Haematology
what does your blood test mean?

This document provides some reading material to be used in preparation for the “Haematology – what does your blood test mean?” workshop at RMIT’s Experience Health and Medical Science day.

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What is haematology?

Haematology is the study of the cells and proteins found in blood. A diagnostic haematology laboratory is usually divided up into four main areas:

1) Routine haematology – full blood examinations, morphology + other tests.
2) Coagulation – testing for the proteins and cells involved in clotting.
3) Blood bank – blood and blood product transfusions.
4) Special tests – performed only when required.

There are three main types of cells in your blood:

1) Red blood cells (erythrocytes) transport oxygen around your body and remove waste products and carbon dioxide.
2) White blood cells (leucocytes) are responsible for fighting infections and invading pathogens (e.g. viruses or bacteria).
3) Platelets (thrombocytes) that help your blood clot if you are bleeding.

In the laboratory we use microscopes, experiments and hi-tech laser analysers to detect changes in the number of these cells. The way they look, as well as changes in the concentration of proteins, can then be used to diagnose disease. For example, people who have leukaemia usually have an increase in the number of white blood cells, as well as changes in the way they look under the microscope.

Haematology also includes compatibility testing for blood transfusions. Before a patient can be transfused with blood or blood products we need to know what blood group they are so that they can be transfused with the correct products or blood. If we don’t do this testing before the patient is transfused there is a chance a reaction could occur and these can kill a patient.

Coagulation testing is another important section of the haematology laboratory. Here the patient’s blood can be tested to diagnose clotting disorders like DVT (deep vein thrombosis), bleeding disorders like Haemophilia and to monitor patients who are taking anticoagulant drugs like warfarin or heparin (to prevent blood clotting).

Once we have worked out if there is anything wrong with a patient we can tell the doctor. Laboratories will send out a report of all of the patient’s test results and the treating doctor will then use this information to work out how sick the patient is and, how they will treat the patient e.g. does the patient need to go to hospital, or need antibiotics, or to go and see a specialist or should they go home to bed and rest for a few days.
Haemopoiesis

*Haemopoiesis* is the name given to the process of blood cell production (includes erythrocytes, leucocytes and platelets). The word is derived from the Greek words *haem* meaning ‘blood’ and *poiesis* meaning ‘to form’. Blood cell production starts with the primitive haemopoietic stem cell; all blood cells originate from this stem cell (figure 1).

Where does haemopoiesis occur?

The site of haemopoiesis depends on how old you are. In the developing foetus, haemopoiesis occurs in the yolk sac but by birth it happens only in the bone marrow (figure 2).

The bones in adults involved in haemopoiesis are the sternum (breast plate), vertebrae, cranium, iliac crests (the bones of the pelvis and hips) and the ends of the long bones, e.g. femur.
Function of leucocytes (white blood cells)

Leucocytes may be divided into two categories:

1. Myeloid cells comprising granulocytes (neutrophils, eosinophils and basophils) and monocytes.
2. Lymphocytes comprising B cells and T cells.
   Once lymphocytes are mature, they enter the circulation and migrate to specific regions in the lymph nodes, spleen and lymphoid tissue of the gut and respiratory tract.

The way leucocytes protect the body from infection is complex and closely regulated by the immunoglobulin (antibody) and complement systems. Leucocytes and protein factors work together to protect the body from pathogens (e.g. viruses or bacteria). Neutrophils are non-specific phagocytic cells and will attack anything foreign in the body whereas lymphocytes are involved in antibody production, or they can directly attack and destroy foreign cells. Leucocytes can also help the body look for cells that maybe malignant (i.e. cancer cells).

Blood components

Blood differs from all of the other tissues in the body in that it has a large liquid component, which gives it characteristic fluid properties. Like all other tissues, blood is composed of a number of different cells, each with a unique function. These cells are erythrocytes (red blood cells), leucocytes (white blood cells) and platelets. Because blood is a fluid, it has to be contained in a closed system otherwise it would leak out in your body. This closed system is called the vascular system (blood vessels).

Function of blood

- Transport of oxygen
- Transport of essential nutrients required for metabolism
- Transport of waste materials
- Transport of cells involved in the defending the body against pathogens and toxins
- Heat exchange mechanism

These functions are all part of the process of homeostasis i.e., the maintenance of a balanced steady state. Your heart is an extremely efficient pump, which is capable of recirculating our total blood volume every 10-12 minutes. In an average adult the blood volume is approximately 5 litres. Occasionally the vascular system (blood vessels) may “spring a leak” and blood will be lost, this is called bleeding or haemorrhage.

Blood is a tissue consisting of cells suspended in a fluid called plasma. Figure 3 shows a sample of blood that has been collected into an anticoagulant (substance to stop blood from clotting) and then centrifuged to separate the red cells and plasma. The proportion of cells and plasma in a healthy adult is indicated below although the amounts will vary slightly depending on the age and sex of the individual. Adult males usually have more red cells than adult females.
Erythrocytes (red blood cells/RBC):

These appear at the bottom of the tube as they have the greatest density i.e. the heaviest. Red blood cells are mainly involved in the transport of oxygen and removal of carbon dioxide.

Buffy coat (leucocytes/WBC and platelets/PLT):

On top of the red cells is a greyish-white layer which is called the 'buffy coat. This layer consists of the leucocytes or white cells and platelets. Leucocytes are part of the body’s defence mechanism, whilst platelets are the cellular part of the haemostatic (clotting) system.

Plasma:

Above the buffy coat is a pale straw-coloured fluid called plasma. Plasma contains hundreds of different substances - proteins, vitamins, hormones and minerals. There are some substances present in plasma, which are of interest to the haematology laboratory. These are the coagulation proteins, which are involved in the clotting of blood.

Serum:

If blood is allowed to clot and then left for a while, the clot will shrink and a straw coloured fluid appears above the clot. This fluid is called serum. For some laboratory tests, serum is the specimen of choice. Serum is almost the same as plasma except there are no clotting proteins present.

Collection of blood samples for testing in the laboratory

Blood samples for testing are collected from the venous circulation using a needle and syringe or the vacutainer system. The process of collecting blood from a patient is called venesection. If a sample of blood is removed from the body and placed in a tube the blood will eventually solidify; this process is called clotting. Anticoagulants can be used to stop the clotting process. The common anticoagulants used are Ethylenediamine tetra acetic acid (EDTA), sodium citrate and heparin.

Ethylenediamine tetra acetic acid (pink/purple top tube):

Ethylenediamine tetra acetic acid (EDTA) is a powder and blood collected into it does not clot. On collection blood must be gently mixed so that the EDTA is dissolved in the blood. EDTA prevents blood from clotting by binding or chelating (binding) the calcium ions.
from the blood (calcium ions are necessary for blood to clot). Blood collected into EDTA is used for routine haematology tests.

**Figure 4: EDTA tube**

*Trisodium Citrate (light blue top tube):*

Trisodium citrate is a liquid anticoagulant that is used to collect blood for coagulation studies. Samples must be gently mixed immediately after collection. Trisodium citrate chelates calcium ions from the blood (calcium ions are necessary for clotting).

**Figure 5: Trisodium citrate tube**

*Heparin (green top tube):*

Heparin is an anticoagulant used for biochemistry tests and some specialised haematology tests. Heparin prevents blood coagulation by inhibiting the action of thrombin. Thrombin is an activated coagulation protein that converts fibrinogen to fibrin. Fibrin formation occurs when blood clots.

**Figure 6: Heparin tube**
What do blood cells look like?

Erythrocytes

On a stained blood film, normal red cells are mainly round in appearance however in-vivo (in your body) red cells have a biconcave ‘donut’ shape. When examining red cells there are three features that must be assessed. These are size, colour and shape.

![Figure 7: Normal red blood cells](image)

Red cells that are smaller than normal are called microcytic. Red cells that are larger than normal are macrocytic (figure 8). Microcytosis is seen in iron deficiency anaemia and thalassaemia. Macrocytes are seen in vitamin B_{12} and folate deficiency and liver disease. Variation in shape and colour of red cells occur in many diseases especially when anaemia is present.

![Figure 8: Variation in red blood cell size](image)

Neutrophils

Neutrophils are the most common white blood cell in blood. Sometimes in inflammatory and infectious conditions there is an increase in neutrophils (neutrophilia), the granules may appear more heavily stained and dark blue (toxic granulation) and vacuolation (holes) in the cytoplasm is present. Neutrophils have a multi-lobed nucleus (3-5 normally).

![Figure 9: Neutrophil](image)
Band form neutrophil

In normal blood, a small percentage of circulating neutrophils are known as band forms. These are slightly immature neutrophils. An increase in band forms is often referred to as a left shift and is common in infection.

Basophils

Basophils are the least common cells in the blood and are characterised by prominent, large densely stained blue-black granules. Often the granules overlie and obscure the nucleus, which contains 2-3 lobes.

Eosinophils

Eosinophils derive their name from the strong affinity granules in their cytoplasm have for the stain, eosin. This stain gives the granules their bright orange red colour. These cells are often increased in allergies and parasitic infections (figure 12).
Monocytes

Monocytes have abundant cytoplasm that stains a blue-grey colour. The nucleus is often irregular, indented or kidney shaped and may be folded over upon itself (figure 13).

![Figure 13: Monocyte](image13.png)

Lymphocytes

This is a typical small lymphocyte (figure 14). It is smaller than any of the other leucocytes (slightly larger than a red cell) and is composed almost entirely of nucleus with only a rim of cytoplasm. The cytoplasm is bright blue and sometimes contains a few scattered reddish granules.

![Figure 14: Small lymphocyte](image14.png)

In viral infections such as Infectious Mononucleosis (glandular fever), many lymphocytes are large and irregularly shaped. These cells are called reactive lymphocytes or atypical lymphocytes (figure 15).

![Figure 15: Atypical lymphocyte](image15.png)
Platelets

In stained blood films, platelets appear as small blue/purple bodies scattered between the red cells and are involved in the clotting process (figure 16).

Figure 16: Platelets

Compatibility Testing

Determining a patient’s blood group and detecting antibodies to red cell antigens is one of the most important tests undertaken in diagnostic pathology laboratories. Donor blood is given to seriously ill patients on the basis of the results provided by scientists. If errors in blood grouping or antibody detection have been made, reactions can be very serious and could result in the death of the patient.

ABO and Rhesus blood groups

The ABO and Rhesus (Rh) blood group systems are two of the most important blood group systems involved in compatibility testing as the antigens in these systems are highly immunogenic.

ABO blood group

Discovered in 1901 by Karl Landsteiner. Inheritance of the ABO antigens follows the normal Mendelian inheritance patterns. One ABO gene is inherited from your mother & one from your father resulting in expression of your ABO blood group. Four different blood group expressions are possible in this system as indicated in Table 1. The frequencies of each blood group in the Australian Caucasian population are shown.

Table 1: ABO blood groups and frequency in the Australian Caucasian population.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>39.0%</td>
</tr>
<tr>
<td>B</td>
<td>11.4%</td>
</tr>
<tr>
<td>O</td>
<td>46.1%</td>
</tr>
<tr>
<td>AB</td>
<td>3.5%</td>
</tr>
</tbody>
</table>
Antibodies to ABO group antigens

Naturally occurring antibodies to the A and B antigens are produced from about the 4th month after birth and remain with the individual throughout life. Table 2 shows the naturally occurring antibodies in individuals with the different ABO groups.

These antibodies are capable of attaching to the corresponding antigen on a red cell surface and directly producing haemagglutination reactions. Since everybody, except those with the AB blood group, has antibodies to the A and/or B antigens, it is essential that red cells of the same blood group as the recipient is transfused. If mismatched red cells are transfused they will be immediately targeted by the recipient's antibodies and destroyed. The consequence of this destruction may result in the death of the recipient.

<table>
<thead>
<tr>
<th>ABO group</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>anti-B</td>
</tr>
<tr>
<td>B</td>
<td>anti-A</td>
</tr>
<tr>
<td>O</td>
<td>anti-A and -B</td>
</tr>
<tr>
<td>AB</td>
<td>none</td>
</tr>
</tbody>
</table>

The ABO blood group is determined using potent antibodies to the A and B antigens. If red cells have the corresponding antigen to the antibody they will agglutinate. This test is called the forward group and provides the ABO group of the individual being tested.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Anti-A</th>
<th>Anti-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = positive reaction, 0 = negative reaction

Rh blood group system

Two different groups discovered the Rh blood group system in the early 1940s.

The most important of the Rh antigens is the Rh D antigen as this is the next most immunogenic blood group antigen after A and B. The frequency of the Rh D antigen varies between racial groups. The frequency in caucasians is 85% whereas most individuals of oriental background are almost always Rh D antigen positive.

To determine if a person has the Rh D antigen, their cells are mixed with potent antibodies to the D antigen. If red cells have the corresponding Rh D antigen to the Rh D antibody they will agglutinate.
Table 4: Reactions of Rh D blood groups.

<table>
<thead>
<tr>
<th>Rhesus D antigen</th>
<th>Anti-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh D positive</td>
<td>+</td>
</tr>
<tr>
<td>Rh D negative</td>
<td>0</td>
</tr>
</tbody>
</table>

As blood groups are genetically determined characteristics they can be used to distinguish between individuals.