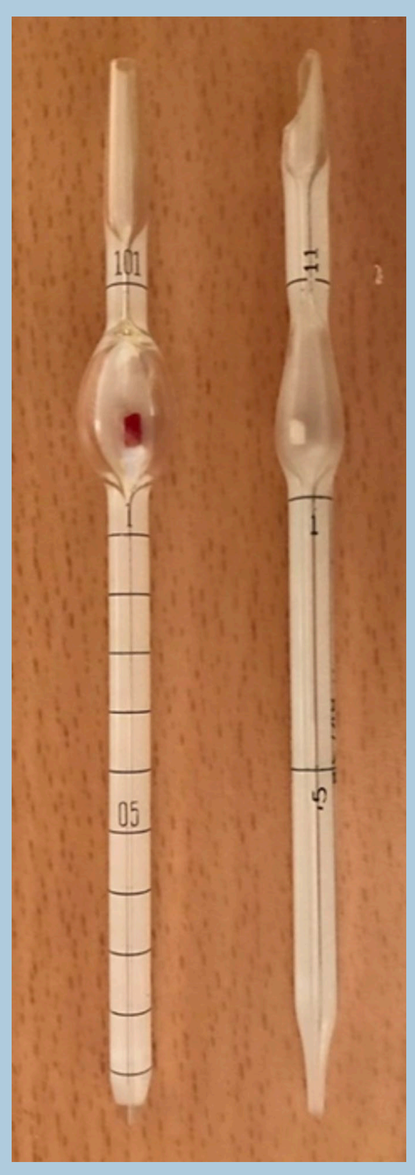
Blood testing: a role in haematology, coagulation and blood transfusion

While the term haematology was first used in the mid-18th century, it was not until the late 19th and early 20th centuries that the laboratory disciplines that encompass study of the cellular composition of blood, its clotting, and potential transfusion emerged.

Haemoglobin determination

- 1900: The Theodor Tallqvist method. A drop of whole blood was placed on a strip of blotting paper and compared with a set of colour standards on paper.
- 1901: The first colourimetric tests developed by WR Gowers and Haldane converted haemoglobin into measurable coloured solution and compared with a standard.
- 1902: Sahli's acid haematin method. Measurements of haemoglobin were taken from graduations on the sampling tube. The volume of acid used to match a coloured standard equated to haemoglobin concentration. Alternatively, coloured pigments were compared with a set of 'colour charts' to establish a quantitative value.
- 1942: The alkaline haematin method was developed where whole blood was added to sodium hydroxide and boiled. Alkaline haematin pigment is generated and compared to coloured standards. Drawbacks included the time taken to generate the pigment, which was too unstable or not representative of all forms of haemoglobin. This was solved using potassium cyanide/ferricyanide (in the form of Drabkin's reagent developed from a technique originally described by Sadie in 1920). Methods of quantitation evolved to use spectrophotometers. Cyanmethaemoglobin absorbs light at 540 nm and development of stable standards allowed for simple quantitation following Beer-Lambert's principles.

The Lovibond Comparator (IBMS Instrument Collection).



Diluting pipettes for red cell counting (left) and white cell counting (right).

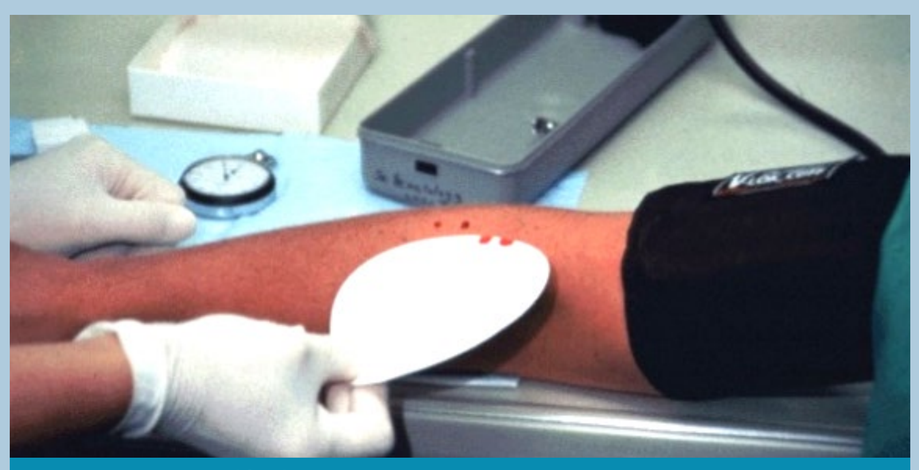
Blood cell counting

- Originally performed on diluted blood samples in a haemocytometer (counting chamber), a device invented in 1881 by Richard Thoma.
- The centre disc is divided into 400 squares, further divided into 25 groups of 16 fields. Diluted blood is put onto the disc, a coverslip applied and the cells counted.
- Thoma realised that different dilutions, and diluents, were needed for counting the different cells:
  - Red cells – Hayems Fluid (modified normal saline)
  - White cells – Turks Solution (Diluted glacial acetic acid)
  - Platelets – 1% ammonium oxalate
- Many iterations of the haemocytometer culminated in the Improved Neubauer haemocytometer. The Improved Neubauer method involves nine large etched squares each measuring 1 mm^2. The central square is further etched into 25 squares.
- Uses of haemocytometers:
  - Thoma, Improved Neubauer, Burker-Turk – Red cell, white cell and platelet counts on diluted whole blood.
  - Fuchs Rosenthal – Red cell and white cell counts in cerebrospinal fluid.
  - Platelets are counted in the whole central square.

Haemoglobinometer with graduated tube, standard solution, 20 cmm pipettes, N/10 hydrochloric acid and a cleaning brush (IBMS Instrument Collection).

Coagulation screening

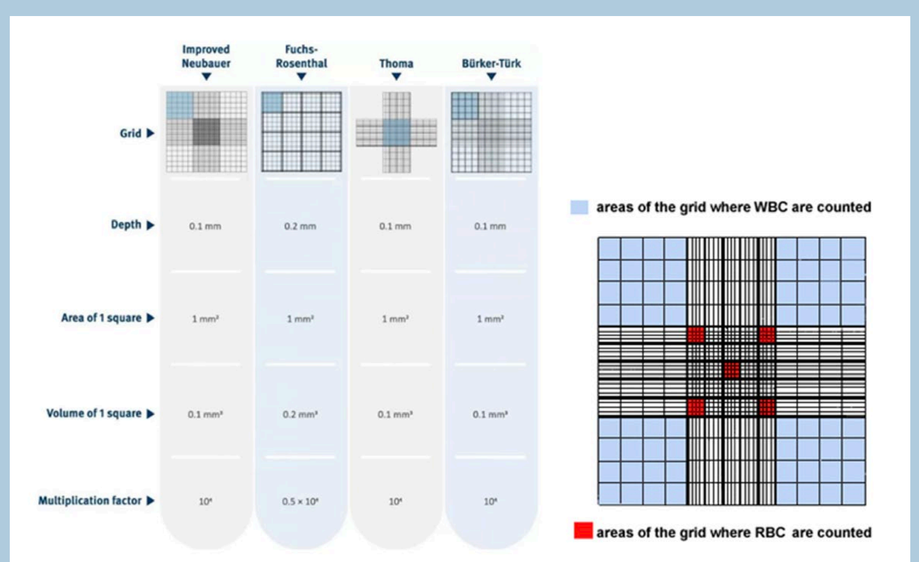
- 1901: Tests to measure haemostasis originated when Milian developed The Bleeding Time as a measure of platelet function. By this time, both fibrinogen and thrombin had been well characterised.
- 1911: Early manual methods to investigate bleeding disorders centred on the time taken to generate *in vitro* clots of whole capillary blood that were visible by eye. These tests tended only to be sensitive to severe fibrinogen deficiency.



Assessing a patient's bleeding time.

Plasma clotting times

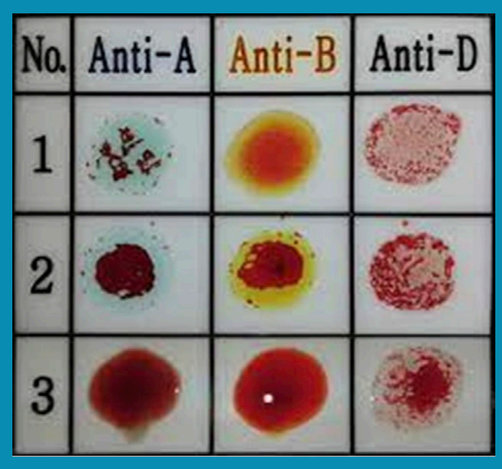
- 1935: Quick developed the prothrombin time (PT), which was based on the ability of a recalcified thromboplastin reagent to clot plasma. This was performed using glass tubes in a 370°C waterbath. This was further developed by Biggs and Douglas using citrated whole blood and a number of activators that could differentiate between haemophilia A and haemophilia B (The Thromboplastin Generation Test).
- 1953: Langdell and colleagues developed the partial thromboplastin time (PTT), which provided a simpler laboratory test to diagnose haemophilia.
- PT- and PTT-based tests: These could be used to differentiate disorders of what became known as the extrinsic and intrinsic coagulation pathways. The PTT was further modified to the activated partial thromboplastin time (APTT), which used negatively charged additive such as kaolin or cephalin.
- Thromboplastin: Over time, its source has evolved, from using human brain, rabbit brain to the current use of recombinants. The PT is a well-established test to measure the effectiveness of warfarin therapy as part of the International Normalised Ratio (INR).



Counting chamber details.

Initiating automation

- 1953: Having discovered the Coulter principle in the late 1940s, Wallace H Coulter was awarded a patent, and this heralded the development of subsequent automation. Coulter was influenced by the US attacks on Hiroshima and Nagasaki, which motivated him to improve and streamline cell counting for use in large-scale screening, as would be have been necessary in the event of a nuclear war.



Blood grouping by mixing antisera and whole blood on a tile surface.

Blood grouping

Early human blood transfusion experiments, generally involving animals, resulted in fatalities. By 1667, a law banning such experiments was passed.

- 1818: James Blundell performed the first successful human-to-human blood transfusion.
- 1901: Karl Landsteiner identified that fatalities were linked to red cell clumping caused by mixing of incompatible blood. Building on earlier work by Creite and Landon, he named and standardised the ABO blood groups.
- Blood Grouping by Hanging Drop: With a reference to bacterial methods, a drop of blood diluted to 5% in 0.6% saline was added to a drop of serum and observed for 'clumping'. The use of tubes by Moss followed soon after in 1910.
- The use of microscopes was frowned upon initially due to false positives, now known as rouleaux.
- 1939: Riddell described the use of tiles, whereby drops of antisera and whole blood were placed on a ceramic surface. This called for very careful technique in order to prevent cross-contamination or spillages.

Crossmatching / compatibility testing

- 1908: Moreschi described the antiglobulin reaction to visualise antigen-antibody reactions. Antigen and antibody react with each other, then, after washing to remove any unbound antibody, antiglobulin reagent is added and binds between the antibody molecules bound to the antigen. This facilitates the observable agglutination and is the origin of the Indirect Antiglobulin Test (IAT; also known as the Coombs Test) in use today.
- 1909: The first crossmatch was performed by Reuben Ottenberg, acting upon a suggestion made by Hektoen in 1907. Blood samples from the donor and recipient were added in a test tube and observed for clumping.
- 1914–1918: Successful crossmatching had a major impact on treatment of soldiers injured in the First World War, during which blood transfusions saved many lives.

Blood Lines:
A Resource Not To Be Taken In Vein
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