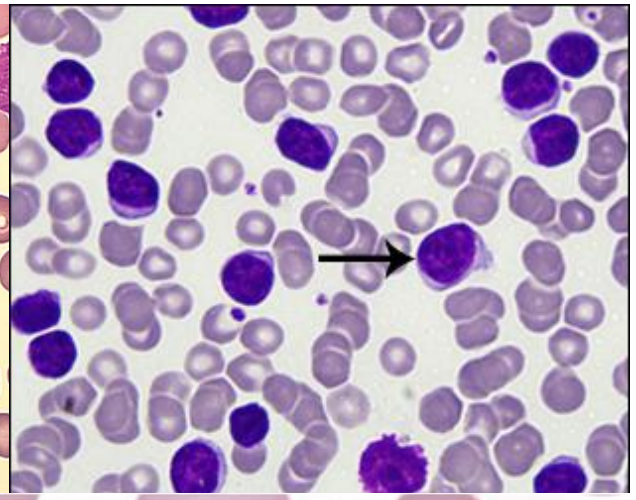
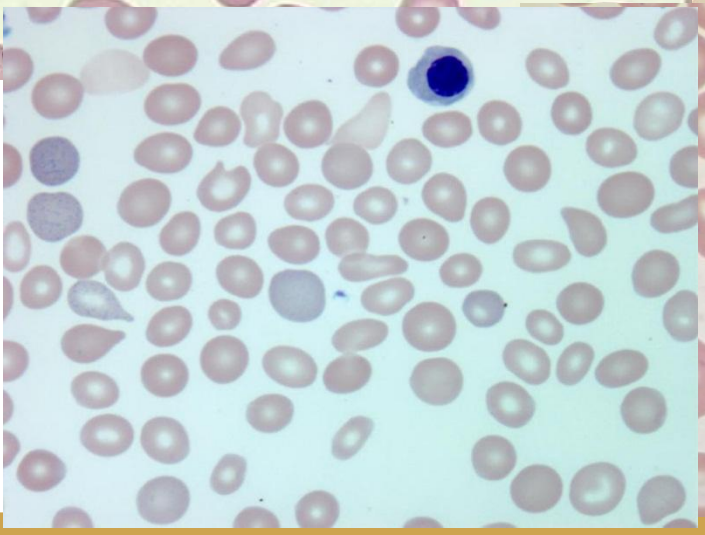
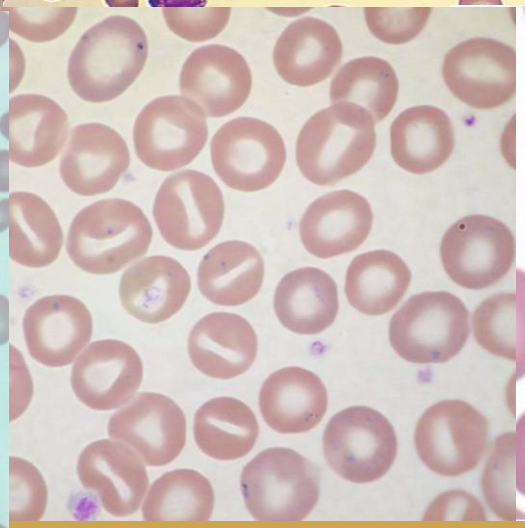
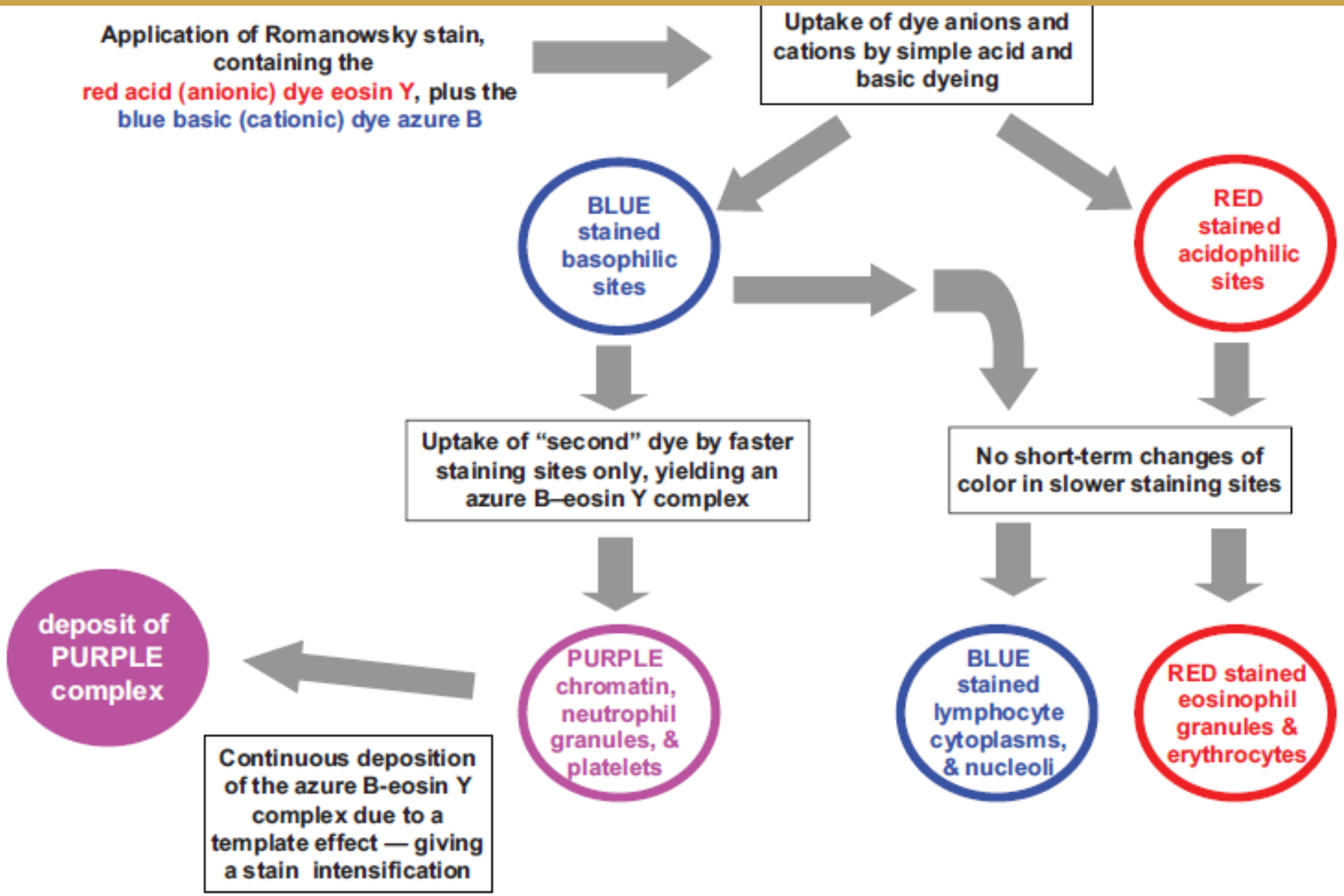
What are the colors you see?


SIR H N Reliance Foundation Hospital AND RESEARCH CENTRE



- Polychromasia is a distinctive feature, useful to distinguish morphologically related cells.

- Romanowsky giemsa effect (RGE): Selective complex formation producing highly polychrome images.

Romanowsky stains

- Giemsa
- Jenner
- Wright
- Field
- Leishman
- May-Grunwald-Giemsa,
- Wright-Giemsa,
- Jenner-Giemsa,
- Azure B-EosinY

Inherent instability of Romanowsky stains

- Precipitation of insoluble salts
  - minimized by minimum standing time
  - low dye concentration
  - low temperature
  - presence of large proportion of organic solvents- methanol, glycerol
  - excess basic dye concentration

- Inherent Dye impurity
  - Azure B

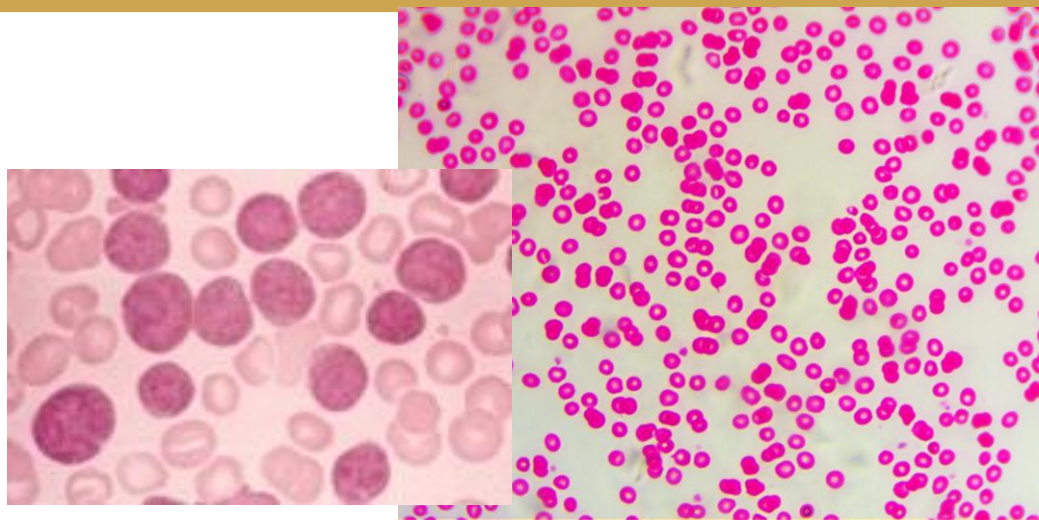
Variables involved

- Composition of solvent- unstable purple color with high concentrations of lower alcohols (methanol content)
- Ratio of acidic & basic dyes- Too blue/pink
- Fixative- formalin prevents purple coloring of nuclear chromatin, blue

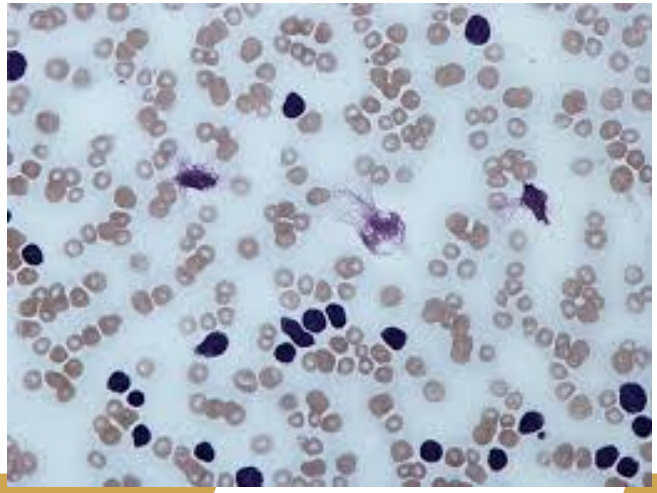
alcohol fix- shattered preparation, aldehyde- coherent structure

- pH value of phosphate buffer is very important- ideal 6.8-8
- Staining time based on specimen thickness
- Temperature

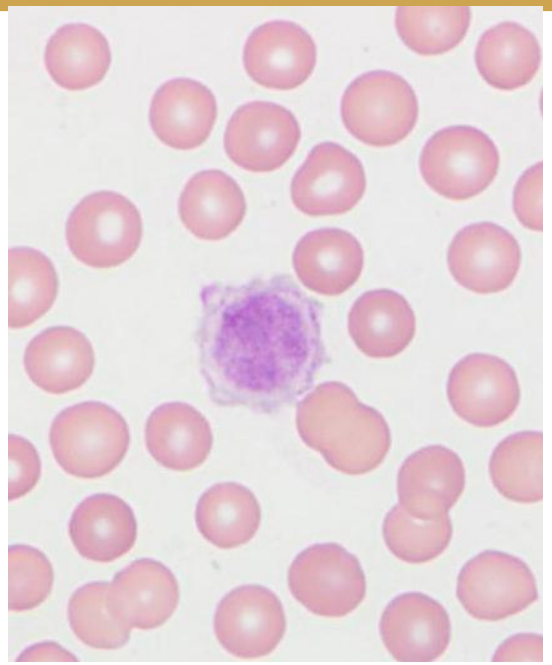
- Too PINK:
  - Improper azure B/eosin Y ratio
  - Impure dye
  - Low pH



- Too BLUE:
  - Improper azure B/eosin Y ratio
  - Stock stain exposed to light
  - Excess staining time
  - Thick film
  - Inadequate time in buffer solution



- Too PALE:
  - Old solution
  - Weak/Impure dyes
  - High temperature
- Nuclei too dark
  - Stain too concentrated
  - Incorrect staining time



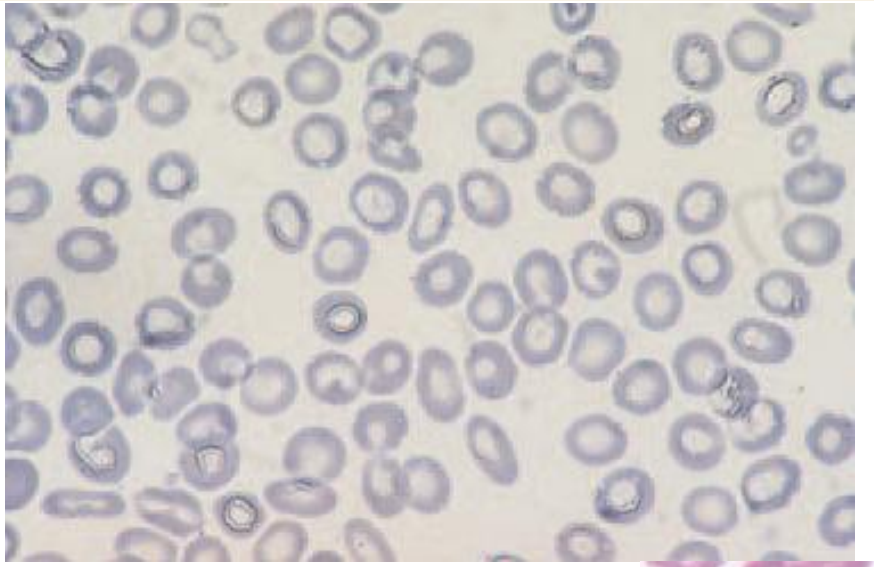
Artefact

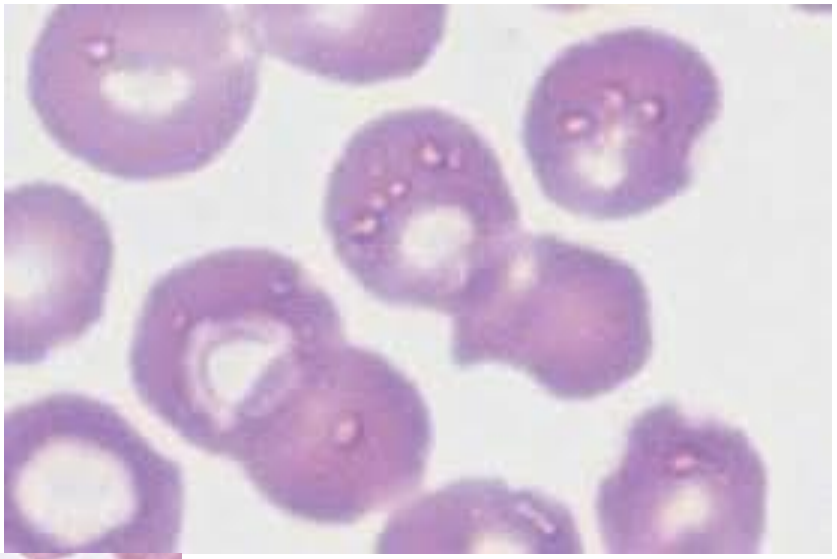
- Year introduced: 1992
- Any visible result of a procedure, caused by the procedure itself and not by the entity being analyzed.
- Abnormalities on blood smears can either be
  - Pathological
  - Artefact
- It is important to correctly identify artefacts so that they are not incorrectly interpreted as a pathological process.
- Can cause confusion and problems in diagnosis

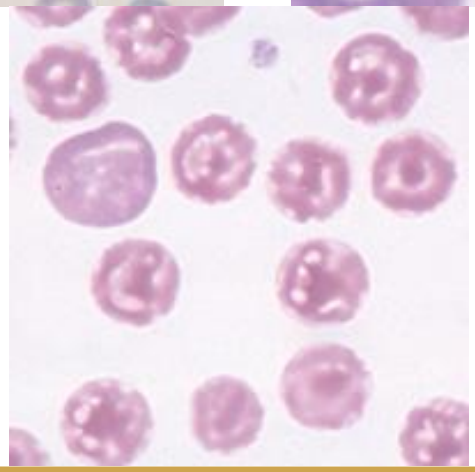
- Poor spreading techniques
- Slow drying in humid conditions- poorly or irregularly spread blood films, often with poor morphology.
- Insufficient or late fixation, and water containing fixing solutions.
- Tailing artefact- Poor distribution of leukocytes
- May result in RBCs that appear as spherocytes and increased WBCs, such as monocytes and neutrophils, in the tails. An incorrect differential will result. Always scan the film under low power to detect this aberration.

Fixation artefact

- Water in the methanol used for fixation of the blood film- refractile rings in red cells and makes it impossible to assess red cell morphology.
- Excessive water on the slide or in the stain producing refractile edges on erythrocytes

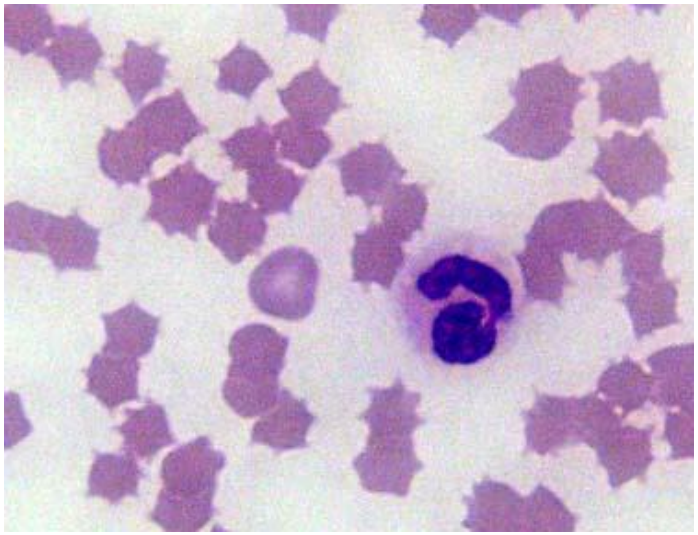






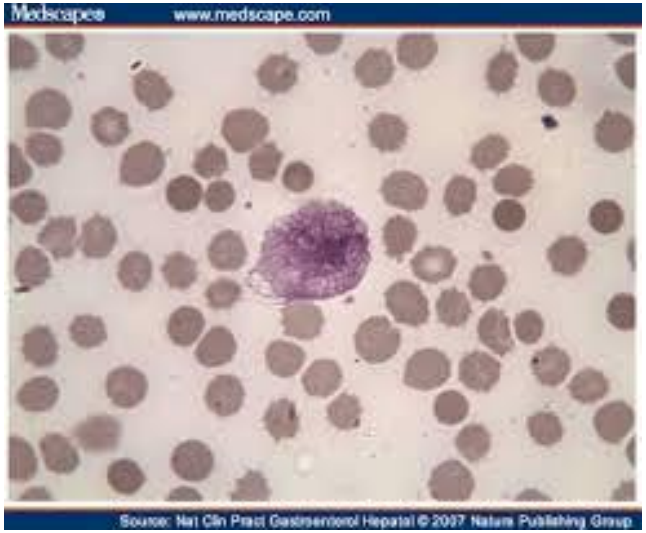
Storage artefact

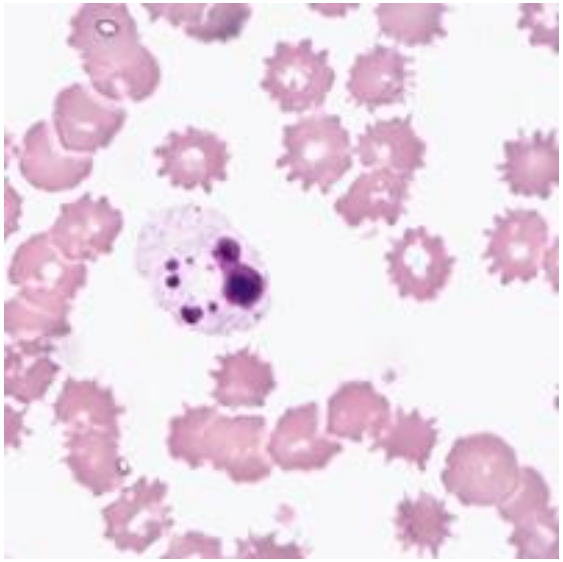
- White cells become fragile and may form smear/ smudge cells
- Neutrophil nuclei round up and form homogeneous round masses or a single mass, similar to NRBC.
- Red cells undergo an echinocytic change or crenation.
- Pseudo toxic changes, pseudoechinocytosis, and Platelet degranulation



Heat artefact

- Red cells bud off vesicles.
- Microspherocytes seen.
- White cells disintegrate.
- Proteins coagulate, producing weakly basophilic particles, similar in size to platelets.





CAUSES OF ARTEFACTS IN BLOOD FILM

- ***In vivo factors***
- Antibodies to blood cells- Agglutination (platelets, RBC's and WBC's)
- Increased plasma volume- Pseudo anemia
- Decreased plasma volume- Pseudopolycythemia
- Treatment related- Platelet Agglutination and pseudo–Pelger- Hue¨t anomaly

- ***Ex vivo factors***
- Anticoagulant
  - EDTA - Agglutination of leukocytes; agglutination, satellitism, degranulation of platelets; and precipitation of proteins
  - Citrate, oxalate, heparin- Agglutination of leukocytes and platelets
  - Nuclear lobulations, degeneration, pyknosis, rupture, Cytoplasmic granulation, vacuolization
- Overfilling of tubes- Pseudopolycythemia, pseudothrombocytopenia, and pseudoleukopenia
- Temperature - Agglutination of platelets, erythrocytes, and leukocytes

Poor slide preparation

May miss:

- Polychromasia
- Agglutination/rouleaux
- Poikilocytes, spherocytes, blister cells
- Parasites
- Degree of cytopenia/cytosis (tailing)
- Rare blast (thick/ thin slide, distorted WBCs)
- Inclusions

CAN LEAD TO SERIOUS MISTAKES!!

Peripheral blood smear preparation

"An ART"