

Safe Blood and Blood Products

Module 3

Blood Group Serology



World Health
Organization

Safe

Blood

and

Blood

Products

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Serology**



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Organization

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Preface

Safe Blood and Blood Products is a series of interactive learning materials developed by the World Health Organization (WHO). The materials have been designed for use in distance learning programmes in blood safety, although they can also be used for independent study or as resource materials in conventional training courses and in-service training programmes.

The materials have been produced for staff with responsibility for donor recruitment and retention, and for the collection, testing and issue of blood for transfusion. They comprise the following modules:

- Introductory Module: *Guidelines and Principles for Safe Blood Transfusion Practice*
- Module 1: *Safe Blood Donation*
- Module 2: *Screening for HIV and Other Infectious Agents*
- Module 3: *Blood Group Serology*.

The English edition was first published in 1993. French, Spanish, Russian, Chinese and Portuguese editions have since been produced and the materials have also been translated into a number of national languages.

This second, updated edition of the materials has been developed to reflect changes in transfusion medicine and laboratory technology since the publication of the first edition.

Distance learning in blood safety

Since the publication of *Safe Blood and Blood Products*, WHO has held a series of regional and sub-regional workshops for senior blood transfusion service personnel from over 100 countries on establishing national distance learning programmes in blood safety. Programmes have since been established in every region of the world, using the WHO learning materials.

Part of the follow-up to the workshops has been the production of *Establishing a Distance Learning Programme in Blood Safety: A Guide for Programme Coordinators*. This provides a practical guide to the planning, implementation and evaluation of a distance learning programme in blood safety.

Other WHO learning materials

The Clinical Use of Blood consists of an open learning module and pocket handbook which provide comprehensive guidance on transfusion and alternatives to transfusion in the areas of general medicine, obstetrics, paediatrics and neonatology, surgery and anaesthesia, trauma and acute surgery, and burns. They are designed to promote a reduction in unnecessary transfusions through the wider use of plasma substitutes and more effective prevention and treatment of the conditions that may make transfusion necessary.

WHO has also published recommendations on *Developing a National Policy and Guidelines on the Clinical Use of Blood* which encourage the use of the learning materials in education and training programmes to promote effective clinical decisions on transfusion.

Additional learning materials in the *Safe Blood and Blood Products* series that are available or in development by the WHO Department of Blood Safety and Clinical Technology (WHO/BCT) include:

- *Costing Blood Transfusion Services*
- *The Blood Cold Chain*
- *Safe Blood Collection*
- *Blood Components Production.*

More detailed information on these materials and other documents and publications related to blood transfusion is available from WHO/BCT, which also issues regular reports on evaluations of the operational characteristics of many commercially available screening assays for transfusion-transmissible infections.

Information can be obtained from the BCT section of the WHO website at <http://www.who.int/bct> or by contacting WHO/BCT at WHO Headquarters or WHO Regional Offices.

Dr Jean C. Emmanuel
Director, Blood Safety and Clinical Technology
World Health Organization

1

Introduction to Module 3

The purpose of this section is to introduce you to Module 3: *Blood Group Serology* which focuses on blood grouping and compatibility testing.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Explain the purpose of Module 3.
 - 2 Identify a personal “supporter” who can assist you throughout your work on this module.
 - 3 Assess your current knowledge, skills and experience in relation to the objectives of this module.
 - 4 Make a realistic Study Plan for your work on this module.
-

1.1 THE DISTANCE LEARNING MATERIALS

Module 3: *Blood Group Serology* is part of a series of distance learning materials, *Safe Blood and Blood Products*, developed by the World Health Organization Blood Transfusion Safety Team (WHO/BTS). These materials have been designed to provide access to training for staff working in blood transfusion services, hospital blood banks and public health laboratories who have limited opportunities to attend conventional training courses.

The other modules in this series are:

- Introductory Module: *Guidelines and Principles for Safe Blood Transfusion Practice*
- Module 1: *Safe Blood Donation*
- Module 2: *Screening for HIV and Other Infectious Agents*.

You should already be familiar with the way in which this distance learning programme operates from your work on the Introductory Module and other modules in the series. If you have not yet read the Introductory Module, it is essential to do so before studying this module so that you understand how the programme is organized. In particular, make sure that you read Section 1 which explains the role of your trainer and supporter and how to use the learning materials, especially Section 1.2 on pages 5–8 which describes the following features of the modules:

- module objectives
- sections
- learning objectives
- activities
- action list and action plan
- summary
- self-assessment
- progress check
- glossary
- appendices
- offprints.

Using Module 3

You should find this module useful if you work in a blood transfusion service, hospital blood bank or public health laboratory and are responsible for ABO and Rhesus (Rh) blood grouping and compatibility testing. You should also find it helpful if you are involved in the selection and issue of blood and blood components.

You may also find Module 3 of interest if you are a senior member of the laboratory technical staff or of the medical staff, such as a hospital medical superintendent, and are responsible for training or supervising staff who are involved in any aspects of serology. In this case, the module will offer basic refresher and updating material for your own use as well

as a comprehensive resource that you can integrate into your own training programmes.

Module 3 may contain some material which is new to you. Take as much time as you need to read through each section and mark anything that you find difficult. Then go back to those parts and reread them until you feel sure that you are able to understand them. If you still find them complicated or are unable to complete some of the activities, seek help from your trainer, your supporter or another senior colleague. Don't be afraid to ask for assistance since what you are learning is extremely important and will directly benefit the centre in which you work.

Some sections may contain material which is already familiar to you. If so, read them through carefully as a means of revision and answer the self-assessment questions to make sure that your knowledge and practice are completely up to date. Complete the activities – they will provide you with a further check on your understanding and will help you to identify any improvements that you can make in your practice.

1.2 BEFORE YOU BEGIN THIS MODULE

You should already have completed the Introductory Module and may also have worked through other modules in this distance learning programme. During this period, you should have been in regular contact with your trainer. You should already have had an opportunity to discuss the work you will be undertaking on Module 3 but, if this has not yet been possible, contact your trainer before you begin this module.

When you started working through the Introductory Module, you were asked to identify someone, ideally your supervisor, who would act as your personal “supporter”. You should have been able to find someone who was willing to meet with you regularly to discuss your progress and provide assistance and support, particularly when you were developing and implementing your Action Plan. You now need to choose a supporter for your work on this module – perhaps the same person or another senior colleague who has experience in blood grouping and compatibility testing.

ACTIVITY 1

Think about the people with whom you work, particularly your supervisor and other senior colleagues, who could support you while you are working through Module 3. Identify one person whom you think would be willing to spend some time talking to you periodically about your work on this module and helping you with any problems that you might face. Remember that it is important to choose someone who is prepared to discuss your ideas about ways of improving the service and to assist you in planning and implementing any changes that you identify as being needed as a result of your work on this module.

Check that the person you have identified is prepared to help you. If you have chosen a different supporter from the person you selected

for your work on other modules, explain how the learning programme operates and what the role of the supporter involves. Show this module to your supporter so that they become familiar with its content and approach. When you are preparing your Study Plan, arrange regular meetings to discuss your progress.

If you have any difficulty in finding a suitable supporter in your workplace, talk to your trainer who will help to find someone to support you.

Even though your supporter will be your main source of assistance, you will also find it helpful to discuss your work on this module with your colleagues, particularly those who are also involved in blood grouping and compatibility testing.

1.3 MODULE 3: BLOOD GROUP SEROLOGY

Blood transfusions are usually life-saving, but there are occasions when they cause degrees of morbidity and even mortality. Regrettably, some of these deaths are caused by poor laboratory practice, such as errors in blood grouping or compatibility testing, perhaps because the person performing the tests does not understand the scientific principles or is unable to interpret the results accurately. On other occasions, it is because blood or plasma has been selected or issued incorrectly.

This module is therefore designed to help you to strengthen your knowledge and skills in blood group serology to ensure that blood is always safely and correctly issued for transfusion by your blood bank.

Section 1: *Introduction to Module 3* outlines the contents of the module and contains activities to help you to prepare for your work on it.

Section 2: *The Components and Functions of Whole Blood* describes the constituents of blood and explains their importance.

Section 3: *Basic Blood Group Immunology* focuses on antigens, antibodies, the antibody immune response and antigen–antibody reactions.

Section 4: *The ABO Blood Group System* considers the importance of the ABO blood group system in blood transfusion practice. It explains basic genetics and the inheritance of blood groups, the development of A and B red cell antigens, the subgroups of the A antigen and high-titre antibodies.

Section 5: *The Rh Blood Group System* considers the importance of the Rh (Rhesus) blood group system in blood transfusion practice. It explains the genetics of the Rh system, the importance of correct RhD typing and testing for the D^u antigen.

Section 6: *Compatibility Testing and Issuing Blood* focuses on compatibility testing and the procedures for selecting and issuing blood, both routinely and in emergencies. It also covers procedures for investigating transfusion reactions, record-keeping and managing stocks of blood and plasma.

Section 7: *Techniques for Blood Grouping and Compatibility Testing* focuses specifically on the main techniques used in blood grouping and compatibility testing. Instructions for performing these techniques are provided in Appendix 1.

Section 8: *Action Plan* is the final section in which you are asked to review all the ideas you have included in your Action List and to prepare an Action Plan as a basis for improving working practices in your laboratory. You will find the Action List for Module 3 on page 96.

1.4 MODULE OBJECTIVES

There are six overall objectives for this module which specify what you should be able to do as a result of reading the text, answering the self-assessment questions, completing the activities and preparing your individual Action Plan.

When you have finished working through this module, you should be able to achieve the following objectives:

Section 2

Explain the functions of the main components of blood and their significance in blood transfusion practice.

Section 3

Explain the red cell antigen–antibody reaction and the factors that affect it.

Section 4

Explain the ABO blood group system and use the results of cell and reverse ABO grouping tests to identify the blood groups of donors and patients.

Section 5

Explain the Rh blood group system, and identify when to use RhD positive or RhD negative blood and when to test for the weak D (D^u) antigen.

Section 6

Explain the importance of compatibility testing and develop and maintain appropriate procedures and records for the safe request, selection and issue of blood under routine and emergency conditions.

Section 7

Explain the principles of the main techniques used in blood grouping and compatibility testing and perform them safely and accurately.

ACTIVITY 2

Before you begin work on Section 2, you will find it helpful to assess your current level of knowledge, skill and experience in relation to the module objectives and to decide what you want to achieve by working through the module. Look carefully at the module objectives and, for each one, decide whether you have:

<i>Module objectives</i>	<i>Rating (1–4)</i>	<i>Comments</i>
<p><i>Section 2</i> Explain the functions of the main components of blood and their significance in blood transfusion practice.</p> <p><i>Section 3</i> Explain the red cell antigen–antibody reaction and the factors that affect it.</p> <p><i>Section 4</i> Explain the ABO blood group system and use the results of cell and reverse ABO grouping tests to identify the blood groups of donors and patients.</p> <p><i>Section 5</i> Explain the Rh blood group system, and identify when to use RhD positive or RhD negative blood and when to test for the weak D (D^u) antigen .</p> <p><i>Section 6</i> Explain the importance of compatibility testing and develop and maintain appropriate procedures and records for the safe request, selection and issue of blood under routine and emergency conditions.</p> <p><i>Section 7</i> Explain the principles of the main techniques used in blood grouping and compatibility testing and perform them safely and accurately.</p>		

- 1 A high level of knowledge, skill and experience.
- 2 A reasonably good level of knowledge, skill and experience.
- 3 Some knowledge, skill and experience.
- 4 Little or no knowledge, skill or experience.

The objectives are repeated in the table above. Note down your rating (1, 2, 3 or 4) for each objective and add any comments you wish to make. Note any objectives that relate to areas of work that you do not currently undertake.

You have now identified the areas that will be mostly revision for you and the areas to which you need to pay particular attention. The module objectives are designed to help you to assess your own progress. When you reach the end of the module, you will be asked to look back at them to check whether you feel that you have achieved them. The most important question to ask yourself then is whether you feel that you can do your job better as a result of your work on this module. If you feel that you would like to improve your knowledge, understanding and skills

further, think carefully about the topics you would like to learn more about. Then talk to your supporter, supervisor or trainer about how you can achieve this.

1.5 PLANNING YOUR STUDY

Since you should already have completed the Introductory Module and, perhaps, other modules, you should be able to make a reasonable estimate of the amount of time that you will need to spend on Module 3. Some of the activities may be time-consuming, but remember that you will be able to complete most of them during the course of your normal work.

ACTIVITY 3

Look quickly at the other sections in this module to get an idea of the content, level and approach and to assess how much of the material is likely to be new to you. Also look at some of the activities to assess the kind of work that will be involved.

Try to estimate how much time you will need to study each section, including answering the self-assessment questions and completing the activities. Remember that you will also need to allocate time to meet with your supporter and trainer and to prepare your Action Plan. Then talk to your supervisor about the amount of time you could be allocated each week, or each month, for your work on Module 3.

Now fill in the Study Plan on page 8. Copy the ratings of your knowledge, skills and experience from Activity 2 as they are an indication of how much time you will need to spend on each section. Then add the dates by which you plan to complete each section, taking into account your current knowledge, skills and experience in relation to each module objective and the time you are likely to have available for study. When you have arranged dates for meetings or other contact with your trainer and supporter, add these to your Study Plan.

SUMMARY

- 1 Module 3 focuses on blood grouping and compatibility testing and the correct issue of blood for transfusion.
- 2 You should identify a personal supporter to provide ongoing support while you work through this module.
- 3 Before starting work on Module 3, you should review your knowledge, skills and experience in relation to the module objectives.
- 4 A realistic Study Plan will help you to organize your work on this module.

STUDY PLAN

Section	Rating (1–4)	Planned completion date	Meeting dates	
			with trainer	with supporter
Section 2 The Components and Functions of Whole Blood				
Section 3 Basic Blood Group Immunology				
Section 4 The ABO Blood Group System				
Section 5 The Rh Blood Group System				
Section 6 Compatibility Testing and Issuing Blood				
Section 7 Techniques for Blood Grouping and Compatibility Testing				
Section 8 Action Plan				

Notes

PROGRESS CHECK

Before moving on to Section 2, spend a few minutes thinking about whether you have achieved the learning objectives for Section 1. These were to:

- 1 Explain the purpose of Module 3.
- 2 Identify a personal “supporter” who can assist you throughout your work on this module.
- 3 Assess your current knowledge, skills and experience in relation to the objectives of this module.
- 4 Make a realistic Study Plan for your work on this module.

If you feel confident that you have understood everything, turn to Section 2. If you feel that you need more information about Module 3 or the learning programme as a whole, contact your trainer to discuss anything you are unsure about or talk to your supporter.

2

The Components and Functions of Whole Blood

The purpose of this section is to help you to understand the constituent parts of blood and their functions.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Describe the main components of blood.
 - 2 Explain the function of these components of blood and their importance in transfusion practice.
-

plasma: The fluid part of blood which carries the cells and other substances such as proteins, clotting factors and chemicals.

erythrocyte: The red blood cell (the most numerous blood cell), which contains the red pigment haemoglobin and is responsible for transporting oxygen to the body tissues.

leukocyte: A family of nucleated white cells involved in fighting infection and making antibodies.

thrombocyte: A blood platelet, which plays a major role in the blood clotting mechanism.

coagulation: Clotting of blood which takes place when blood is collected into a dry container or reaches an open wound.

reticuloendothelial system: A collection of endothelial cells that produce macrophages or large mononuclear cells. They are found in the bone marrow, liver, spleen and lymph glands.

haemoglobin: A red fluid found in the red blood cells which is made up of iron (haem) and polypeptide chains (globin).

2.1 WHOLE BLOOD

Blood is a complex fluid consisting of different blood cells suspended in a yellowish liquid called **plasma**. The blood cells comprise a mixture of red cells (**erythrocytes**), white cells (**leukocytes**) and platelets (**thrombocytes**). The plasma contains many different proteins, chemical substances, clotting (**coagulation**) factors and numerous metabolic substances.

Blood serves as a transport medium for carrying all its different components to the different organs of the body.

2.2 RED BLOOD CELLS (ERYTHROCYTES)

Red cells appear under the microscope as biconcave discs (see Figure 1). They are extremely small, with a diameter of 7.2 microns. There are approximately 5 million of these cells in each cubic millimetre (mm³) of blood ($5 \times 10^{12}/L$). They are made in the bone marrow and, when they are mature, enter the bloodstream where they have a lifespan of approximately 120 days. After this, they break down and are removed by cells of the **reticuloendothelial system**. These cells are highly specialized and are scattered throughout the body. They are found mainly in the bone marrow, liver, spleen and lymph glands.

Red cells are filled with a substance called **haemoglobin** and their primary function is to carry oxygen to the body tissues.

2.3 HAEMOGLOBIN

Haemoglobin is a large complex molecule made up of iron-containing molecules called haem which are attached to polypeptide chains called globin. Haemoglobin is a red fluid found in the red blood cells. It has the ability to combine reversibly with oxygen and carbon dioxide. Its main function is to transport oxygen to the various tissues to provide the body with its energy and heat. Oxygen is taken up in the lungs and pumped by the heart to the tissues. After it has been used, the oxygen is replaced by unwanted carbon dioxide which is carried back to the lungs by the red cells and a small amount by plasma. Here it is given up and replaced with fresh oxygen ready to start the next circulatory cycle.

Haemoglobin levels are measured in grams of haemoglobin per decilitre of blood. Men have a slightly higher average haemoglobin level than women: men average between 13.5 and 17.0 g/dl and women between 12.0 and 16 g/dl.

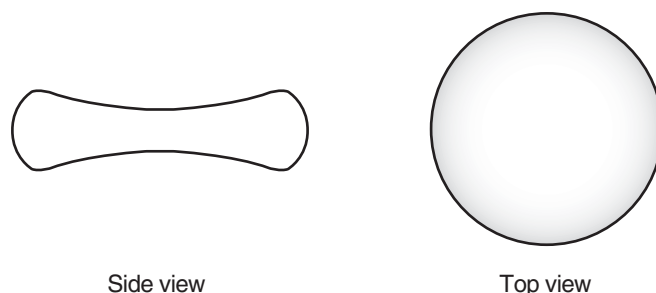


Figure 1: Side and top view of a normal red cell, showing its biconcave form

The minimum haemoglobin levels accepted by blood transfusion centres in many countries are as follows:

- males: 13.5 g/dl
- females: 12.5 g/dl.

Haemoglobin levels can be measured in different ways, but the best method is either a colorimetric or a photometric technique.

The level can also be estimated, as opposed to being measured, by comparing its specific gravity with the specific gravity of a prepared copper sulfate solution of known strength. This technique is widely used in blood transfusion practice as it is suitable for use in places where electricity supplies are poor or are not available. Acceptable donor haemoglobin levels are determined by confirming that a drop of blood can sink in a copper sulfate solution of a specific gravity of 1.055 for men and of 1.053 for women. Appendix 1 in the Introductory Module contains an example of a standard operating procedure for the preparation of copper sulfate solution and Appendix 5 in Module 1 contains examples of standard operating procedures for haemoglobin screening using the copper sulfate method.

The WHO Haemoglobin Colour Scale is another simple method of estimating haemoglobin levels (see Module 1, Appendix 6). It is an inexpensive clinical device that provides a reliable method for screening for the presence and severity of anaemia.

granulocyte: A white blood cell (leukocyte) containing neutrophil, eosinophil or basophil granules in the cytoplasm.

lymphocyte: A type of white blood cell formed in the lymph nodes. There are two kinds of lymphocyte: B lymphocytes which produce circulating antibodies and T lymphocytes which are responsible for the cellular immune response.

monocyte: A large leukocyte which ingests bacteria and other foreign bodies.

neutrophil: A member of the family of white cells involved in fighting infection.

eosinophil: A member of the family of white cells involved in fighting infection.

basophil: A member of the family of white cells involved in fighting infection.

ACTIVITY 4

What are the minimum haemoglobin levels that are accepted among blood donors in your donor clinic? Briefly describe the methods that are used to measure them.

2.4 WHITE BLOOD CELLS (LEUKOCYTES)

Leukocytes are a family of nucleated cells consisting of **granulocytes**, **lymphocytes** and **monocytes**. There are three different forms of granulocyte: **neutrophils**, **eosinophils** and **basophils**.

Under normal conditions, granulocytes are derived solely from the bone marrow. Small numbers of lymphocytes are produced in the bone marrow, but the main supply comes from the lymphatic tissue and thymus. The site of production of the monocyte is still uncertain; it is probably produced at any reticuloendothelial tissue site, particularly the spleen.

The normal number of circulating leukocytes in the blood is far less than the number of red cells. In a normal healthy adult, there are between 4000 and 11 000 leukocytes per mm³ (4.0–11.0 x 10⁹/L) of blood, which are made up as follows:

- neutrophils: 1500–7500 per mm³ (1.5–7.5 x 10⁹/L)
- eosinophils: 0–400 per mm³ (0–0.4 x 10⁹/L)

- basophils: 0–200 per mm³ (0–0.2 x 10⁹/L)
- lymphocytes: 1000–4500 per mm³ (1.0–4.5 x 10⁹/L)
- monocytes: 0–800 per mm³ (0–0.8 x 10⁹/L).

Unlike red cells, leukocytes are nucleated cells and they have a much shorter lifespan. Granulocytes live for between three and five days. The lifespan of lymphocytes is not so clearly defined and could be from a few days to many years. Monocytes, however, are known to leave the circulatory system after a few days.

The major role of the granulocytes is to fight infection. Lymphocytes play a very important part in making antibodies against any foreign antigen and in fighting viral infections. Monocytes are often referred to as scavenger cells because they ingest bacteria and other foreign bodies. This process of ingestion is called **phagocytosis**.

phagocytosis: The process by which certain white cells ingest bacteria and other foreign bodies.

fibrin: Fine protein strands produced when soluble fibrinogen is acted upon by thrombin in the process of blood coagulation.

serum: The fluid surrounding red cells that have been allowed to clot.

fibrinogen: A substance involved in the process of blood coagulation.

2.5 BLOOD PLATELETS (THROMBOCYTES)

Blood platelets are much smaller than red or white cells and their number varies from 150 000 to 500 000 per mm³ (150–500 x 10⁹/L) of blood in normal adults. They play a major role in our blood clotting mechanism. They work by releasing a substance at the site of an injury or wound and combine with other clotting factors in the plasma to produce fine protein strands (**fibrin**). In turn, fibrin forms a fine mesh or network which traps the red blood cells to produce clotting and prevent further bleeding. If stored correctly, blood platelets can have a laboratory shelf-life of up to five days before transfusion.

2.6 BLOOD COAGULATION

Blood serum and plasma

Normally, when blood is collected into a dry syringe and put into a dry tube, coagulation or clotting takes place to form a semi-solid mass of blood cells. The fluid surrounding this semi-solid mass is called **serum**. If, however, blood is collected into a tube containing an anticoagulant – a substance that prevents clotting – the non-clotted cells sediment to the bottom of the tube. The fluid surrounding these non-clotted cells is called **plasma**.

Serum can therefore be defined as the fluid surrounding red cells that have been allowed to clot, whereas plasma is the fluid surrounding red cells that have been prevented from clotting.

Mechanism of coagulation

The complete process of blood coagulation is extremely complex and it is outside the scope of this module to explain the intrinsic (surface contact) and extrinsic (tissue injury) pathways of coagulation in detail. In simple terms, however, damage or injury to a blood vessel will trigger the coagulation pathway or cascade, resulting in the change of soluble **fibrinogen** to fibrin, which forms a stable clot and prevents further bleeding.

2.7 REASONS FOR BLOOD TRANSFUSION

Blood and blood products are used for a number of purposes, but the three main reasons for blood transfusion are:

- to correct anaemia (a low haemoglobin level)
- to replace blood lost by bleeding, either during surgery or because of an accident
- to replace other constituents of blood, such as coagulation factors.

ACTIVITY 5

Identify the reasons for the transfusion of the last 20 units of blood issued by your blood bank. What were the most common reasons for transfusion? You should be able to discover this information from your records or by consulting your colleagues.

If you cannot obtain this information, note down the reasons for transfusion on the next 20 occasions that blood is issued by your blood bank.

SUMMARY

- 1 Whole blood contains a mixture of red cells (erythrocytes), white cells (leukocytes) and platelets (thrombocytes), suspended in plasma.
- 2 Red cells are filled with haemoglobin. Their primary function is to carry oxygen to the body tissues.
- 3 White cells (leukocytes) are a family of nucleated cells consisting of granulocytes, lymphocytes and monocytes.
- 4 Blood platelets (thrombocytes) play a major role in the blood clotting mechanism.
- 5 Serum is the fluid surrounding red cells which have been allowed to clot. Plasma is the fluid surrounding the red cells that have been prevented from clotting.
- 6 There are two ways in which the coagulation pathway may be triggered: intrinsic (surface contact) and extrinsic (tissue injury).

SELF-ASSESSMENT

- 1 *What is the average lifespan of the red cells within the circulatory system?*
- 2 *What is the main function of haemoglobin?*

- 3 *What is the function of lymphocytes?*
 - 4 *What are the three main reasons for transfusing blood?*
-

PROGRESS CHECK

Before moving on to Section 3, spend a few minutes thinking about whether you have achieved the learning objectives for Section 2. These were to:

- 1 Describe the main components of blood.
- 2 Explain the function of these components of blood and their importance in transfusion practice.

If you feel confident that you have understood everything in this section, turn to Section 3.

If you feel that you need to spend more time on this section, go back to the parts that are most unfamiliar or that you have found difficult. You may find it helpful to talk to other people, such as your supporter or other senior colleagues, if there is anything you are still not sure about.

3

Basic Blood Group Immunology

The purpose of this section is to help you to gain a clear understanding of antigens, antibodies, antigen–antibody interaction and the factors affecting this interaction. Section 3 simply provides background information which you will use in later parts of the module and so there are no activities in this section.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Define antigens and antibodies.
 - 2 Explain the primary and secondary immune response and the appearance of naturally-occurring antibodies.
 - 3 Explain antigen–antibody reactions and the factors affecting them.
 - 4 Describe the principles of the techniques that lead to red cell sensitization and agglutination.
-

antigen: Any substance recognized as foreign by the body which stimulates the immune system to mount a response against it.

antibody: A protective protein produced by the immune response of an individual to stimulation by a foreign protein. It recognizes antigen on foreign red cells and may cause in vivo agglutination and subsequent haemolysis.

immunoglobulin: An antibody molecule synthesized by specialized lymphocytes in response to an antigen.

gammaglobulin: The class of serum proteins that includes antibody molecules.

3.1 ANTIGENS

An **antigen** is any substance that, when introduced into a body and recognized as foreign, will bring about an immune response. This might result in the production of an antibody that will react specifically with that antigen in some observable way.

3.2 ANTIBODIES

An **antibody** is a product of an immune response and will react with that antigen in some observable way.

Antibodies are **immunoglobulins** (Ig) and are found in the **gammaglobulin** part of plasma proteins. There are five categories of immunoglobulins: IgG, IgM, IgA, IgD and IgE. In this module, we shall concentrate on IgG and IgM.

Antibodies are proteins that are formed from amino-acid molecules held together by peptide bonds; these are referred to as amino-acid chains. The IgG antibody has only four of these chains: two of them are small and are referred to as 'light chains', while the two larger chains are referred to as 'heavy chains'. This antibody is, however, very small when compared with the IgM antibody which is made up of 10 light chains and 10 heavy chains. Look at Figure 2 which shows the difference between the two antibody molecules.

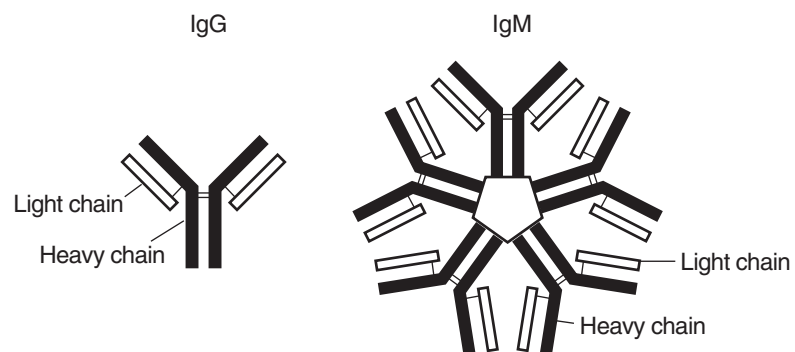


Figure 2: Simplified structure of the IgG and IgM molecules

IgG antibodies

IgG antibodies make up approximately 73% of our total immunoglobulins. They have a molecular weight of only 150 000. They can readily cross the placenta and consequently are often associated with a condition known as **haemolytic disease of the newborn** (HDN). This can occur when maternal antibody crosses the placenta and attacks the fetal red cells which possess the corresponding antigen.

IgG antibodies do not cause **agglutination** of red cells containing their antigen when suspended in saline; they have the ability only to coat or **sensitize** them. The life span of the IgG immunoglobulin is approximately 60–70 days.

IgM antibodies

IgM antibodies comprises approximately 8% of our total immunoglobulins. They are much larger antibodies than IgG antibodies, with a molecular

haemolytic disease of the newborn: A disease in which maternal antibody crosses the placenta and attacks the fetal red cells which possess the corresponding antigen.

agglutination: The clumping together of red cells.

sensitized cell: A cell coated with antibody, but not agglutinated.

complement: A protein present in normal human serum. It is often involved in blood group reactions and immunological disorders.

haemolysis: The breaking down (lysis) of the red cell membrane which liberates the contents: haem and globin. Haemolysis results from the reaction between a haemolytic antibody and its specific red cell antigen in the presence of complement.

primary antibody response: The response that the body makes when meeting a foreign antigen for the first time.

secondary antibody response: The increase in titre of an antibody when meeting its antigen for the second time.

weight of 900 000. They cannot cross the placenta and so they do not cause haemolytic disease of the newborn. They readily agglutinate red cells suspended in saline and have a life span of only 10 days. The IgM antibody often activates **complement** during an antigen–antibody reaction and, as a result, will cause **haemolysis** of the red cells rather than agglutination.

Table 1: Differences between IgG and IgM antibodies

	IgG	IgM
Approximate % of total immunoglobulins	73	8
Molecular weight	150 000	900 000
Agglutinate red blood cells in saline	No	Yes
Cross placenta	Yes	No
Activate complement	Yes	Yes
React optimally at	37°C	4°C
Antibody type	Immune	Naturally-occurring

3.3 ANTIBODY IMMUNE RESPONSE

When the body is first exposed to a foreign antigen, it makes what is called a **primary response**. This response develops slowly and it may be many months before any antibody is demonstrable. Once the primary response has been stimulated, a second exposure of the individual to the same antigen will result in a **secondary response**. This response is much more dramatic in its action and will often produce very large amounts of antibody in a short period of time. The primary response is often associated with small amounts of IgM antibody, while the secondary response will produce mainly IgG (see Figure 3).

'Naturally-occurring' antibodies and immune antibodies

If we look at the serum of a normal healthy adult person, we find ABO blood group antibodies. In contrast, the serum of a cord or newborn baby has no ABO antibodies, or at most, very small amounts. However, further examination of the baby's serum 12–20 weeks later will show that

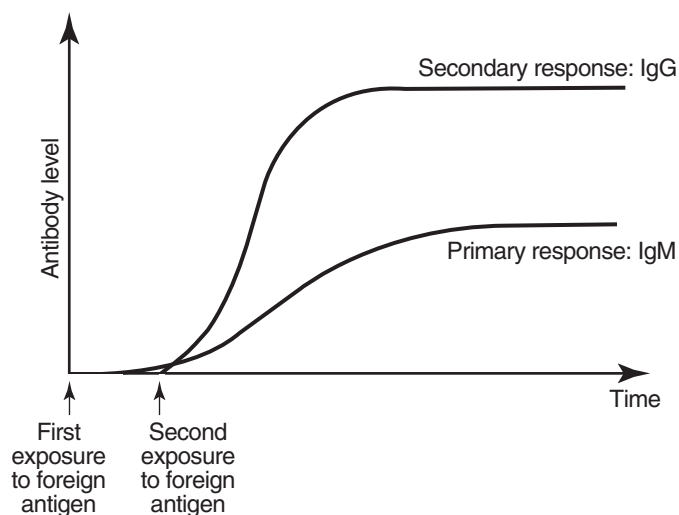


Figure 3: Primary and secondary antibody responses

naturally-occurring antibody: An antibody that appears in the bloodstream without any known antigenic stimulus.

moderate amounts of the antibody are present. These appear without any apparent immunization of the baby with A or B blood group antigen. These antibodies are therefore commonly referred to as **naturally-occurring**, which means they have appeared without any known antigenic stimulus. As we have seen, an antibody is a substance that is produced as the result of the introduction of an antigen, so the term 'naturally-occurring' can be misleading. It is now known that AB antigens that are very similar to those of the human blood groups can be found on bacteria, viruses and many foods. Hence, these so-called 'naturally- occurring' antibodies are the result of antigens entering the body and stimulating the appropriate antibody, which is usually IgM. Immune blood group antibodies are usually IgG and are produced in response to a foreign red cell antigen. This can be the result of a blood transfusion or, in the case of a pregnant woman, a leak of blood from the fetus in her circulation.

3.4 RED CELL ANTIGEN-ANTIBODY REACTIONS

Most of the techniques used in the blood bank laboratory to detect reactions between antigens and antibodies are based on agglutination techniques and occasionally on techniques looking for red cell lysis (haemolysis).

Agglutination is the clumping of red cells which is caused by an antibody attaching to antigens on more than one red cell, producing a net or lattice that holds the cells together. There are two stages in producing agglutination:

Stage 1

The antibody binds to its red cell antigen as soon as it comes into contact with it. This does not cause agglutination of the cell, but simply coats or sensitizes the cell.

Stage 2

A lattice is formed, producing the clump or agglutination. This is a continuation of Stage 1 in which, provided that the conditions are suitable, the antibody can cause physical agglutination of the cells.

IgM antibodies are large and have 10 antigen-combining sites. They can both sensitize and agglutinate cells directly. Figure 4 shows red cells being agglutinated by IgM antibodies.

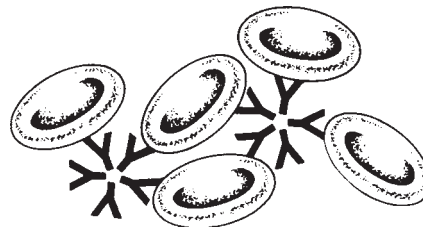
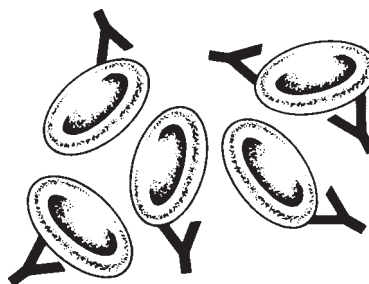


Figure 4: Reaction of red cells with IgM antibody leading to agglutination of the cells

IgG antibodies are smaller and do not directly agglutinate cells. Instead, they coat or sensitize the cells (see Figure 5 on page 20).

The following indirect techniques can be used to see that this coating has taken place and that an antigen-antibody reaction has occurred.

Figure 5: Red cells sensitized with IgG



- 1 The use of albumin (or other charged polymers).
- 2 The use of anti-human globulin reagents.
- 3 The use of proteolytic enzymes.

Haemolysis of red cells is caused by some IgM antibodies and a few IgG antibodies. After the antibody binds to the antigen, the complement pathway (see page 22) can be activated and this leads to the red cells being ruptured and lysing. Lysis therefore also indicates a blood group antigen–antibody reaction and, like agglutination, must be recorded when performing tests.

Factors affecting red cell antigen–antibody reactions

Red cell ionic charge

Red cells in the normal physiological state never make direct physical contact with each other in the body (**in vivo**) or in a test tube (**in vitro**) because each cell carries a negative electrical charge. Since like charges repel and unlike charges attract, the red cells are in a constant state of repulsion and never come into contact with each other. The distance that they are kept apart is very small, but it is enough to prevent the small IgG molecules reaching across the gap between the cells and bringing about their agglutination, as shown in Figure 5. However, the larger IgM molecules link the red cells together, as shown in Figure 4. IgM antibodies can therefore cause cells to agglutinate directly, but IgG antibodies attach to their antigens, coating or sensitizing the red cells.

The negative charge on the red cell is produced by groups of neuraminic acid on the red cell membrane. The repulsive force holding the cells apart is sometimes called the ‘zeta potential’.

Temperature

Different antibodies have different preferred temperatures of reaction. For example, ABO blood group antibodies react best at the temperature of 4°C, while Rh antibodies react best at body temperature, i.e. 37°C.

pH

The optimum pH for most blood group antibodies is between 6.5 and 7.5. Reactions are inhibited when the pH is too acid or too alkaline.

Freshness of serum and red cells

The best reactions can always be obtained when using fresh serum and red cells. For this reason, it is advisable to use freshly prepared red cells and to store serum that is not being used immediately at –20°C or colder.

in vivo: A reaction occurring within the body as, for example, in autoimmune haemolytic anaemia.

in vitro: A reaction occurring outside the body: that is, a test tube reaction.

Antibody to antigen ratio

The ratio of antibody to antigen is important. The more antibody that is present in relation to the number of antigens on the red cells, the stronger the reaction. It is therefore important to ensure that the strength of the red cell suspension is prepared accurately because too heavy a suspension might mask the presence of a weak antibody. The most suitable strength of cell suspension, when used for agglutination tests, is 2–4%.

Ionic strength

The rate at which the antigen–antibody reaction occurs is considerably increased when the ionic strength of the medium in which the red cells are suspended is decreased. Using **low ionic strength saline (LISS)**, the incubation period of the anti-human globulin test can be reduced to 15 minutes.

low ionic strength saline (LISS): The titre of most blood group antibodies is enhanced when the ionic strength of the saline is lowered from its normal strength to 0.2% saline in 7% glucose. Most low ionic strength saline is now produced commercially.

anti-human globulin test: A test using antiglobulin reagent to detect the presence of human globulin on sensitized red cells.

anti-human globulin reagent (AHG): A blood grouping reagent that reacts specifically with human globulin.

Agglutination of antibody coated red cells

As we have seen on pages 19–20, there are three methods commonly used in blood banking to test whether an antigen–antibody reaction has occurred.

Use of albumin

Large charged molecules such as albumin will bring red cells closer together so that IgG antibodies can bind to antigens on adjoining cells and form agglutinates. The albumin will not cause non-coated cells to clump.

Albumin is sometimes added to reagents to enhance their agglutinating activity or is used in the two-stage albumin addition (layering) test. This test is described in Section 7 and in Appendix 1. In this test, cells and serum are incubated to allow antibodies to coat the cells. Albumin is then added to agglutinate these coated cells.

Use of anti-human globulin reagents

The **anti-human globulin test** is a test using anti-human globulin reagent to detect the presence of human globulin on sensitized red cells. It has three stages:

Stage 1: Sensitization or coating

Cells and serum are incubated to allow any antibodies present in the serum to bind to the antigens in the red cells.

Stage 2: Washing

These cells are washed several times in a large volume of saline to remove any protein or immunoglobulins not coated onto the cells.

Stage 3: Addition of anti-human globulin reagent

Anti-human globulin reagent (AHG) is added to the washed cells. If the cells are coated with IgG antibodies (or C3 component of complement), they will be agglutinated by the anti-human globulin reagent binding to the IgG antibodies

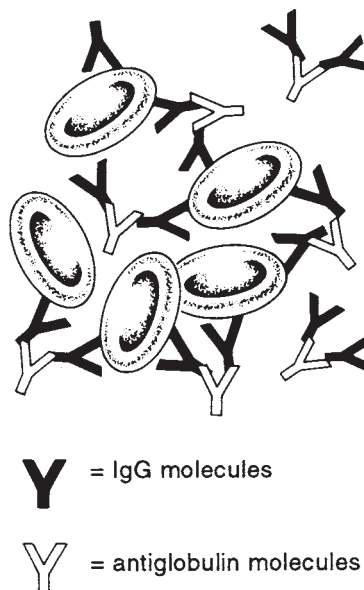


Figure 6: IgG sensitized red cells agglutinated by antiglobulin serum

coating the cells. If there are no antibodies on the cells, there will be no agglutination.

The anti-human globulin test is described more fully in Section 7 and Appendix 1.

Figure 6 above shows IgG sensitized red cells agglutinated by anti-human globulin serum. The solid antibodies are the blood group molecules, while the outlined molecules are the anti-human globulin antibodies.

Use of proteolytic enzymes

The overall negative charge on red cells keeps them apart. This is caused by the presence of chemical groups, called neuraminic acid, on the surface of the cells. **Enzymes** such as papain and bromelain have the ability to remove some of the neuraminic acid, which reduces the negative charge. This brings the red cells closer together, allowing IgG antibodies to agglutinate the cells. IgM antibodies also agglutinate enzyme-treated cells and their reaction might be stronger, as with anti-A and anti-B.

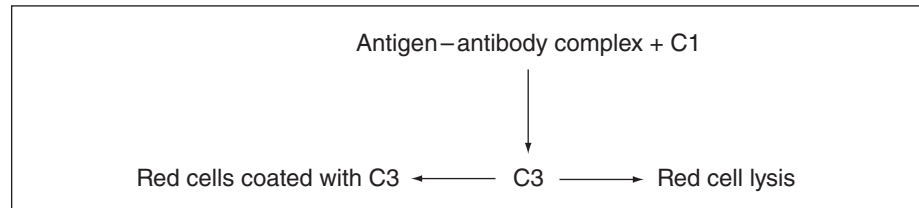
Warning: Enzyme treatment removes some antigens from the cells. The use of enzyme techniques will therefore not detect all antibodies. For this reason, they should be regarded as additional techniques, not as a replacement for the basic techniques (saline, albumin and anti-human globulin) that are described in Section 7 and in Appendix 1.

enzyme: A substance that has the ability to remove some of the protein and chemical surrounding the red cells, thereby lowering the forces of attraction around the cells (zeta potential). This causes the cells to become more sensitive to agglutination and allows an IgG antibody to agglutinate red cells suspended in saline.

3.5 COMPLEMENT

As stated earlier, some antibodies cause cells to lyse. This is because the antigen–antibody complex activates a complex sequence of components called complement that leads either to the lysis of the red cells or to the coating of the red cells with the component C3. Complement is a protein present in normal human serum. It is often involved in blood group reactions and immunological disorders.

Figure 7: Simplified complement pathway



Some cells coated with C3 will be removed as they pass through the liver, but others will remain in the circulation and are detected when a direct anti-human globulin test is performed.

The components of complement are labile and quickly denature; complement activity declines on storage and is destroyed by heating serum at 56°C for 30 minutes.

The complement pathway is complex, but Figure 7 above outlines the important stages.

SUMMARY

- 1 An antigen is any substance that, when introduced into a body and recognized as foreign, brings about an immune response that might result in the production of an antibody that will react specifically with that antigen in some observable way.
- 2 An antibody is a protective protein that is produced by the immune response of an individual to stimulation by a foreign protein and reacts with an antigen in some observable way.
- 3 Antibodies are immunoglobulins. There are five categories of immunoglobulins: IgG, IgM, IgA, IgD and IgE.
- 4 On exposure to an antigen, the body's primary response is often associated with small amounts of IgM antibody, while the secondary response mainly produces IgG.
- 5 In the first stage of red cell agglutination, the red cells are sensitized or coated by the antibody binding to antigens. The second stage is the physical agglutination or clumping of the red cells.
- 6 IgM antibodies can sensitize and agglutinate red cells.
- 7 Red cells coated with IgG antibodies will not agglutinate unless one of the following three indirect techniques is used:
 - albumin (or other charged polymers)
 - anti-human globulin reagents
 - proteolytic enzymes.
- 8 The antigen-antibody complex activates a complex sequence of components called complement that leads either to lysis of the red cells or to the coating of the red cells with the complement C3.

SELF-ASSESSMENT

- 5 *What is the difference between the primary and secondary immune response?*
 - 6 *Why are ABO antibodies not present in the serum of cord and newborn babies when the antibodies appear without any obvious stimulation around 12 weeks later?*
 - 7 *What are the two stages in producing agglutination?*
 - 8 *Briefly describe each stage of the anti-human globulin test.*
-

PROGRESS CHECK

Before moving on to Section 4, spend a few minutes thinking about whether you have achieved the learning objectives for Section 3. These were to:

- 1 Define antigens and antibodies.
- 2 Explain the primary and secondary immune response and the appearance of naturally-occurring antibodies.
- 3 Explain antigen–antibody reactions and the factors affecting them.
- 4 Describe the principles of the techniques that lead to red cell sensitization and agglutination.

If you feel confident that you have understood everything in this section, turn to Section 4.

If you feel that you need to spend more time on this section, go back to the parts that are most unfamiliar or that you have found difficult. You may find it helpful to talk to other people, such as your supporter or other senior colleagues, if there is anything you are still not sure about.

4

The ABO Blood Group System

The purpose of this section is to help you to understand the ABO blood group system and its importance in blood transfusion.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Explain the basic genetics of the ABO blood groups and use them to predict the possible blood groups of family members.
 - 2 Use the results of cell and reverse ABO grouping tests to identify the blood group of a donor or patient and determine the possible percentage frequency of A, B, AB and O blood groups in your locality.
 - 3 Identify the presence of the subgroups of the antigen A from the results of cell and reverse ABO grouping tests.
 - 4 Explain the importance of high-titre antibodies.
-

4.1 THE ABO BLOOD GROUPS

Early in the twentieth century, a most important discovery was made in blood transfusion when Karl Landsteiner showed that by cross-testing one blood sample with another, some samples would mix successfully with no visual signs of reaction while others would react strongly, causing agglutination, which is a massive clumping of the red cells (see Figure 8).

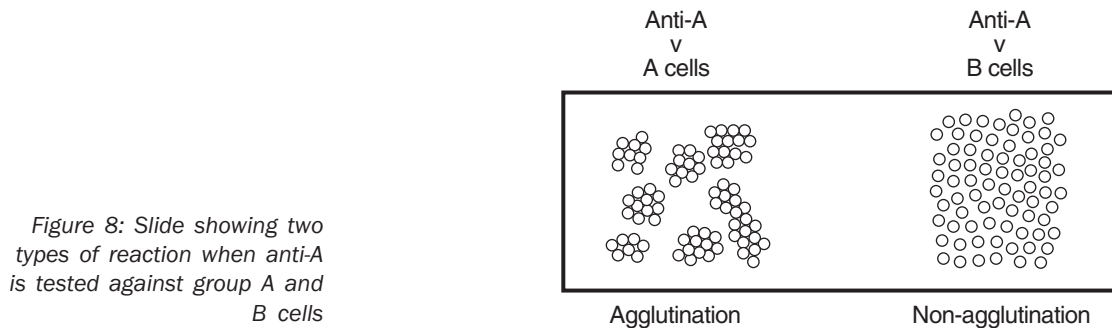


Figure 8: Slide showing two types of reaction when anti-A is tested against group A and B cells

This agglutination was attributed to the presence of an antigen on the red cells and an antibody in the serum. By following up this observation, it was shown that two different red cell antigens exist, which are called antigen A and antigen B. Within the ABO groups, it is possible for the red cells to have either of these antigens on their surface, or both, or neither.

Cells that only have the A antigen are called group A. Those that only have the B antigen are called group B. Cells that have both the A and B antigen are called group AB. It is also possible for the cells to lack both of these antigens and, in such cases, the group is O.

In a similar way, two different antibodies exist in the serum. One, which reacts specifically with group A cells, causing them to agglutinate, is called anti-A. The other, which reacts specifically with group B cells in the same way, is called anti-B.

The presence of anti-A and anti-B antibodies in the serum differs according to the AB antigens present on the red cells (see Table 2):

- those with A antigen on the red cells (group A) have anti-B in the serum
- those with B antigen on the red cells (group B) have anti-A in the serum
- those with both A and B antigens on the red cells (group AB) do not have any anti-A or anti-B in the serum
- those without A or B antigens on the red cells (group O) have both anti-A and anti-B in the serum.

Table 2

	Antigen on cells	Antibodies in serum
Group A	A	anti-B
Group B	B	anti-A
Group AB	A and B	none
Group O	none	anti-AB

ACTIVITY 6

Complete the first table below to show the reactions of anti-A and anti-B with the red cells of group A, group B, group AB and group O. Use a plus sign to indicate the reactions that will give agglutination and use a minus sign to indicate the reactions that will not.

Group	Anti-A	Anti-B
A		
B		
AB		
O		

Complete the second table below to show the reactions of A, B, AB and O serum of groups with A, B and O cells. Use a plus sign to indicate the reactions that will give agglutination and use a minus sign to indicate the reactions that will not.

Group	A cells	B cells	O cells
A			
B			
AB			
O			

Check your answers with those given in the Activity Checklists and Answers on page 101.

4.2 BASIC GENETICS OF THE ABO BLOOD GROUPS

gene: The basic unit of inheritance which is carried on the chromosome.

chromosome: A thread-like structure which carries genes and is present in the nucleus of cells.

All of our features and characteristics are controlled by **genes** which exist as units of inheritance within the nuclei of our living body cells. These genes are carried on our **chromosomes** of which each cell contains 23 pairs, or 46 in total. We inherit one of each of these pairs from each parent.

Unlike nucleated body cells, our reproductive cells (spermatozoa and ova) possess only single chromosomes. On fertilization, these combine to re-form as pairs in the living cells of the embryo. Among our inherited characteristics there is a gene responsible for the specificity of our ABO blood group; in other words, we inherit two blood group genes. For the ABO blood groups, the chromosome from the mother carries one of A, B

or O gene. Similarly, the other chromosome from the father carries one of A, B or O gene.

Two definitions are important as applied to blood groups:

genotype: The genes inherited from each parent which are present on the chromosomes.

phenotype: The observable effect of the inherited genes: the blood group itself.

A **genotype:** the genes inherited from each parent's blood group which are present on the chromosomes.

A **phenotype:** the observable effect of the inherited genes: that is, the blood group itself.

The A and B genes are dominant over the O gene and the phenotype A can therefore arise from either the AO or the AA genotype. Similarly, the phenotype B can arise from either the BO or the BB genotype. Table 3 shows the possible combinations of genes and the blood groups they confer.

Table 3

Genotype	Blood group (phenotype)
AA	A
AO	A
BB	B
BO	B
AB	AB
OO	O

Figure 9 shows an ABO group family tree. You will see from this that the mother is group A (genotype AO) and the father is group B (genotype BO). The possible ABO genotypes of their children could be AB (group AB), AO (group A) BO (group B) or OO (group O).

ACTIVITY 7

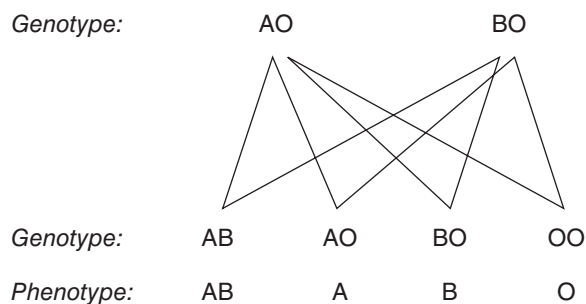


Figure 9: ABO blood group family tree

Prepare a blood group family tree for the following mothers and fathers showing the genotypes and possible groups of their children:

- 1 Mother group OO: Father group AB
- 2 Mother group BO: Father group BO
- 3 Mother group OO: Father group BO

Check your answers with those given in the Activity Checklists and Answers on pages 102.

4.3 DEMONSTRATING ABO BLOOD GROUPS

Anti-A, by definition, will react only with the group A antigen; similarly, anti-B will react only with the group B antigen. It therefore follows that if you wish to determine whether or not the AB antigens are present on the red cells, you will have to test them against potent and specific anti-A and anti-B. Using these two reagents, you can determine the ABO cell group.

You cannot satisfactorily determine a complete blood group by testing only the red cells. In effect, this only tells you half the group: i.e. the cell group. It is also necessary to carry out a **reverse grouping test** by testing the serum against known group A and group B cells.

reverse grouping test: A test to detect ABO antibodies in serum or plasma.

As we have already seen, the antibodies in the serum relate closely to the antigens present on the red cells: a person of group A will have anti-B in the serum and a person of group B will have anti-A in the serum. Therefore, when performing an ABO group, the cell group and reverse group are complementary to each other and, in effect, one will confirm the other. For example, if your tests show A antigen on the red cells and anti-B in the serum, you can feel very confident that this grouping is correct. However, if your red cells test shows the presence of the B antigen and your serum test shows anti-B, you should be alerted to an error either in the cell grouping or in the reverse grouping. The whole test will then have to be repeated.

ACTIVITY 8

The table below shows the type of result you can expect when performing a cell and reverse ABO grouping test. From the results shown, name each of the blood groups.

Check your answers with those given in the Activity Checklists and Answers on page 99.

	Cells tested against		Serum tested against		Group
	Anti-A	Anti-B	A cells	B cells	
1	pos	neg	neg	pos	
2	neg	neg	pos	pos	
3	pos	pos	neg	neg	
4	neg	neg	pos	pos	
5	neg	pos	pos	neg	
6	neg	neg	pos	pos	
7	pos	pos	neg	neg	
8	pos	neg	neg	pos	
9	neg	pos	pos	neg	
10	pos	neg	neg	pos	

The percentage of the population belonging to each blood group varies with racial type. Table 4 on page 30 shows how the percentage frequency of ABO blood groups differs between racial groups.

Table 4: The percentage frequency of ABO blood groups in different racial groups

Group	Whites	Blacks	Oriental
A	40	27	28
B	11	20	27
AB	4	4	5
O	45	49	40

ACTIVITY 9

Look at the results of 100 blood grouping tests and record the number (and therefore the percentage) that were group A, group B, group AB and group O.

How do your results compare with the percentage frequencies in Table 4? Check your records or consult your colleagues to identify whether your results are typical of your locality.

Use of group O cells when carrying out a serum group

Group O cells need to be included when testing the serum because some donors may have antibodies in their serum other than anti-A or anti-B. These antibodies are not naturally expected to be present and so they are referred to as 'irregular antibodies'. They occur as a result of earlier immunization, either during pregnancy in the case of a woman, or through a previous blood transfusion. The presence of these irregular antibodies may be demonstrated by using group O cells in your testing procedure.

4.4 DEVELOPMENT OF THE A AND B RED CELL ANTIGENS AND ANTIBODIES

The A and B blood group antigens develop in strength from early fetal life through to adolescence. At birth, they are weaker than in adults and weaker than expected reactions may be found with anti-A and anti-B.

The normal anti-A and anti-B are extremely weak at birth, and these antibodies might not be demonstrable until the baby reaches approximately three months of age. It is therefore accepted that, when performing blood grouping on samples from the umbilical cord or from newborn babies, only the red cell groups need to be carried out.

4.5 THE SUBGROUPS OF THE ANTIGEN A

In 1911, it was shown that the ABO system was a much more complex system than had previously been believed when it was observed that the blood group A could clearly be divided both serologically and genetically into two distinct subgroups: A_1 and A_2 . Likewise, it was recognized that group AB could be subdivided into A_1B and A_2B .

Approximately 80% of the group A population are of the subgroup A_1 . The remaining 20% are A_2 . The same percentages also apply to the group AB.

Since the discovery of A_1 and A_2 , many more different subgroups of A have been reported and 12 groups are now known, all showing varying serological and chemical characteristics. The majority of these do not play a significant role in everyday serology. It is possible for people of subgroups A_2 and A_2B to have anti- A_1 in their serum, but this is usually weak and is of no importance in selecting donor blood for transfusion. Weak forms of the B antigen are rare, but may be found in Chinese populations. Figure 10 summarizes the subgroups of the antigen A.

Figure 10: Summary of the subgroups of the antigen A

A subgroup	Subgroup frequency	Antibodies always present	Antibodies sometimes present
A_1	80%	anti-B	none
A_2	20%	anti-B	anti- A_1 in 2% of bloods
A_1B	80%	none	none
A_2B	20%	none	anti- A_1 in 25% of bloods

4.6 ANTI- A_1 AND ANTI-AB IN BLOOD GROUPING TESTS

Anti-A

Anti-A consists of a mixture of two antibodies:

- anti-A which agglutinates A_1 , A_2 , A_1B and A_2B cells
- anti- A_1 which agglutinates only A_1 and A_1B cells.

Anti- A_1 can also be obtained from the seeds of the plant *Dolichos biflorus* and can be made into an anti- A_1 grouping reagent.

Figure 11 shows the serological reactions with anti-A, anti-AB and anti- A_1 .

Figure 11: Serological reactions with anti-A, anti-AB and anti- A_1

	A_1	A_2	A_1B	A_2B
anti-A	pos	pos	pos	pos
anti-AB	pos	pos	pos	pos
anti- A_1	pos	neg	pos	neg

Anti-AB

It is usual to include anti-AB as part of standard blood grouping tests on donors to ensure that the weaker group A and group B antigens are not missed. The anti-A and anti-B in this mixture of antibodies has a much greater affinity for these weaker antigens and will react strongly with them, even though the specific anti-A and anti-B might have failed to react. Anti-AB is not required for testing patients' red blood cells, but it is recommended for donor blood grouping.

ACTIVITY 10

What are the possible reasons for the ABO grouping results in the table on page 32?

	Cells tested against			Serum tested against		
	<i>anti-A</i>	<i>anti-B</i>	<i>anti-AB</i>	<i>A cells</i>	<i>B cells</i>	<i>O cells</i>
1	pos	neg	pos	pos	pos	neg
2	neg	neg	neg	pos	pos	pos
3	neg	pos	pos	pos	neg	pos
4	pos	pos	pos	pos	neg	neg
5	weak pos	pos	pos	neg	neg	neg

Check your answers with those given in the Activity Checklists and Answers on page 103.

4.7 IgM AND IgG (NATURALLY-OCCURRING AND IMMUNE) ANTI-A AND ANTI-B

All individuals, except those who are group AB, produce IgM anti-A and/or anti-B. Some, particularly those who are group O, also produce IgG antibodies. It is thought that these are the result of stimulation by A- and B-like antigens commonly found in the environment, food, etc. When someone has IgG anti-AB, the amount of IgM anti-AB is usually high and the term high-titre anti-AB or high-titre O is used. The serum of these individuals will often lyse A and/or B cells in the reverse grouping.

These high-titre antibodies are important in two situations:

- 1 *Transfusion of group O blood or plasma to people who are not group O*

If group O plasma containing high-titre anti-AB is transfused into an A or B person, there could be some red cell destruction – a transfusion reaction. It is therefore preferable not to transfuse a person who is not group O with group O products. If it is unavoidable, select units from donors that do not lyse A and/or B cells in the serum group or remove the plasma aseptically from the red cells (see Appendix 2).

- 2 *In pregnancy, when an O mother has an A or B infant*

It is worth noting if the maternal serum lyses A and/or B cells as IgG anti-AB could have crossed the placenta and destroyed the fetal red cells. At birth, the baby could suffer from the effects of anaemia and jaundice as a result of the red cell destruction. However, haemolytic disease of the newborn (HDN) of this sort is far less severe than that caused by anti-D (see Section 5), and tests for quantifying IgG anti-AB during pregnancy are of little value in predicting whether the baby will suffer. There is, therefore, no value in performing any further tests during pregnancy.

direct anti-human globulin test (DAT): A test used for detecting the presence of human globulin on the surface of cells sensitized in vivo.

indirect anti-human globulin test (IAT): A tube haemagglutination method, commonly referred to as the Coombs test, in which antibodies incapable of causing direct agglutination can be shown to have combined with their specific red cell receptors by testing against an antiglobulin serum.

secretor: A person who possesses the dominant secretor gene and produces blood group specific substance in some body fluids, such as saliva and serum.

ABO incompatibility between mother and baby

If a baby develops jaundice, it is necessary to investigate the reason why it has occurred. ABO incompatibility is only one of many causes of neonatal jaundice. If the baby requires an exchange transfusion, the criteria for selecting the blood will be the same, whatever the cause of the jaundice. However, if it was noted that the mother's serum lysed the reverse group cells, this could indicate that anti-A or anti-B might be the cause. A **direct anti-human globulin test (DAT)** should be performed on the infant's red cells to see whether they are coated with IgG antibody. However, the DAT is often only very weakly positive in ABO disease. A simple test of maternal–infant incompatibility can also be performed.

The simple method for looking for maternal–infant blood group incompatibility is to set up a test using 2–3 drops of fresh maternal serum plus 1 drop of a 5% suspension of the infant's washed red blood cells. Incubate at 37°C for 15 minutes, centrifuge the tube lightly and examine for haemolysis and agglutination. If the cells are totally lysed, this is indicative of ABO HDN. No single test is diagnostic but, if the DAT is positive as well, this is strong evidence for HDN.

If no lysis or agglutination is present, this test can be taken on to be washed and tested with anti-human globulin reagent (AHG). If the DAT on the infant's cells was negative or only weakly positive and the **indirect anti-human globulin test (IAT)** is now positive, this indicates maternal–fetal incompatibility, but it is unlikely to cause severe red blood cell destruction.

Techniques for ABO grouping are described in Section 7 and Appendix 1.

ACTIVITY 11

What procedures do you use when blood grouping samples from the umbilical cord or from newborn babies? Do you group only the red cells?

Do you record high-titre individuals when testing donors? If you don't, identify any action you think should be taken. Discuss it with your colleagues and note down your recommendations on your Action List.

4.8 SECRETOR STATUS

The A and B antigens are found not only on red cells but also, in persons termed **secretors**, in a soluble form in the serum. About 80% of individuals inherit the gene that produces these soluble antigens. In normal transfusion practice, this is of no significance.

SUMMARY

- 1 The two most important factors in the ABO blood group system are the red cell antigens and serum antibodies. These combine to give the four major ABO blood groups.

- 2 A genotype is the genes inherited from each parent which are present on the chromosomes. A phenotype is the effect of the inherited genes: that is, the blood group.
- 3 ABO grouping involves:
 - testing red cells against potent and specific anti-A and anti-B
 - performing a reverse grouping test with A, B and O cells.
- 4 Blood group A is divided into two major subgroups: A₁ and A₂. Blood group AB is divided into A₁B and A₂B.
- 5 Anti-A and anti-B are predominantly IgM antibodies, but IgG forms do exist.
- 6 IgG anti-AB can be important in two situations:
 - transfusing group O blood to people who are not group O
 - in pregnancy, if the mother is group O and the baby is group A or B.

SELF-ASSESSMENT

- 9 *What antigens are present on the red cells and antibodies in the serum of the four main ABO groups?*
 - 10 *What is the reason for using anti-AB in standard blood grouping tests?*
-

PROGRESS CHECK

Before moving on to Section 5, spend a few minutes thinking about whether you have achieved the learning objectives for Section 4. These were to:

- 1 Explain the basic genetics of the ABO blood groups and use them to predict the possible blood groups of family members.
- 2 Use the results of cell and reverse ABO grouping tests to identify the blood group of a donor or patient and determine the possible percentage frequency of A, B, AB and O blood groups in your locality.
- 3 Identify the presence of the subgroups of the antigen A from the results of cell and reverse ABO grouping tests.
- 4 Explain the importance of high-titre antibodies.

If you feel confident that you have understood everything in this section, turn to Section 5.

If you feel that you need to spend more time on this section, go back to the parts that are most unfamiliar or that you have found difficult. You may find it helpful to talk to other people, such as your supporter or other senior colleagues, if there is anything you are still not sure about.

5

The Rh Blood Group System

The purpose of this section is to help you to understand the clinical importance and basic genetics of the Rh (Rhesus) blood group system and the D antigen in particular.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Explain the significance of the Rh system in blood transfusion practice.
 - 2 Explain the basic genetics of the Rh system and predict the possible blood groups of family members.
 - 3 Determine the possible percentage frequency of RhD positive and RhD negative individuals in your locality.
 - 4 Explain the importance of correct RhD grouping, particularly in relation to haemolytic disease of the newborn.
 - 5 Explain the significance of the Rh weak D (D^u) antigen and recognize when to test for D^u .
-

5.1 THE Rh BLOOD GROUPS

With the discovery of the ABO blood group system, it was thought that the earlier difficulties encountered when attempting blood transfusions would now be overcome and that transfusions would become safe and uneventful. This did not prove to be the case, however. While the majority of transfusions were completely successful, occasionally patients receiving ABO compatible blood would suffer an unpleasant transfusion reaction. Similarly, it was not uncommon for a mother to be delivered of an ABO blood group compatible baby which showed gross signs of anaemia. This was believed to have been caused by incompatible blood group antibodies present in the maternal serum crossing the placenta and destroying the fetal red cells, thus leading to haemolytic disease of the newborn.

The clinical importance of Rh

The clinical importance of the Rh blood group system was clearly demonstrated by Levine and Stetson in 1939 when, following the delivery of a stillborn baby, a patient urgently required a blood transfusion. ABO-compatible blood was transfused, following which the patient had a near fatal transfusion reaction. Further laboratory studies showed that the mother's serum contained an irregular antibody that reacted strongly with the ABO-compatible donor red cells and also with the red cells of her fetus. Like the antibody reported by Landsteiner and Wiener, the mother's antibody reacted with approximately 85% of the random Caucasian population. When this antibody was compared with the one earlier discovered by Landsteiner and Wiener, it was shown that the two had similar specificity.

As a result of these findings, not only had a new blood group system been discovered, but a scientific explanation had been provided to help understand the cause of unexplained transfusion reactions and why babies were occasionally born suffering from an anaemia caused by maternal-fetal blood group incompatibility.

5.2 BASIC GENETICS OF THE Rh SYSTEM

Following the discovery of the Rh blood group system, it soon became obvious that this system was much more complicated than the ABO system which had earlier been shown to have only the two antigens, A and B. The Rh system has two genes, *D* and *CE* but, unlike the ABO genes, these code for large proteins in the red cell membrane that can carry more than one antigen. The *CE* gene codes for the following antigen combinations: *ce*, *Ce*, *cE*, *CE*, but the *D* gene only for the D antigen. RhD negative individuals lack a functioning *D* gene.

These two genes are inherited together giving the "haplotypes", as shown in Table 5.

An individual inherits one haplotype from each parent so that a large number of different "genotypes" are possible. Certain of these are more common than others, but there are differences between racial groups. However, it is the presence or absence of the D gene that is the most important. The D gene is sometimes called Rh₁ and anti-D is called anti-Rh₁.

Table 5: Rh haplotypes and shorthand Rh notation ("d" is used to show the absence of the D gene)

	D positive		D negative (d)	
ce	cDe	Ro	cde	r
Ce	Cde	R ₁	Cde	r'
cE	cDE	R ₂	cdE	r''
CE	CDE	R _z	CdE	r ^y

Where a person inherits a D gene, their red cells are positive when tested with anti-D and that person is said to be RhD positive.

Where a person does not inherit a D gene, their red cells are negative when tested with anti-D and that person is referred to as RhD negative.

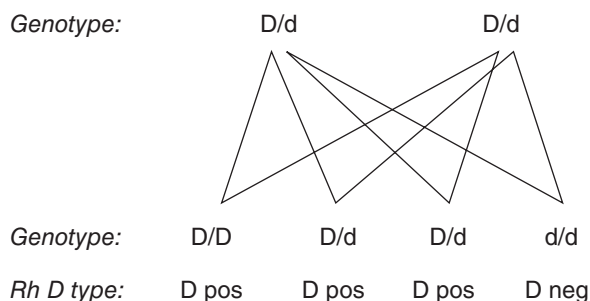
It is not possible to determine whether a person reacting with anti-D is **homozygous** for D (i.e. has inherited a D gene from each parent: D/D) or **heterozygous** (i.e. has inherited a D gene from only one parent: D/d). A person who does not inherit a D gene is D negative.

The family tree in Figure 12 shows that it is possible for two D positive parents to have a child who is D negative. Both parents are heterozygous for D (D/d) and a child who did not inherit the D gene from either parent would be D negative (d/d). The other two possible combinations would result in D positive children.

homozygous: A condition in which two identical allelic genes are carried on homologous chromosomes.

heterozygous: A condition in which non-identical allelic genes are carried on homologous chromosomes.

Figure 12: Family tree showing that two D positive parents can have a D negative child



ACTIVITY 12

Complete the two family trees below, showing the possible genotypes of the children and their RhD type.

Genotype: D/D D/d Genotype: D/d d/d

Genotype:

Genotype:

Rh D type:

Rh D type:

Check your answers with those given in the Activity Checklists and Answers on page 104.

The number of people who are RhD positive varies in different populations in the world. In northern Europe, 85% of the population are D positive, whereas in west Africa 95% are D positive. In parts of China, however, everyone is D positive.

ACTIVITY 13

What proportion of your patients and donors are D positive? What proportion of them are D negative?

Look at the results for 100 RhD blood grouping tests for patients and donors and give the figures as a percentage. How do your results compare with the frequencies given above?

Development of the RhD antigen

Unlike the ABO antigens, Rh antigens are fully developed in early fetal life and remain so throughout adult life. Cord and newborn infants' red cells will therefore Rh type as strongly as normal adult blood.

5.3 IMPORTANCE OF RhD GROUPING

In transfusion work, it is important to ensure that RhD negative patients receive RhD negative blood when being transfused. It is particularly important when transfusing females (with the possible exception of those past childbearing age) because the inadvertent transfusion of RhD positive blood to a RhD negative female child or woman would normally sensitize her to produce anti-RhD. Since anti-D is an IgG antibody, this could cross the placenta in any subsequent RhD positive pregnancy and destroy the fetal red cells, thereby bringing about haemolytic disease of the newborn. It is therefore essential to use a sound and reliable technique when performing an RhD group.

5.4 THE WEAK D (D^u) ANTIGEN

Normally RhD positive red cells are readily agglutinated when tested against an anti-D serum, while RhD negative red cells will not be agglutinated. However, there are some cells that will react as RhD positive when tested with some anti-D reagents, but will give a negative result when tested with others. This could be the case if a person groups as D positive with the anti-D in use, but was grouped as D negative on the last occasion. Cells giving this type of reaction are called weak D or D^u cells.

Weak D or D^u is used to show a weakened expression of the normal D antigen or, in other words, fewer than normal D antigens per red cell. This is an inherited characteristic. Because there are slight differences in anti-D reagents, these D^u cells can give different strength reactions with different reagents.

When testing *patients*, it is not necessary to perform a test specifically to detect weak D if the routine anti-D reagent(s) give a negative result. However, in some places where the incidence of D^u is high, such as in

some African countries, it may be local policy to test for D^u. Otherwise, it is necessary to test for D^u only if the result obtained is different from that previously found or if the reactions between two reagents differ: that is, one is positive and one is negative. If in doubt, call a patient D negative.

When testing *donors*, unless automated grouping machines are used, it is normal practice to test for D^u when samples give a negative result with the test anti-D.

Any donor found to be D^u has a weakly expressed D antigen and is therefore regarded as RhD positive.

There is often concern about the danger of missing a weak D or D^u, but this is insignificant. If a D^u patient is typed as D negative, they will receive D negative blood without adverse effects.

Testing for weak D: the D^u test

D^u testing consists of using an anti-D reagent by an indirect antiglobulin test. If a D^u test is necessary, it must be performed with a reagent that has been standardized for D^u testing. Always check the manufacturer's instructions and follow the method given. If the method does not tell you to use a negative control, a direct antiglobulin test (DAT) is required. If either the negative control or the DAT is positive, the test is invalid and you should therefore regard that patient as RhD negative. Blood from a donor with a positive DAT or negative control test should not be used for transfusion. Positive controls should always be incorporated into antiglobulin tests.

Partial D or D variants

Partial D or D variant is the term used to describe a rare group in people who type as D positive, but who produce anti-D that reacts with all D positive cells except their own and those of other rare individuals with the same partial D type. In these people, a part of the normal D antigen is missing and they can make an anti-D to that missing part. These are sometimes referred to as D categories or D variants. It is *not* possible to recognize these rare types either by routine or D^u testing; they are recognized when they produce anti-D. This is a very rare occurrence, but you should bear it in mind when investigating atypical antibodies.

Techniques for RhD typing are described in Section 7 and Appendix 1.

SUMMARY

- 1 The Rh system has two genes (*D* and *CE*) that are inherited together to give a number of Rh haplotypes.
- 2 It is important to ensure that RhD negative patients receive RhD negative blood when being transfused.
- 3 Testing for weak D or D^u in patients is usually performed only if the reactions between two anti-D reagents differ or if the result obtained is different from that previously found. When testing donors, it is normal practice to test for D^u when samples give a negative result with the initial test anti-D used.

- 4 Only anti-D reagents prepared for D^u testing should be used for D^u testing, together with a negative control or the direct antiglobulin test.

SELF-ASSESSMENT

- 11 *Is the Rh antigen well-developed or poorly-developed in fetal life?*
 - 12 *Why is it particularly important that RhD negative females are correctly RhD typed?*
 - 13 *When would you test for the D^u antigen?*
 - 14 *Would you regard a D^u donor as RhD positive or negative when it comes to transfusing his blood?*
-

PROGRESS CHECK

Before moving on to Section 6, spend a few minutes thinking about whether you have achieved the learning objectives for Section 5. These were to:

- 1 Explain the significance of the Rh system in blood transfusion practice.
- 2 Explain the basic genetics of the Rh system and predict the possible blood groups of family members.
- 3 Determine the possible percentage frequency of RhD positive and RhD negative individuals in your locality.
- 4 Explain the importance of correct RhD grouping, particularly in relation to haemolytic disease of the newborn.
- 5 Explain the significance of the weak D (D^u) antigen and recognize when to test for D^u.

If you feel confident that you have understood everything in this section, turn to Section 6.

If you feel that you need to spend more time on this section, go back to the parts that are most unfamiliar or that you have found difficult. You may find it helpful to talk to other people, such as your supporter and other senior colleagues if there is anything you are still not sure about.

6

Compatibility Testing and Issuing Blood

The purpose of this section is to help you to understand the reasons for compatibility testing and its importance, and the procedures for both routine and emergency testing. It should also help you to establish appropriate procedures for selecting and issuing blood, investigating transfusion reactions, record-keeping and managing stocks of blood and plasma.

The techniques used in compatibility testing are described more fully in Section 7 and in Appendix 1.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Explain the importance of compatibility testing in blood transfusion practice.
 - 2 Establish appropriate procedures for the request of blood for transfusion.
 - 3 Establish appropriate procedures for selecting and issuing blood and plasma routinely and in an emergency.
 - 4 Ensure that those responsible for giving blood to patients are aware of the correct procedures.
 - 5 Develop appropriate procedures to investigate suspected transfusion reactions.
 - 6 Establish and maintain an efficient record-keeping system.
 - 7 Establish and maintain efficient procedures for managing stocks of blood and plasma.
-

6.1 COMPATIBILITY TESTING

The term compatibility testing refers to the set of procedures required before blood can be issued as being safe for transfusion. By ensuring that there are no antibodies in the patient's serum that react with the red cells for transfusion, the donor's blood should not cause any adverse reactions and the red cells will have a maximum survival time following transfusion.

Compatibility testing involves:

- 1 Checking the patient's records for the results of:
 - previous blood grouping
 - the presence of any antibodies
 - details of past transfusions
 - the reason for transfusion.

This information should be given on the blood request form, but it is worth also checking your own laboratory records.

- 2 Performing an ABO and RhD group on the patient's blood sample and checking to ensure that these match any previous results you have.
- 3 Performing an antibody screen, if possible.
- 4 Performing the final compatibility test, the **cross-match**, which is the test between the patient's serum and the donor red cells to detect any antibodies in the patient's serum that react with the donor red cells. This is sometimes referred to as the major cross-match. The minor cross-match is the testing of the patient's red cells with donor serum to detect the presence of any antibodies in the donor's serum that might react with the patient's red cells. In the majority of cases, the minor cross-match is no longer required since the donor's serum is checked for the presence of antibodies when it is grouped.

cross-match (match):

The testing of the patient's serum against the donor red cells and the donor serum against the patient's red cells, before transfusion.

Blood group systems other than ABO and Rh

Other than the ABO and Rh blood group systems, there are some 20 blood group systems, such as Duffy, Kidd and Kell, on human red cells. These might lead to the production of antibodies if a person lacking one of these antigens is sensitized, by pregnancy or transfusion, to that antigen. If these antibodies are not detected in the compatibility test, they could cause severe transfusions reactions.

Safe blood transfusion depends on avoiding incompatibility between the donor's red cells and antibodies in the patient's plasma.

6.2 REQUESTS FOR BLOOD

When blood is required for a transfusion, 5–10 ml of the patient's blood should be collected into a dry tube in order to ensure that serum, in preference to plasma, is available for carrying out the compatibility test.

The blood sample should then be clearly labelled with the patient's full name, hospital reference number and ward and sent immediately to the laboratory, together with a completed blood request form.

The request form should provide the following information about the patient and should be signed by the doctor in charge of the patient or by the person authorized by the doctor to do so:

- date of request
- patient's full name
- patient's date of birth
- patient's sex
- patient's hospital reference number
- patient's ward
- patient's address
- provisional diagnosis
- patient's blood group, if known
- the presence of any antibodies
- history of any previous transfusions
- history of any previous transfusion reactions
- females: number of previous pregnancies
- number and type of units of blood or blood products required
- whether patient's serum should be grouped and held
- reason for transfusion
- date and time required
- signature of the doctor requesting the blood.

Requests for blood for transfusion must never be accepted unless all the patient's details on the sample match those on the request form. If the details do not match, a new sample and form should be requested. An example of a blood request form is given in Figure 13 on page 44. This includes a compatibility test record which should be completed before the blood is issued.

ACTIVITY 14

Is a blood request form used in your hospital? If it is, does it contain all the points listed above? Compare it with the example given in Figure 13. Can you suggest any improvements that could be made to your form or the way that it is used? Note your recommendations on your Action List.

If a blood request form is not yet used in your hospital, talk to your supervisor and medical colleagues about the importance of introducing one. On your Action List, note your recommendations on what it should contain and how it should be used.

Figure 13: Example of a blood request form

EXAMPLE OF BLOOD REQUEST FORM								
HOSPITAL: _____				Date of request: _____				
PATIENT DETAILS								
Family name: _____				Date of birth: _____		Gender: _____		
Given name: _____				Ward: _____				
Hospital reference no.: _____				Blood group (if known): ABO <input style="width: 50px;" type="text"/>				
Address: _____				Rh D <input style="width: 50px;" type="text"/>				

HISTORY								
Diagnosis: _____				Antibodies: Yes/No _____				
Reason for transfusion: _____				Previous transfusions: Yes/No _____				
Haemoglobin: _____				Any reactions: Yes/No _____				
Relevant medical history: _____				Previous pregnancies: Yes/No _____				
REQUEST								
<input type="checkbox"/> Group, screen and hold patient's serum				Whole blood <input style="width: 50px;" type="text"/> units				
<input type="checkbox"/> Provide product				Red cells <input style="width: 50px;" type="text"/> units				
Date required: _____				Plasma <input style="width: 50px;" type="text"/> units				
Time required: _____				Other <input style="width: 50px;" type="text"/> units				
Deliver to: _____								
NAME OF DOCTOR (print): _____				SIGNATURE: _____				
IMPORTANT: This blood request form will not be accepted if it is not signed or any section is left blank.								
LABORATORY USE ONLY								
Donor typing		Compatibility testing					Patient ABO <input style="width: 50px;" type="text"/>	
							Rh D <input style="width: 50px;" type="text"/>	
Donation pack no.	ABO	Rh	AHG	Room temp. saline	Date of match	Time of match	Date of issue	Time of issue
Signature of tester: _____								

6.3 SELECTING BLOOD FOR PATIENTS

When selecting blood for transfusion, it is important that blood of the correct ABO group is used. Red cells that are incompatible with the ABO antibodies in the patient's plasma can cause fatal haemolytic reactions. See Figures 14 and 15 for rules in selecting the correct group.

Red cell components

In red cell transfusion, there must be ABO and Rh compatibility between the donor's red cells and the recipient's plasma.

- 1 Group O individuals can receive blood from group O donors only
 - 2 Group A individuals can receive blood from group A and O donors
 - 3 Group B individuals can receive blood from group B and O donors
 - 4 Group AB individuals can receive blood from AB donors, and also from group A, B and O donors
-

Figure 14: Transfusion rules for red cells in the ABO system

Note: Red cell concentrates, from which the plasma has been removed, are preferable when non-group specific blood is being transfused.

Patients who are RhD negative should receive blood that is also RhD negative to prevent them being stimulated to produce anti-D that, as we have seen in Section 5, can cause transfusion reactions and haemolytic disease of the newborn (HDN). The exception to this rule is when transfusing a baby suffering from HDN (see page 45).

Plasma and components containing plasma

In plasma transfusion, group AB plasma can be given to a patient of any ABO group because it contains neither anti-A nor anti-B antibody

- 1 Group AB plasma (no antibodies) can be given to any ABO group
 - 2 Group A plasma (anti-B) can be given to group O and A patients
 - 3 Group B plasma (anti-A) can be given to group O and B patients
 - 4 Group O plasma (anti-A + anti-B) can be given to group O patients only
-

Figure 15: Transfusion rules for plasma in the ABO system

Severe acute haemolytic transfusion reactions are nearly always caused by transfusing red cells that are incompatible with the patient's ABO type. These reactions can be fatal.

These reactions most often result from errors made in identifying the patient when blood samples are being taken or when blood is being administered. Fatal reactions can also result from errors in the laboratory, such as not using the correct sample or not recording the results correctly.

The blood request form should always include the reason for transfusion so that the laboratory can select the most suitable blood for compatibility testing. Blood must be selected carefully to suit the needs of each individual patient. In general, the oldest units should be used first, but there are the following exceptions.

- 1 Patients receiving large quantities of blood as in “massive” transfusions (more than their total blood volume) should be given the freshest units of blood available.
- 2 Patients receiving large quantities of blood who have a small blood volume, such as neonates receiving exchange transfusions or any infant under five years of age receiving a transfusion, should also be given the freshest blood available.
- 3 Patients over five years old requiring blood for anaemia can be given red cell concentrates within the expiry date (see Appendix 2 for the procedure for preparing red cell concentrates). However, patients receiving regular transfusions, such as those with sickle cell anaemia and thalassaemia, should receive relatively fresh units that are less than seven days old, if available, which will make the time between transfusions longer.
- 4 Blood being issued to another hospital should always include units with a variety of expiry dates, including some fresh units, so that it does not all go out of date at the same time.

The selection of blood for patients with atypical antibodies requires special care. In general, blood that does not contain the antigen that reacts with the patient’s antibody should be selected, but the selection should be discussed with senior colleagues.

Selecting and issuing blood for a massive transfusion

“Massive” transfusion is transfusion of more than the patient’s own blood volume within 24 hours. After 12 units of blood have been transfused, uncross-matched blood of the appropriate ABO and RhD group can be issued because the serum from the original match sample is no longer representative of the patient’s blood. The ABO and RhD group of the units of blood should be checked before issue and all other details entered on the blood bag label and in the records, as usual. After three days, if blood is still required, a new sample should be taken and the blood matched.

Selecting and issuing whole blood or red cells for neonates and babies

When neonates or infants require blood or an exchange transfusion, the freshest blood available, preferably not more than seven days old, should be selected. The choice of group is also important, depending on the reason for the exchange. When the transfusion is simple as, for example, in a “top-up” transfusion for anaemia, blood of the appropriate RhD group that is *ABO compatible with the mother and the baby* should be selected, and cross-matched using the mother’s serum. For an exchange transfusion, the selection of blood depends on the reason for the exchange. There are four reasons for exchange transfusion:

- 1 *RhD haemolytic disease of the newborn*
RhD negative blood, ABO compatible with the mother *and* baby, should be selected and cross-matched with the mother’s serum.

2 *ABO haemolytic disease of the newborn*

Low-titre group O blood of the same RhD group as the baby should be selected and cross-matched with the mother's serum.

3 *Any other cause of neonatal jaundice*

Blood of the same ABO and RhD group as the baby, or low-titre group O of the same RhD group, should be selected and cross-matched with the mother's serum.

4 *Another Rh antibody or a non-Rh antibody, such as anti-c or anti-Kell (anti-K)*

Blood must be selected which does not have the antigen to the particular antibody of the same ABO group as the baby. In these cases, the selection of blood should always be discussed with senior colleagues. In an emergency, however, blood can be selected by cross-matching units of blood against the mother's serum and looking for negative units.

In summary, when selecting blood for neonates and babies for exchange due to RhD HDN or ABO HDN, the simplest solution is to select group O RhD positive blood when both the mother and baby are RhD positive, regardless of the ABO groups. Select group O RhD negative blood if either or both the mother and baby are RhD negative, again regardless of the ABO groups.

Always remember that it is very important to make the correct selection of blood for any kind of exchange transfusion. The decision should be discussed with colleagues and, if possible, staff at the blood transfusion centre. If you are in doubt, select low-titre group O blood of the appropriate RhD group.

When blood or plasma is issued for neonates or babies, it is important to select the correct amount of blood to be transfused. The amounts required are usually small, unless the blood is for an exchange transfusion. Ideally, a paediatric blood bag should be used. This is a normal size blood bag with four or five small satellite bags. The unit of whole blood or red cells can be divided into these four or five separate bags, which can then be used individually. Paediatric blood bags are relatively expensive, however, and are not always available.

If there is little blood available, try to combine as many non-urgent requests as you can and cross-match the same unit of group O blood for all of them. Each time the unit is entered, a new transfusion set must be used in order to prevent the transmission of infection. The blood should be used within 12 hours or discarded, in case the unit has become accidentally contaminated. The same applies to any unit of blood that is returned having been opened.

ACTIVITY 15

Do your procedures for selecting blood differ from those described in Section 6.3? Do you think you could make any improvements to them?

Note down your recommendations on your Action List and discuss them with your supervisor and other senior colleagues.

6.4 SCREENING FOR IRREGULAR ANTIBODIES

Some patients develop antibodies to blood group antigens as a result of a blood transfusion or after a pregnancy when some of the fetal red cells have leaked into the mother's circulation. If the antigens on these cells are seen as "foreign", the patient produces antibodies against them.

The purpose of screening for these antibodies before performing the cross-match is to give you time to find compatible blood for those patients who have formed antibodies. The test should be performed at 37°C, if possible using an antiglobulin technique and red cells that have been selected as having all the main blood group antigens present. Since it is not possible to find these on the cells of one individual, cells from two or three donors are used. Two-cell screening sets usually consist of one from a group O R₁R₁ (CDe/CDe) person and one from a group O R₂R₂ (cDe/cDe) person. With three cells, the additional one is from a group O (cde/cde) person. Each serum should be tested against the antibody screening cells, using a standard indirect antiglobulin test with the cells in either normal saline or low ionic strength saline (LISS).

In some laboratories, an enzyme antibody screen is also performed, but this needs to be standardized carefully so that false-positive reactions do not occur. If an antiglobulin test cannot be performed, an albumin addition test can be used, although it is not as sensitive.

6.5 CROSS-MATCHING

Cross-matching is carried out to ensure that there are no antibodies present in the patient's serum that will react with the donor cells when transfused. Even if the patient's and donor's ABO and RhD groups are known, it is essential to perform a cross-match as the final serological test of compatibility as this will also show if any mistakes have been made in the ABO grouping of the patient or donor. Remember that it is ABO incompatibility between the patient's plasma and donor red cells that causes fatal haemolytic transfusion reactions.

Whenever possible, an indirect antiglobulin test should be used for the cross-match. The techniques used are described in Section 7 and in Appendix 1.

6.6 LABELLING COMPATIBLE UNITS

When the cross-match has been read and it is decided that the donor unit is compatible with the patient, there must be some way of labelling the unit for that patient. It is best to use compatibility labels that can be attached in some way to the unit of blood. New labels can then be written if the blood is not used for that patient.

If adhesive labels are too expensive, luggage or tag labels that can be tied to the unit are available in most countries. If no labels are available

and it is necessary to write on the blood unit itself, this *must* be done in such a way that the group of the unit, the patient's name and the patient's hospital reference number can be clearly seen. These are the three most important facts, along with the expiry date of the blood.

Compatibility labels should be very simple and easy to read so that no mistake can be made when the blood is taken from the refrigerator. The details needed on the label, as shown in Figure 14, are:

- blood pack number
- patient's name
- patient's date of birth
- patient's hospital reference number
- patient's ward
- patient's blood group
- blood group of unit
- expiry date of unit
- date of the cross-match
- signature of the person responsible for performing the cross-match.

These details, for all units cross-matched for the patient at one time, should also be given in a report that can be issued when the first unit of blood is collected. This report should be kept in the patient's notes as a record of the blood transfused.

THIS BLOOD IS COMPATIBLE WITH:

Blood pack number:

Patient's name:

Patient's date of birth:

Patient's hospital reference number:

Patient's ward:

Patient's blood group:

Blood group of unit:

Expiry date of unit:

Date of cross-match:

Signature:

RETURN TO BLOOD BANK PROMPTLY IF NOT USED

Figure 14: Example of a compatibility label

ACTIVITY 16

Look carefully at the compatibility labels used in your blood bank and compare them with the example given in Figure 16. Can you suggest any ways in which your labels could be improved? If you can, note down your recommendations on your Action List.

If you do not have compatibility labels of this kind, talk to your supervisor about the importance of introducing them. On your Action List, note down your recommendations on what they should contain.

6.7 GROUP AND HOLD SYSTEM

Blood is often requested for a patient even though the doctor in charge feels that there is only a slight chance that it will be used. There is a potential risk to a patient's life when there is a delay in carrying out the grouping and compatibility testing when blood has not been requested, but is subsequently needed urgently.

A "group and hold" or "group and save" system can be introduced in an attempt to reduce the number of units of blood that are cross-matched unnecessarily, while at the same time ensuring that blood can be provided for the patient if it is required urgently.

On admission to hospital, the patient's blood is fully ABO and RhD grouped and the serum is screened for irregular antibodies. If irregular antibodies are detected, these need to be identified and compatible blood found and cross-matched. However, if there are no antibodies, as is usually the case, the remaining serum can then be labelled clearly with the patient's name, hospital reference number and ward, and stored at -20°C . If blood is urgently required, the serum can be thawed and used to carry out an urgent cross-match. If the patient has no irregular antibodies, the blood can be released after an "immediate spin", although it is good practice then to incubate the tubes and carry out the indirect antiglobulin test.

This system works well when a "blood ordering schedule" has been devised as a guide to the number of units of blood to be ordered for a particular surgical procedure. This schedule is worked out by reviewing the number of units of blood that have been used for common elective operations over a period of time. In agreement with the surgical team and the blood bank, a list is made of the expected normal usage of blood for these procedures and the number of units of blood to be routinely cross-matched before the operation. For some operations where there is only a small chance of blood being needed, a "group and hold" is requested to avoid having to cross-match blood that will probably not be used. If blood is required, however, it can then be provided quickly.

See Section 7 and Appendix 1 for techniques used in emergency cross-matching.

6.8 SELECTING AND ISSUING BLOOD IN AN EMERGENCY

If you receive a request for blood in an emergency, the procedure to follow depends on the degree of urgency. If it is "extremely urgent" (that is, required within 10–15 minutes), you will not be able to do a cross-match and you must tell the doctor requesting the blood that he or she is responsible for the effects of any transfusion given since cross-matched blood cannot be provided in less than half an hour. If it is not possible

to wait this long and uncross-matched blood is needed, the following procedure should be used.

- 1 Carry out an ABO and RhD group on the sample, using rapid techniques. Select blood of the appropriate ABO and Rh group.
- 2 Label the blood with as many of the patient's details as are known and write "UNCROSS-MATCHED BLOOD" clearly on the label. An example of a typical label is given in Figure 17.
- 3 Take samples from the bleed line or pilot tube of the units so that the group can be checked and a compatibility test carried out later.
- 4 Issue the blood as normal.

When blood is not required for at least half an hour, there is usually time to perform a cross-match using a low-ionic strength indirect antiglobulin test.

WARNING: UNCROSS-MATCHED BLOOD

Blood pack number:

Patient's name:

Patient's date of birth:

Patient's hospital reference number:

Patient's ward:

Patient's blood group:

Date:

Signature:

RETURN TO THE BLOOD BANK PROMPTLY IF NOT USED

Figure 17: Example of a label for the use of unmatched blood in emergency

The person requesting the blood should sign the request form to show that he or she is responsible for the consequences of a transfusion of uncross-matched blood. If there is no signature on the form or there is no request form at all, try to contact the person urgently to explain that a request form and signature are required. If this is not possible, enter the person's name in the record book and record that uncross-matched blood was issued at his or her request.

If no sample is received or there is no time to do a rapid group, you should, wherever possible, issue group O RhD negative (haemolysin-free) blood until a sample arrives or a rapid group can be done.

This procedure takes time, but it must be carried out to ensure the safety of the patient. It is in circumstances like these, when time is short, that mistakes are made. In an emergency, extra staff from another part of the

blood bank or laboratory should, wherever possible, be asked to help. The most important thing to remember in an emergency is:

DON'T PANIC!

Remember that, in these cases, there will nearly always be time to take a sample and do a rapid group. Most emergency cases require volume replacement at first and can be given crystalloid solutions, such as saline or Ringer's lactate, which are available in most hospitals. This gives you precious minutes to make the checks that may save the patient's life.

Other important points to remember in emergencies are as follows:

- 1 Always make sure that your senior colleagues know when there is an emergency of any kind.
- 2 If your blood is delivered from a blood transfusion centre or another hospital, make sure that its staff are told about the emergency as soon as possible, especially if you think that more blood will be required.
- 3 If you collect blood from your own donors, make preparations to contact and bleed more donors.
- 4 Always make sure that you keep in contact with staff who are with the patient so that you are aware of potential problems *before* they actually happen.
- 5 Ensure that full and accurate records are kept. It is always important to keep up-to-date records in the blood bank, but it is particularly important when uncross-matched blood is issued. You must make sure that all decisions made by the clinicians are clearly recorded, including the names and signatures of those involved.

6.9 ISSUING BLOOD OR PLASMA FROM THE BLOOD BANK

The person who issues blood or plasma for transfusion has one of the most important jobs in the blood bank. It is that person's responsibility to make sure that the blood or plasma is the correct group, that it has been selected and cross-matched for the particular patient and that it appears to have been stored safely. In other words, it is that person's responsibility to ensure that the blood or plasma is as *safe* as possible for the patient. What must you do to ensure that it is safe?

- 1 Insist that the person taking the blood or plasma from the blood bank brings some documentation to identify the patient who will receive the blood transfusion, including the patient's name, hospital reference number and ward and, where possible, blood group.
- 2 Check this with the patient's details on the blood request form in the blood bank, on the compatibility label on the unit of blood or plasma and in the compatibility register,

ensuring that the patient's name and hospital reference number are the same. The *hospital reference number* is the most important thing to check because it is unique to that patient, whereas there may be other patients with the same name or a similar name.

- 3 Check that all other tests, including anti-HIV, hepatitis B and syphilis, have been performed and are negative.
- 4 Check that the blood group on the request form (if it has been included) is compatible with the blood group on the label of the unit of blood or plasma and in the compatibility register.
- 5 Check the expiry date on the unit of blood or plasma to make sure that it is being issued on, or before, the date of expiry.
- 6 Inspect the unit of blood or plasma for any signs of deterioration, as shown in Figure 18.
- 7 Enter the date and time of issue in the compatibility register.
- 8 Record the name of the person taking the blood or plasma and make sure that this person signs the register.

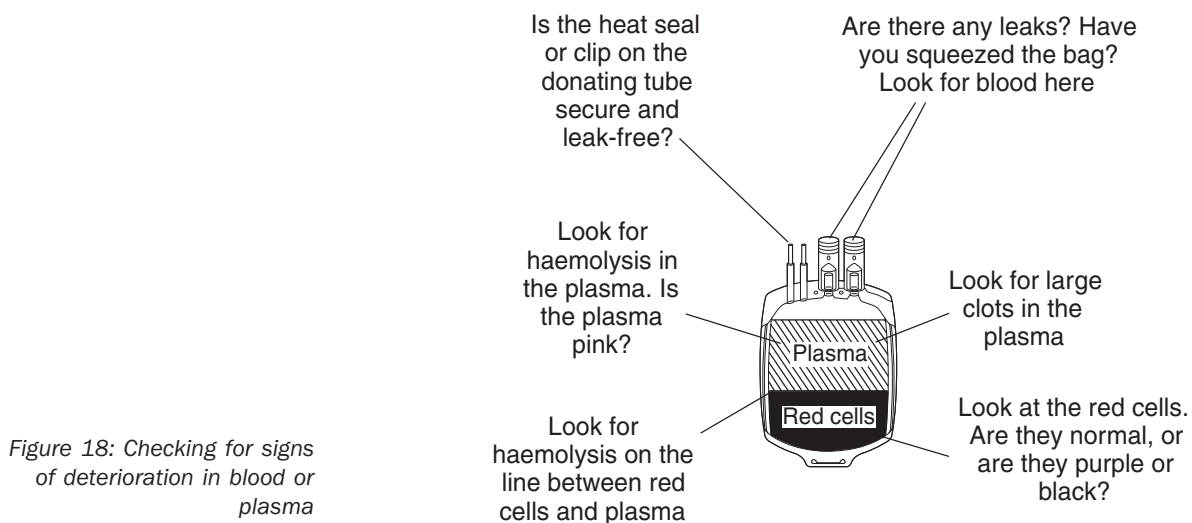


Figure 18: Checking for signs of deterioration in blood or plasma

If your blood bank does not use blood request forms that will enable you to check the patient's name, hospital reference number and other details, ask the person collecting the blood to bring the patient's notes or a piece of paper with the patient's name and hospital number written on it. However, using request forms will make it easier for you to ensure that blood is issued correctly and will also save you time because information about each patient will be presented in a standardized way.

It is important to have a system for checking that you have the right unit of blood for the right patient. If your hospital does not give patients a hospital reference number, check the patient's date of birth as well as their name. If that is not available, try using the patient's address.

If request forms are not yet used in your hospital, you must make sure that all the information you need is in your compatibility register. If necessary, find out the information you need by going to the ward yourself. If there are any problems about doing this, show this module to the doctors and explain how important it is to make sure that their patients receive the right unit of blood and why blood request forms should be used.

You should be able to do everything else on the list without needing further information. All you need is time. Figure 19 provides a simple checklist for the issue of blood or plasma.

BEFORE YOU ISSUE BLOOD OR PLASMA

- 1 Check that the person collecting the blood or plasma has brought documentation to identify the patient.
- 2 Check the patient's:
 - name
 - hospital reference number
 - ward
 - blood group
 with
 - the blood request form
 - the compatibility label
 - the compatibility register.
- 3 Check that all other tests, including anti-HIV, hepatitis B and syphilis, have been performed and are negative.
- 4 Confirm that the blood or plasma is compatible by checking the blood group on:
 - the blood request form
 - the compatibility label
 - the compatibility register.
- 5 Check the expiry date of the blood or plasma.
- 6 Inspect the blood or plasma for any signs of deterioration.
- 7 Enter the date and time of issue in the compatibility register.
- 8 Obtain a signature in the compatibility register from the person collecting the blood.

Figure 19: Checklist for the issue of blood or plasma

ACTIVITY 17

What procedure is followed in your blood bank when blood is issued?

Look at the checklist in Figure 19 and compare it with the system that is used in your blood bank. Do you think there are any ways in which your procedure could be improved? If so, note your recommendations down on your Action List.

Make your own checklist for the issue of blood, taking any local policy guidelines into account. Alternatively, you could use the checklist in Figure 17 (a separate copy is included as an Offprint with this module).

Put your checklist on the wall of the blood bank and make sure that everyone reads it and follows the procedure.

If you have a hospital transfusion committee, show it to them so that everyone involved in blood transfusion knows the procedure for the safe issue of blood from your blood bank.

Storing blood outside the blood bank

If the ward or operating theatre does not have a refrigerator that is appropriate for storing blood, the blood should not be released from the blood bank until immediately before it is going to be transfused. Failing this, it should be issued in a blood transport box or an insulated cold box to keep the temperature within the range of +2°C to +6°C. Since a cold box will probably have a maximum storage time of only four hours, the blood must be returned to the blood bank if it is not used within that time. When using a cold box, the units of blood must not be allowed to come into direct contact with the ice-packs as this will cause haemolysis of some of the red cells, which could be fatal for the patient.

Blood left outside the correct storage temperature for more than 30 minutes is considered unsafe for transfusion. It must be immediately returned to the blood bank, clearly labelled to show that it has been left without correct refrigeration for more than the allowed period of time.

The storage of blood and plasma is covered in detail in Section 5 of the Introductory Module.

6.10 WHEN BLOOD OR PLASMA IS GIVEN TO THE PATIENT

Giving blood or plasma to patients is not normally the responsibility of the blood bank. The person who starts the transfusion of the blood or plasma has the last – and most important – responsibility in the sequence of checks from blood collection to transfusion. It is therefore essential that everyone involved in giving blood or plasma to patients knows and uses the following procedure to check, at the bedside, that the correct blood is being given to the correct patient.

- 1 Check that the name, hospital reference number and ward in the patient's notes are identical with the name, number and ward on the compatibility label on the blood or plasma and on the blood request form. The *patient's name* and hospital reference number are the most important checks. Check the name given in the patient's notes by asking the patient to give their name. If the patient is unable to speak, ask a relative to tell you the patient's name.
- 2 Check that the blood group on the compatibility label on the unit of blood or plasma is identical with the blood

group on the request form and in the patient's notes, if it is recorded there.

- 3 Check the expiry date on the unit of blood or plasma to make sure that it is being transfused on, or before, the date of expiry.
- 4 In the patient's notes, record the date and time of transfusion, the number of units of blood or plasma given and the blood or plasma unit numbers.
- 5 Sign the patient's notes.

It is also important to remember that the patient's basic clinical signs, including pulse, blood pressure and temperature, should be checked before, during and after the transfusion.

Figure 20 provides a simple checklist for giving blood to a patient. If possible, reproduce these instructions so that you can give out a copy with each unit of blood or plasma to make sure that the person giving the blood or plasma follows this procedure. If this is not possible, display copies in each place where blood is transfused.

The final check at the patient's bedside is the last opportunity to detect an identification error and prevent a potentially incompatible transfusion, which could be fatal.

BEFORE YOU GIVE BLOOD OR PLASMA TO A PATIENT

- 1 Confirm the patient's:
 - name
 - hospital reference number
 - ward
 by asking the patient or a relative to confirm the patient's name and by checking:
 - the patient's notes
 - the compatibility label
 - the blood request form.
- 2 Confirm that the blood or plasma is compatible by checking the blood group on:
 - the patient's notes
 - the compatibility label
 - the blood request form.
- 3 Check the expiry date of the blood or plasma.
- 4 In the patient's notes, record:
 - the date of transfusion
 - the time of transfusion
 - the number of units of blood or plasma given
 - the blood or plasma unit numbers.
- 5 Sign the patient's notes.

Figure 20: Checklist for giving blood or plasma to a patient

ACTIVITY 18

Talk to some of the doctors in your hospital to find out the procedure that is followed when blood is collected from the blood bank and given to a patient. How does it compare with the checklist shown in Figure 20? If you can suggest any ways in which the procedure could be improved, note down your recommendations on your Action List.

Discuss your recommendations with your supervisor and with members of the medical staff. Then make your own checklist for giving blood or use the one given in Figure 18 (a separate copy is included as an Offprint with this module).

Give out a copy each time that blood or plasma is issued from the blood bank. If this is not possible, put a copy on the wall in each place where blood is transfused.

Try to make sure that everyone involved in giving blood reads it and follows the procedure.

If you have any difficulty in finding out what happens to the blood that you issue or in trying to change the procedure, show this module to the doctors who order blood and plasma. Explain that you are trying to make blood transfusion as safe as possible for their patients and ask for their cooperation in following this procedure.

6.11 TRANSFUSION REACTIONS

Fortunately, the majority of blood transfusions take place without any adverse effects on the patient. Occasionally, however, patients will react to transfused blood even though the laboratory tests carried out before transfusion showed the blood to be compatible.

The severity of reaction that a patient suffers can vary from a mild reaction, which leads to little more than a headache with a slight rise in body temperature, to the more severe haemolytic form which, in rare cases, can be fatal.

Transfusion reactions fall mainly into three categories:

- febrile reactions
- allergic reactions
- haemolytic reactions.

Febrile reactions

Febrile reactions lead to headache followed by a sudden chill, shivering and a rise in body temperature. These reactions are rarely severe and respond rapidly to medical treatment.

urticaria: The appearance of weals on the skin with rash.

Allergic reactions

Severe allergic reactions, sometimes called anaphylactic reactions are comparatively rare. In such cases, the patient can suffer **urticaria** of the

laryngeal oedema:

Swelling of the larynx creating difficulty in breathing.

haemoglobinaemia:

Free haemoglobin in the bloodstream (plasma).

macrophage: A phagocytic cell type found in the bloodstream as well as tissues. Macrophages ingest bacteria and cell debris.

skin, moderate bronchial spasm and possible **laryngeal oedema**. Reactions of this kind are rare and respond rapidly to medical treatment.

Haemolytic reactions

Haemolytic reactions are the most severe of the three types of transfusion reaction and are initiated by:

- antibody in the patient's serum reacting with its corresponding antigen on the donor red cells
- antibody in the donor plasma reacting with its corresponding antigen on the patient's red cells.

Haemolytic transfusion reactions can occur either intravascularly or extravascularly.

Intravascular reactions cause haemolysis of the red cells within the circulatory system, with subsequent jaundice and **haemoglobinaemia**. These reactions are brought about mainly by antibodies of the IgM type, the most dangerous of these being the specific anti-A and anti-B of the ABO system. Many reactions of this type are fatal, with the death of the patient resulting either from uncontrolled bleeding or from renal failure.

Extravascular reactions are rarely as severe as intravascular reactions, although they can cause considerable discomfort to the patient. Fatal reactions are rare. This type of reaction is caused by IgG antibodies which bring about destruction of the red cells via the **macrophages**. This sometimes results in a sudden drop in the patient's haemoglobin level, often up to 10 days after the transfusion.

Laboratory investigation of transfusion reactions

All transfusion reactions must be reported and investigated as soon after the reaction as possible in order to determine their cause.

When it is known that a patient is having a transfusion reaction, it is important to ensure that:

- 1 The transfusion is stopped.
- 2 The medical officer is informed.
- 3 The necessary samples are taken for the laboratory. These should include:
 - an immediate post-transfusion clotted and non-clotted blood sample from the patient
 - red cell and plasma residues from the transfused donor blood
 - the first specimen of the patient's urine following the reaction.

The patient's pre-transfusion sample should already be in the laboratory.

The extent of the investigation that you are able to carry out will be determined largely by the facilities in your laboratory and your own

technical expertise. However, you should be able to carry out the following procedures:

- 1 Fully record:
 - the type of reaction that the patient suffered
 - the length of time after transfusion that the reaction occurred
 - the volume of blood that was transfused.
- 2 Examine the patient's pre- and post-transfusion plasma for evidence of jaundice and/or haemoglobinaemia.
- 3 Perform a direct antiglobulin test on the pre- and post-transfusion red cells.
- 4 Repeat the compatibility test of the patient's serum against the donor red cells, using both pre- and post-transfusion specimens, including a check on the patient's and donor's ABO and RhD groups.
- 5 Check the donor plasma against the patient's red cells by an indirect antiglobulin technique to exclude antibodies in the donor plasma reacting with the patient's red cells.
- 6 Perform a Gram stain on the contents of the units transfused to check for bacterial contamination.
- 7 Check the post-transfusion sample of the patient's urine for evidence of free haemoglobin or red cells.
- 8 Recheck *all* your records to ensure there are no errors due to the incorrect entry of data or a mix-up of samples.

You should be able to perform all these tests and checks. There are many other tests, such as antibodies to white cells and platelets, measurement of bilirubin on pre- and post-transfusion samples and tests for methaemalbumin and for bacterial contamination. These tests can be carried out only in a relatively advanced laboratory, however, and it may be necessary to refer to a laboratory of this kind if the severity of the reaction justifies it.

It is important to remember that *all* reactions should be investigated, wherever possible. This will not only establish the cause of the reaction, but also help to ensure the safety of the patient by preventing a recurrence if further transfusions are necessary. It will also serve as a quality control on your transfusion practice and record-keeping.

ACTIVITY 19

What are your laboratory's procedures for investigating transfusion reactions? How do they compare with the procedures given above? If you can identify any improvements that could be made to your procedures, note down your recommendations on your Action List.

If there are no established procedures for investigating transfusion reactions in your laboratory, talk to your supervisor about developing a system and note down your recommendations on your Action List.

6.12 RECORD-KEEPING

Good record-keeping is an essential part of the quality system in safe blood transfusion practice. A written record is required of every stage of the process from the time that the blood donor is recruited, through the testing and processing of the donated blood, to its eventual transfusion or disposal.

It is also essential to have a written “standard operating procedure” or SOP that specifies the exact method to be followed for every procedure that is used, such as performing ABO and RhD grouping. This ensures that all members of staff know what to do, how to do it and that they all perform a particular procedure in the same, correct, way each time. Only approved forms, worksheets and registers should be used. Results should not be written on a piece of paper and the results transcribed later, as this leads to errors.

It cannot be said too often that blood transfusion practice is 10% work – and 90% checking that work. In the laboratory, two technicians are better than one for the safe testing of blood – one to read the results and the other to write them down. The two technicians can then change places to check the results.

The required checks cannot be made, however, if the results are not written down or are not recorded in the correct way. It is insufficient, for example, to write the words “Pos” or “Neg” meaning “RhD positive” or “RhD negative” next to a donor’s or patient’s name. Once the result has been determined, you should either write “Rh pos” or “Rh neg”. If two anti-Ds are used, the two results must be recorded separately; you should write “RhD neg” only when you have checked that the two results are the same.

Checks should be made *every time* a test is carried out and *every time* a sample or a unit of blood moves on to another part of the system. These checks are essential to ensure the safety of the patient. In addition, accurate and up-to-date records can help you to manage your blood bank in a efficient way.

In some countries, a shortage of paper or expensive printing facilities cause problems in trying to keep good records. If this is a problem in your country or area, show this module to your supervisor and ask him or her to make sure that in your laboratory you have at least:

- blood request forms
- compatibility labels
- registers for test results.

Laboratory documentation

Computerized records or laboratory registers are the best means of keeping a full record of both blood donors and patients. Such records must be completed accurately and kept up to date and confidential. It is preferable to have two separate registers, one for donors and one for patients.

Donor blood grouping register

The donor blood grouping register needs to record not only the number of the unit of donated blood and the donor's ABO and RhD group, but also the fate of the unit and, if it is transfused, the name of the recipient.

Only units of blood that have been tested and found negative for infectious agents should be accepted for compatibility testing and entered in this register.

The donor blood grouping register should always include the following information:

- 1 Date
- 2 Number of the donor unit
- 3 Donor's ABO and RhD group
- 4 Type of blood or blood component
- 5 Expiry date of the unit or component
- 6 Signature of the person performing the grouping
- 7 Date of release for matching or disposal
- 8 Fate of the unit: that is, whether it is passed as suitable for transfusion or discarded
- 9 Name of the patient for whom it is selected for matching
- 10 Date of selection for matching.

An example of what a donor blood grouping register should contain is shown in Figure 21 on page 62. Note that you would probably need to use two facing pages in a large register or ledger to record the necessary information.

Compatibility register (patient testing register)

The compatibility register or patient testing register needs to include not only the patient's name, ABO and RhD group, but also the reference number of the units of blood matched with it and the blood donor's group.

The compatibility register should include the following information:

- 1 Date
- 2 Patient's full name
- 3 Patient's hospital reference number
- 4 Patient's date of birth
- 5 Patient's hospital ward
- 6 Name of the consultant or doctor in charge of the patient
- 7 Diagnosis/reason for transfusion
- 8 Patient's ABO and RhD group
- 9 Results of the irregular antibody screening test, if performed
- 10 Donor blood:
 - date

DONOR BLOOD GROUPING REGISTER											
Date	Donor number	ABO GROUP						Rh GROUP			Donor group
		Anti-A	Anti-B	Anti-AB	A cells	B cells	O cells	Result	Anti-D	Anti-D	

Type of component	Expiry date	Date of release	Fate of unit	Patient's name	Date

Figure 21: Example of a donor blood grouping register

- reference numbers of the donor units
 - donor's ABO and RhD group
 - expiry date of donor units
 - type of blood or blood component
- 11 Results of cross-matching
 - 12 Date and time of the cross-match, plus the signature of the person performing it
 - 13 Date and time of the issue for transfusion, plus the signature of the person collecting the blood or plasma
 - 14 The fate of the unit: whether it was transfused or returned. If it was not used, the date and time the blood or plasma was returned to the blood bank and whether or not it is suitable for reissue, plus the signature of the person returning it.

An example of what a compatibility or patient testing register should contain is shown in Figure 22 on page 64. Note that you would probably need to use two facing pages in a large register or ledger to record the necessary information.

It might be necessary to keep a separate register so that you can record the details of blood grouping tests (and, if performed, antibody screening tests) on those patients who do not require blood transfusion.

ACTIVITY 20

Look carefully at the donor blood grouping register and patient testing registers used in your blood bank and compare them with the examples given in Figures 21 and 22. Can you suggest any ways in which they could be improved? If you can, note down your recommendations on your Action List.

If you do not keep test registers of this kind, talk to your supervisor about the importance of introducing them. Note down your recommendations on your Action List.

It is important to remember that the forms, labels and registers included in this section are only examples and that you must decide on the most appropriate content and format for the documentation in your blood bank.

6.13 HOSPITAL BLOOD STOCK MANAGEMENT

Blood, plasma and platelet concentrates are precious and should rarely have to be discarded because they are out of date. In a well managed laboratory blood bank, the intake and issue of blood are equally balanced so that blood is not allowed to stay for too long in the refrigerator before compatibility testing.

COMPATIBILITY REGISTER									
Date	Patient's name	Patient's number	Date of birth	Hospital	Ward	Doctor in charge	Reason for transfusion		

ABO GROUP RESULTS					RH GROUP RESULTS			ANTIBODY SCREEN			Patient's group		
Anti-A	Anti-B	Anti-AB	A cells	B cells	O cells	Result	Anti-D	Anti-D	Result	1		2	Auto

COMPATIBILITY TEST										
Date	Donor number	Donor group	Room temp.	Albumin AHG	Result	Date and time of	match Matched by	Expiry date	Type of	component

Date issued	Time issued	Signature of person taking blood	Fate of unit	Date and time of return	Suitable for re-issue	Signature

Figure 22: Example of a compatibility register (patient testing register)

Some blood will inevitably be returned unused and this should, if passed as suitable, be used as soon as possible. If you receive a good supply of blood and sometimes some of it goes out of date without being used, use your records to work out how much less blood might have been sufficient. There may have been another patient in another hospital who really did need that blood.

There are many simple ways of making sure that blood and blood components do not have to be discarded because they are out of date, but the easiest is to ensure that the stocks are rotated every day. Each day, the stocks should be reviewed in the following way.

- 1 Remove and discard whole blood or red cells that have passed their expiry date.
- 2 Return to stock any unit of whole blood or red cells that has been matched and not used, as long as it shows no signs of deterioration and has been passed as being suitable for reissue.
- 3 Bring forward “older” units to the front of the stock in the refrigerator and put fresher units at the back to make sure that the units with the shortest time before expiry are used first.

As you have seen in the other modules, it is essential to keep records throughout the whole process of blood transfusion practice, from the recruitment of the donor to the time when the blood or plasma is transfused into the patient. These records are essential for the safety of both donors and patients, but accurate records will make your blood transfusion practice more efficient, as well as safe. For example, your records will help you to calculate how much blood and plasma you need in your blood bank each day, week, month and year.

Calculating blood and plasma requirements

Whether you collect blood yourself or receive it from a blood transfusion service or another hospital, you should be able to estimate how many units of blood and plasma of each group you need each week. This will help to ensure that you do not order too much or too little blood or plasma. Too much means that some units will go out of date before being used. Too little is even worse as it may result in patients not receiving blood or plasma when they most need it.

You therefore need to make an accurate assessment of how much blood you are likely to need. To do this, you need to keep an “inventory”. An inventory of your blood and plasma stocks is a list of the number of units of blood or plasma that pass through your blood bank in a certain period of time. It includes all the blood or plasma that is discarded for any reason, as well as the units that are transfused. Your own blood bank inventory will depend on many things, such as:

- the size of your hospital and the number of patients
- the number of obstetric deliveries
- the number of patients with anaemia
- the number and kind of operations carried out.

In order to make your own inventory, you should also find out your current *average* weekly usage of blood and plasma. To do this, you need to:

- 1 Count the number of units of blood and plasma that have been used each week in your blood bank over the last year, or at least during the last six months.
- 2 Make a table showing the number of units used each week and divide it into ABO and RhD groups.
- 3 Take away the highest week's figure in each group. You must do this to avoid getting a false figure as a result of an occasional crisis. For example, if there was a bad road traffic accident one week and large amounts of blood and plasma were needed, you should not include that week's figures.
- 4 Add up the total number used in each group, leaving out the highest week's figure in each group.
- 5 Divide the total by the number of weeks (minus one for the highest week).

This will give you an estimate of the average weekly usage of blood and plasma for each blood group.

Using these figures you can plan how much blood you need to collect from your donors or order from your blood transfusion service or another hospital, because you now know how much blood you should have in your blood bank each week.

Let us suppose, for example, that a total of 2000 units were used in your blood bank during the last year and that they were supplied by the blood transfusion service. Of those 2000, the number of units per group was as follows:

<i>Number of units</i>	<i>Blood group</i>
600	A RhD positive
20	A RhD negative
700	O RhD positive
50	O RhD negative
500	B RhD positive
20	B RhD negative
100	AB RhD positive
10	AB RhD negative

ACTIVITY 21

Calculate the weekly average usage for each blood group given above. Check your answers with those given in the Activity Checklists and Answers on pages 107.

You now know how many units you would be likely to need each week. Let us suppose that it is the day that you order blood from the blood transfusion service and you have counted the number of units in stock. There are:

<i>Number of units</i>	<i>Blood group</i>
6	A RhD positive
1	A RhD negative
5	O RhD positive
1	O RhD negative
6	B RhD positive
0	B RhD negative
1	AB RhD positive
1	AB RhD negative

ACTIVITY 22

Using the figures for average weekly usage that you worked out in Activity 21, decide how many units of each group you would need to order for the next week. Check your answers with those given in the Activity Checklists and Answers on page 108.

You now know how many units you would need to order from the blood transfusion service for the following week.

ACTIVITY 23

What method is used to calculate how much blood or plasma you need each week in your blood bank?

Using the method described above, which you practised in Activities 21 and 22, work out how much blood and plasma you need each week in your own blood bank. If you currently use a different method to calculate your blood requirements, compare your figures with the results obtained from this method.

If you can identify any ways of improving the accuracy of your method for calculating blood requirements, note down your recommendations on your Action List.

voluntary non-remunerated donor: A donor who gives blood, plasma or other blood components freely and voluntarily without receiving payment in the form of money or a substitute for money.

Do you order blood and plasma from a blood transfusion service or another hospital? If so, it is obviously sensible to place a regular weekly order rather than waiting until you have run out of a particular group of blood or plasma. However, it may be more difficult to plan ahead and maintain adequate stocks if you collect the blood you need from your own donors, particularly if you have to depend on family or family replacement donors. This is why it is so important to build up a panel of **voluntary non**

remunerated donors who give blood on a regular basis because it will enable you to plan the systematic collection of blood from donors with known ABO and RhD groups. When an emergency arises, it will also enable you to contact particular donors with the required groups, knowing that they will be likely to respond.

Module 1: *Safe Blood Donation* describes in detail how to set up a panel of regular voluntary non-remunerated donors. If your blood bank does not have enough people or time to organize a donor recruitment campaign, you could contact a local organization such as the Red Cross or Red Crescent Society and encourage them to help in recruiting voluntary donors who are willing to give blood regularly. You can demonstrate why their help is needed if you have records of the number of units of blood collected and used over the last year (or longer, if possible) and the number of times you needed blood in an emergency and it was not available.

If you have never kept any records of the number of units and the groups of blood you collect and issue, now is the time to start. There are many kinds of records that you could keep, but the most important are:

- the number of units collected or received
- the number of units transfused
- the number of patients transfused
- the outdate rate
- the number of times an operation or transfusion is cancelled because of a lack of suitable blood.

Number of units collected or received

A record should be kept of the number of units collected or received by your blood bank each year. This should include a weekly record of the amount of blood collected from donors and the amount of whole blood, red cells, plasma and other blood products received from a blood transfusion service or another hospital blood bank.

Number of units transfused

Probably one of the easiest and most important records to keep is the total number of units of whole blood, red cells, plasma or other blood products transfused each week, month and year. It will show you how much the blood usage varies from week to week and whether there are particular times of the year when it is particularly high or low.

The simplest way to present information about total blood usage is to draw a chart like the one shown in Figure 23.

Figure 23 shows an increase in the number of units of blood transfused in a particular year. There may be several reasons for this, but the most important thing is to know that it is happening so that you can take whatever action may be necessary. For example, you may need extra staff, equipment and reagents for the blood bank in order to carry out the additional testing that will be required. You will be able to make a more convincing case for this if you show your senior colleagues a chart like Figure 23 rather than simply saying that the blood bank is busier than it used to be.

Figure 23: Total blood usage over a period of 12 months

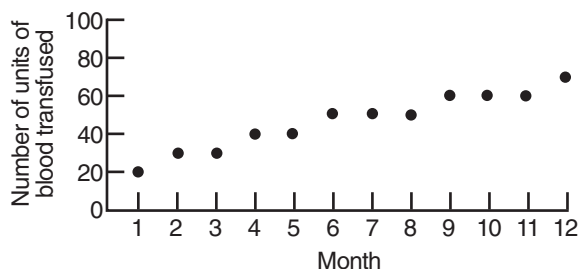
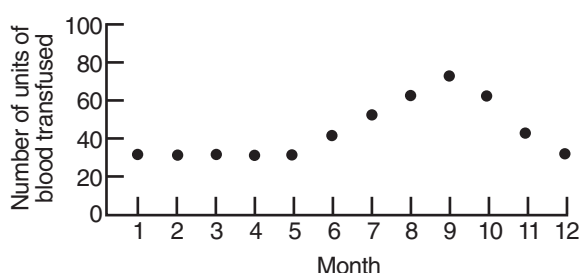


Figure 24 shows another chart where the blood usage in a particular year was particularly high in month 9. Each year, the chart shows a similar rise at the same time of year. Can you suggest why the blood usage might increase at the same time each year?

Figure 24: Total blood usage over a period of twelve months, with a peak in month 9



The increase in blood usage is obviously due to some seasonal change that occurs each year. In tropical countries, this is most likely to be the result of an increase in anaemia caused by malaria. If this happens in your blood bank and you regularly have a blood shortage at a particular time of the year, you should show a chart of blood usage to senior colleagues and discuss what action can be taken *before* you run out of blood.

Number of patients transfused

A record should also be kept of the total number of patients who have received any amount of whole blood, red cells, plasma or other blood products each year. This record should include the sex, age and diagnosis of each patient so that you can see whether there is an increase in the need for blood in a certain age group or for a particular clinical problem. For example, if the number of units transfused is high at certain times of year, as shown in Figure 24, you can look at the diagnosis of the patients who have been transfused and work out why there has been an increase at that time of year and whether there is likely to be a similar increase at the same time next year.

Outdate rate

The outdate rate is calculated by dividing the number of units collected or received by your blood bank by the number of units which go out of date.

Example

Number of units collected or received in a year = 2000

Number of units going out of date = 100

Outdate rate = 5%

It is important to calculate this figure for each group so that you can work out how many units of each group to order each week. For example, if the outdate rate for A Rh positive is higher than 10%, you should reduce the number of units of A Rh positive blood ordered or collected.

Cancellation of operations or transfusion because of a lack of blood

Calculating the number of times that an operation or transfusion is cancelled or delayed because of a lack of blood will be difficult unless you keep a record of each occurrence. If a request for blood is received and it cannot be met, it should always be recorded. This is an extremely important figure when calculating the amount of blood required in your blood bank.

If there are any occasions when this happens, it is important to show your records to your senior colleagues in the laboratory and to the doctors who request blood, asking for their help in persuading the hospital or transfusion service to send more blood, or to plan ways of bleeding more donors in your own hospital.

ACTIVITY 24

Which of the following records are kept in your blood bank?

- *the number of units collected or received*
- *the number of units transfused*
- *the number of patients transfused*
- *the outdate rate*
- *the number of times an operation or transfusion is cancelled because of a lack of suitable blood.*

How is this information used to help in planning and managing stocks of blood and plasma? If you can suggest any ways in which this information could be used more effectively, note down your recommendations on your Action List.

If any of these records are not kept in your blood bank at present, develop a simple system to record this information to assist you in calculating blood requirements in the future. Note down your recommendations on your Action List and discuss them with your supervisor and other senior colleagues to ensure that this information is used to assist in the efficient planning and management of stocks of blood and plasma.

SUMMARY

- 1 Compatibility testing involves:
 - checking the patient's records
 - carrying out an ABO and RhD group on the patient's blood sample

- screening for antibodies
 - cross-matching the patient's serum with the donor red cells.
- 2 Blood should not be issued unless a blood request form has been correctly completed by the person prescribing it.
 - 3 The blood request form should include the reason for the transfusion so that the most suitable blood can be selected for compatibility testing.
 - 4 The oldest units of blood should be used first, with the following exceptions:
 - patients receiving massive transfusions
 - patients under five years of age
 - patients receiving regular transfusions
 - hospitals receiving supplies of blood.
 - 5 Screening for irregular antibodies before cross-matching enables compatible blood to be found for patients who have formed antibodies.
 - 6 A cross-match is performed to ensure that there are no antibodies present in the patient's serum that will react with the donor red cells when transfused.
 - 7 When the cross-match has been completed and the donor unit is found to be compatible with the patient, the unit of blood must be clearly labelled.
 - 8 Where blood is requested, but may not be required, the "group and hold" procedure should be followed to minimize the number of units matched while ensuring that blood can be provided for the patient, if needed.
 - 9 Non-cross-matched group O RhD negative blood may be used when blood is urgently required and there is no time for an emergency compatibility test.
 - 10 Blood or plasma should not be issued without confirmation that the correct group has been selected and matched for the correct patient and that it shows no signs of deterioration.
 - 11 Blood or plasma should not be given to a patient without confirming, at the bedside, that it has been issued for the correct patient.
 - 12 There are three main types of transfusion reaction:
 - febrile reactions
 - allergic reactions
 - haemolytic reactions.

All transfusion reactions must be reported and investigated as soon as possible in order to determine the cause.
 - 13 Accurate records must be kept at all times, including:
 - blood request form
 - compatibility test record

- donor blood grouping register
 - compatibility register (patient testing register).
- 14 Stocks of blood and plasma should be rotated each day to ensure that they are used before they are out of date.
- 15 In order to calculate blood and plasma requirements, records should be maintained of:
- the number of units collected or received
 - the number of units transfused
 - the number of patients transfused
 - the outdate rate
 - the number of times an operation is cancelled because of a lack of blood.

SELF-ASSESSMENT

- 15 Which blood would you select for the following patients?
- a neonate requiring an exchange transfusion
 - a three-year-old child requiring a transfusion for anaemia due to malnutrition
 - a woman with postnatal anaemia
 - a patient who is haemorrhaging and has received more than 12 units of blood
 - a 15 year-old patient receiving regular transfusions for thalassaemia.
- 16 If the patient's and donor's ABO and RhD groups are known, why is it necessary to perform a cross-match?
- 17 Why is it necessary to investigate a reported transfusion reaction?
- 18 What information do you need to calculate the outdate rate?
-

PROGRESS CHECK

Before moving on to Section 7, spend a few minutes thinking about whether you have achieved the learning objectives for Section 6. These were to:

- 1 Explain the importance of compatibility testing in blood transfusion practice.
- 2 Establish appropriate procedures for the request of blood for transfusion.
- 3 Develop and maintain appropriate procedures for selecting and issuing blood and plasma routinely and in an emergency.

- 4 Ensure that those responsible for giving blood to a patient are aware of the correct procedures.
- 5 Develop appropriate procedures to investigate suspected transfusion reactions.
- 6 Establish and maintain an efficient record-keeping system.
- 7 Establish and maintain efficient procedures for managing stocks of blood and plasma.

If you feel confident that you have understood everything in this section, turn to Section 7.

If you feel that you need to spend more time on this section, go back to the parts that are most unfamiliar or that you have found difficult. You may find it helpful to talk to other people, such as your supporter or other senior colleagues, if there is anything you are still not sure about.

7

Techniques for Blood Grouping and Compatibility Testing

The purpose of this section is to thoroughly familiarize you with the principles of the major techniques that are used in everyday blood group serology. Precision is essential in your work and this can be achieved only by applying good laboratory technique and adequately controlling all the tests that you carry out. The interpretation of results is equally important and this will be emphasized throughout the section.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Accurately perform the main blood grouping techniques and their controls.
 - 2 Use an appropriate scoring system to decide the strength of reactions.
 - 3 Recognize and avoid the common pitfalls in blood grouping, particularly in relation to poor technique.
 - 4 Explain the importance of quality control of reagents and techniques.
 - 5 Perform both routine and emergency compatibility tests.
-

7.1 INTRODUCTION

The techniques used in blood group serology are basically simple when compared with many of the technical procedures carried out in other branches of pathology. However, the results obtained are often more significant than in other tests. An error in ABO or RhD grouping a patient or in missing an incompatibility in a cross-match can have the most serious effects on the patient and may even be fatal. It is therefore essential to have a precise understanding of the principles described in this section.

For ease of reference, the instructions for performing the following techniques covered in Section 7 are provided in Appendix 1:

- 1 Washing cells and making red cell suspensions
- 2 Slide (tile) technique
- 3 Immediate spin (IS) technique
- 4 Saline room temperature technique
- 5 Albumin addition technique for Rh typing
- 6 ABO and RhD grouping in tubes
- 7 ABO and RhD grouping using a microplate
- 8 D^u (weak D) testing
- 9 Indirect anti-human globulin test (IAT)
- 10 Low ionic strength indirect anti-human globulin test (LISS/IAT)
 - 10.1 LISS suspension method
 - 10.2 LISS addition method
- 11 Direct anti-human globulin test (DAT)
- 12 Preparation of control IgG coated cells for the anti-human globulin test
- 13 Cross-matching
 - 13.1 One-tube cross-match: immediate spin and IAT
 - 13.2 One-tube cross-match using LISS addition
 - 13.3 Cross-matching using an albumin addition and saline room temperature technique
 - 13.4 Emergency cross-matching.

7.2 HEALTH AND SAFETY

All handling of blood samples or any other biological material is a potential health hazard. Make sure, therefore, that your personal hygiene is of a high standard and that you fully understand how to deal with spillages of biological material, contaminated laboratory equipment and the safe disposal of laboratory waste.

Laboratory safety has been covered in detail in Section 3 of the Introductory Module. Read this carefully and make sure that you understand it before proceeding with your practical techniques.

7.3 EQUIPMENT

Figure 25 below lists the minimum equipment required for routine serological testing.

EQUIPMENT REQUIRED FOR ROUTINE SEROLOGICAL TESTING

- Bench-top centrifuge
- Refrigerator for storing reagents, ABO cells and blood samples
- Deep freezer for storing serum samples
- Light box or white tile
- Water-bath at 37°C (or heated block or incubator)
- containers for saline
- Plastic wash bottles
- Thermometers
- Pasteur pipettes
- Glass tubes for indirect antiglobulin tests (75 x 12mm)
- Tubes (glass or plastic) for grouping (50 x 7 mm)
- Racks for test tubes
- Glass microscope slides
- Wooden applicator sticks
- Waterproof markers for glass and plastic tubes
- Hand lens (magnification x2 to x5)
- pH indicator papers
- Supply of distilled or deionized water
- Microplates (optional)
- Centrifuge with holder for microplates (optional)

Figure 25: Checklist of equipment required for routine serological testing

ACTIVITY 25

Make a list of the equipment used in your laboratory for routine serological tests. Compare this with the list shown in Figure 25. Are there any items required for routine serological testing that are not available in your laboratory? If there are, note down your recommendations on your Action List and talk to your supervisor about the importance of this equipment being made available.

7.4 SALINE

It is very important that the saline used in red cell work has a pH of 6.5–7.5. If it is outside this range, some antibodies will not combine with their antigen and false-negative results will occur. If it is within this range and it is to be used on that day, no buffer will be required.

However, most saline is used over a 2–3 day period and, since the pH falls on storage, it is necessary to add some buffer salts. This can be done by using buffer tablets pH 7 or by adding buffer solutions.

See Appendix 1 for the preparation of saline and buffer solutions. A detailed standard operating procedure for the preparation of saline solutions and phosphate buffer solutions is included in Appendix 1 of the Introductory Module.

7.5 THE PASTEUR PIPETTE

The piece of apparatus most commonly used in blood grouping is the Pasteur pipette. This is a piece of 5 mm glass tubing drawn out to a tip which delivers a small drop approximately 0.02 ml in volume. Plastic pipettes are also available which are usually disposed of after being used.

The volume of the drop delivered by the pipette will vary depending on the angle at which the pipette is held. You should therefore always hold it at the same angle to ensure that you invariably use the same size drops. The vertical position seems to be the most popular angle for using the pipette. Where possible, a clean pipette should be used for each new sample, because traces of unwanted serum can lead to false results. However, if this is not possible, it is essential to wash the pipette thoroughly between successive tests to ensure that all traces of foreign material are washed out of it. The correct way to wash a pipette is to:

- rinse it in a beaker of water and shake it out
- rinse it in a beaker of saline, taking saline up into it, and shake it out
- rinse it in a second, clean beaker of saline and shake it out.

Using a glass pipette can be dangerous as it is easy to “stab” yourself with it. Plastic pipettes are much safer to use. If you do stab yourself with any object in the laboratory, always report the incident and seek advice on first aid.

7.6 GLASS SLIDES, TEST-TUBES AND MICROPLATES

Three manual methods can be used when performing blood grouping:

- glass microscope slides or white porcelain tile
- glass or plastic test-tubes
- microwell plates (microplates).

Glass slides or white porcelain tile

The glass slide or white tile technique (see Technique 2 in Appendix 1) is insensitive and leads to errors. It should therefore be used only to perform a simple cell blood group before a full grouping is carried out using either the tube or microplate technique.

Test-tubes

Test-tubes can be made of glass or plastic. Tubes used for blood grouping are usually 50 x 7 mm in size, although 75 x 12 mm tubes are usually used for anti-human globulin tests. The advantage of the tube technique is that it allows for long incubation without any evaporation of the tube's content.

Always clean tubes thoroughly before reusing them, by washing them well, then rinsing them in distilled or deionised water and drying them.

Microwell plates or microplates

Microwell plates are rapidly replacing test-tubes in many laboratories. A microplate consists of a small tray with 96 small wells, each of which can hold between 200 and 300 microlitres of reagent, as shown in Figure 26.

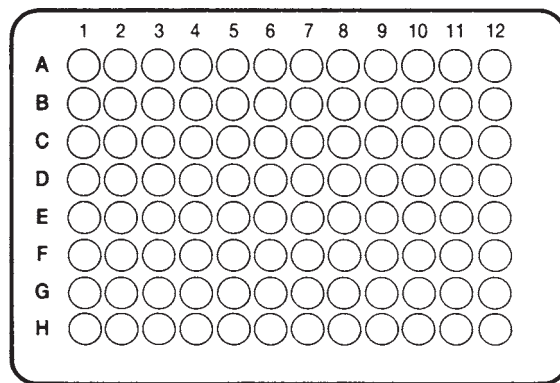


Figure 26: A standard 96-well microplate

Three types are available, as shown in Figure 27:

- a V-type well
- a flat-bottom well
- a U-type well.



Figure 27: Standard V-type, flat-bottom and U-type wells

The U-type well is generally used in red cell serological work because it is easier to read the results.

The advantage of the microwell method is that one microplate can do the work of 96 test-tubes so it uses far less antisera and is very cost-effective.

Microwell plates can be reused, but you must clean and dry them very thoroughly to make sure that all foreign protein is removed. It is advisable to avoid using a plate for different tests by ensuring that the wells that have had anti-A in them one day will have anti-A in them again on the next day.

7.7 READING REACTIONS

Slide or tile technique

Mix the serum and cells to an area with a diameter of 2 cm. Then gently rock the slide or tile, looking for agglutination. Read the reactions within two minutes, otherwise the drying of the reagents will give a false-positive reaction.

Tube technique

Allow the cells to settle to the bottom of the tube. This takes at least 45 minutes unless the tube is lightly centrifuged. Take one tube at a time, hold it over a well-illuminated white background, a light box with a diffuse light or a concave mirror. Look for haemolysis. If it is present, record it.

Hold the tube at an angle and shake it gently to dislodge the cells from the bottom. Look for agglutination. It will be easier to read the result if you use a hand lens (magnification x2 or x5). Record the result immediately, before reading the next tube.

Microplate technique

As with the tube technique, allow the cells to settle or lightly centrifuge the plate. Look for haemolysis and then gently dislodge the cells from the bottom of the wells. You can do this either by using a purpose-made microplate shaker or by tapping the side of the plate with the palm of your hand. The reactions can then be read directly or with the aid of a purpose-made microplate reading-mirror. There are other methods of reading reactions in microplates, but centrifugation is required first. The above method is simple but effective.

7.8 DEFINING THE STRENGTH OF REACTION

When reading your results, it is necessary to have some method of showing the difference in the strength of reactions. This is usually done by giving a score to the strength of the reaction obtained. For example:

4 +	= Complete agglutination of all cells
3 +	= Majority of cells agglutinated, with some free cells
2 +	= Definite agglutination with naked eye reading
1 +	= Weakly positive with the naked eye; clearly positive using a hand lens or concave mirror
Negative	= No agglutination
L	= Lysis of red cells

7.9 BLOOD GROUPING SERA

Blood grouping sera, or antisera, have, in the past, been prepared from naturally-acquired or immune polyclonal antibodies from human or sometimes animal blood. Most ABO and RhD-grouping reagents are now

made from monoclonal antibodies obtained from in vitro cultures of cells secreting antibodies; these cells are called hybridomas.

The advantage of monoclonal reagents is that they provide an almost unlimited supply of identical antibodies that are very specific and potent and are free from any unwanted or contaminating antibodies that may give false positive results. They are also free from viruses such as HIV and hepatitis. However, they must be stored (usually at 2°C to 6°C) and used strictly according to the manufacturers' instructions and by no other technique, as they are prepared in a carefully standardized diluent and are susceptible to changes in pH, etc.

Some monoclonal grouping reagents are blended; an IgG antibody is mixed with an agglutinating IgM antibody to produce a "blended" reagent. It is always essential to follow the manufacturers' instructions in the package insert, paying particular attention to incubation times and temperatures.

7.10 RED CELLS FOR GROUPING TESTS

Red cells used in blood grouping need to be free of excess plasma or serum and any small clots that may lead to false-positive results if they are confused with agglutinates. Washing the cells once usually achieves this and also removes haemolysed cells. The supernatant of the last wash should be free of haemolysis.

The washed cells are then suspended in saline to give the correct cell suspension, usually 2–3%. Accurate cell suspensions are essential and practice is needed to make suspensions correctly. To obtain an approximate 3% suspension, add 1 drop of well-packed (well-centrifuged) red cells to 30 drops of saline (see Technique 1 in Appendix 1).

When testing a large number of samples, there is a risk that tubes, however well labelled, might become mixed up if the cells are washed and that errors might occur as a result. If you are testing fresh samples, it is therefore acceptable to prepare the 2–3% cell suspension directly from the sample, but be careful to take red cells into the pipette without too much serum. If the sample is from an anaemic patient, wash the cells once to remove excess serum. Also, if a sample looks haemolysed, wash the cells before use.

Preparation and storage of A, B and O cells for reverse grouping

Cells for reverse grouping may be prepared daily from fresh samples and a mixture of cells from two individuals is preferable. If possible, use A RhD negative, B RhD positive and O RhD positive. These can then also be used to control both the ABO and RhD grouping sera.

The reverse grouping cells should be washed at least once before making a 2–3% cell suspension. If they are kept at 4°C when not in use, they can be used for two days. If not, they should be prepared daily. They should be discarded if they look haemolysed or discoloured.

Before these red cells are used routinely, they should be tested with anti-A, anti-B and anti-D to ensure they give the expected reactions: strong positive and clear-cut negative reactions.

ACTIVITY 26

Turn to Appendix 1 and look at Technique 1.

Take five previously typed blood samples and, using Technique 1, prepare a 3% cell suspension of each. You will need these cell suspensions in the next activity.

Ask your supervisor to check that you have prepared the red cell suspensions correctly.

7.11 STORAGE OF GROUPING REAGENTS (ANTISERA)

Monoclonal grouping reagents are usually stored at 4°C, but it is important to read the manufacturer's instructions and follow them carefully. Antisera that have to be kept frozen should not be repeatedly thawed and refrozen as this leads to deterioration. Ideally, sera should be thawed only once. After thawing, mix well as the proteins tend to separate from the water.

For any reagent (antisera or red cells) obtained from outside your laboratory, you need to keep a record of the following information:

- when it arrived
- where it was obtained from
- the conditions under which it was stored
- when it was put into routine use
- the batch number
- the expiry date
- any comments or problems you have with the reagent.

7.12 QUALITY CONTROL OF GROUPING REAGENTS BEFORE USE

Antisera or monoclonal grouping reagents should be tested against a set of controls before they are put into routine use to ensure that they will give the correct results (see below). Once in use, they must be tested against a set of controls with each batch of tests, or at least once a day.

These reagents should:

- give clear positive reactions with cells bearing the corresponding antigens
- give clear negative reactions with cells without the corresponding antigens
- not lyse the red cells
- not produce rouleaux.

These reagents should *not* be used if any of the following are present:

- haemolysis
- precipitate or particles
- gel formation.

7.13 ABO AND RhD GROUPING

ABO and RhD grouping are generally performed at the same time.

For ABO grouping, a saline technique at room temperature is generally used for both the cell and reverse group.

RhD grouping reagents vary in their method of use. Some monoclonal reagents are used by the same technique as ABO reagents, and these are ideal for use in microplates since the ABO and RhD grouping can be performed together in the same plate. Other RhD antisera have to be incubated at 37°C; these are generally more suited to tube techniques.

Grouping of blood donors

Donors should be grouped on each occasion they donate using anti-A, anti-B and anti-AB for the cell group and A, B and O cells for the reverse group.

At least one anti-D reagent should be used. Samples that are negative with the anti-D may be further tested for weak D (D^u), using a reagent designed for that purpose.

Grouping of patients

Patients' red cells should be tested with anti-A and anti-B and the serum or plasma should be tested with A, B and O cells. One anti-D should be used. Although there is no need to perform a D^u test on the negative samples, this is the policy in some laboratories.

As the sera of infants do not contain anti-A or anti-B, the reverse grouping is not required when testing infants less than three months old.

ABO and RhD grouping in tubes

The technique for ABO and RhD grouping in tubes is described in Technique 6 in Appendix 1.

ABO and RhD grouping using a microplate

The technique for ABO and RhD grouping using a microplate is described in Technique 7 in Appendix 1.

Testing for weak D (the D^u test)

The technique for D^u testing is described in Technique 8 in Appendix 1.

ACTIVITY 27

Read Techniques 2–8 in Appendix 1.

Using either Technique 6 or Technique 7, perform an ABO and RhD grouping test on the previously typed samples (the cell suspensions you prepared in Activity 26). Note down your results, using the scoring system described in Section 7.8 on page 79.

How do your results compare with those that had been obtained previously? Do they agree with the original ABO and RhD grouping tests? If they don't, try to explain the reason for this.

Ask your supervisor to check your results.

7.14 ERRORS ENCOUNTERED IN BLOOD GROUPING

You need to be aware of the pitfalls that can occur in blood grouping that may lead to either a false-positive or a false-negative result. Have you encountered any of the following relatively common pitfalls?

Poorly standardized or stored reagents

It is very important that all new batches of grouping reagents and antisera are fully tested before being used. Antisera can deteriorate if not stored properly to give false-negative results and so they should always be stored at the temperature recommended by the manufacturer. Infected sera can lead to false positive results.

Rouleaux formation

Rouleaux formation is often referred to as **pseudo-agglutination** because it can give the appearance of agglutination. It requires a trained eye to detect it. Figure 28 shows true agglutination and Figure 29 shows **rouleaux formation**. This phenomenon is usually found in the serum of patients whose albumin/globulin ratio is abnormal. If you are in doubt about pseudo-agglutination when reading your tests, consult your senior technician. Rouleaux formation will usually disperse if 1 drop of hypertonic saline (1.5%) is added, whereas true agglutination will not disperse.

pseudo-agglutination:

False agglutination which is usually due to an upset in the albumin/globulin ratio.

rouleaux formation: A type of reaction where the red cells form together, appearing like agglutination. It is therefore a false agglutination. See pseudo-agglutination.

Contaminated blood samples

Contaminated blood samples can often lead to erroneous results. A contaminated sample can usually (but not always) be detected by its unpleasant odour when opening the tube. In addition, you can see haemolysis when preparing the cells for grouping. It is much safer to request a further fresh sample from the patient when dealing with a contaminated sample.

Figure 28: True agglutination
(as seen microscopically)

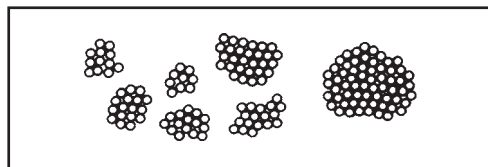
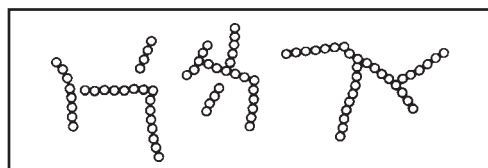


Figure 29: Rouleaux formation
(as seen microscopically)



Wharton's jelly

Wharton's jelly is the substance that surrounds the body and umbilical cord of a newborn infant and is often encountered in cord blood samples. It occurs when a cord blood sample is taken directly from the umbilical cord when it is cut instead of being taken from the umbilical vein using a syringe. This jelly-like substance contaminates the blood sample and can give rise to very strong rouleaux. To remove Wharton's jelly, wash the cells several times in saline that has been warmed to 37°C.

Auto-antibodies and cold-reacting antibodies

The blood of some patients contains antibodies that react with the A, B and O cells in the reverse group. If this occurs, incubate the reverse group at 37°C before reading. Since many of these antibodies react below body temperature, you will then find the true ABO reactions. Sometimes these antibodies are very potent and cause haemolysis at 37°C. If this is the case, perform a direct anti-human globulin test (see Technique 11 in Appendix 1). Report a positive result to the patient's doctor as the patient may have a condition known as autoimmune haemolytic anaemia.

Poor technique

The majority of errors in blood grouping still result from bad technique, particularly:

- using the wrong sample
- failure to place antisera or cells in the correct tube
- failure to appreciate the importance of temperature and time in incubation
- transcription errors, such as writing down the incorrect result or copying data incorrectly.

The majority of errors can be prevented if good technique is applied and the recommended controls are carried out.

7.15 METHODS OF DETECTING IMMUNE RED CELL ANTIBODIES

Most transfusion-related deaths are caused by ABO incompatibility so you should always take very great care in grouping both the donor and the patient and in ensuring that the patient receives ABO-compatible blood. However, some patients develop immune (usually IgG) antibodies after transfusions or pregnancies. It is important that these antibodies are detected and blood that is compatible with them is found for transfusion.

Immune antibodies can be detected by testing the sera of patients with selected antibody, screening red cells or when cross-matching blood of the same ABO and RhD type as the patient. The best technique for this is the indirect anti-human globulin test (Coombs test). Wherever possible, this should be used for the cross-match. If your supplies of anti-human globulin (Coombs) reagent are limited, reserve the indirect anti-human globulin test (IAT) for cross-matching blood for patients who either have been pregnant or have had previous transfusions. An albumin addition technique (see Section 7.19) can be used for other patients.

The cross-match should detect not only immune antibodies but also any ABO incompatibility that may be present because the wrong unit of donor blood was selected. This can be performed using a saline room temperature technique in addition to the IAT or albumin technique, or by using the one-tube, immediate spin and IAT technique.

Techniques for detecting immune red cell antibodies are described in Appendix 1.

7.16 THE INDIRECT ANTI-HUMAN GLOBULIN TEST USING TUBES

The indirect anti-human globulin test (IAT) is a tube haemagglutination method, commonly referred to as the Coombs test, in which antibodies incapable of causing direct agglutination can be shown to have combined with their red cell antigens by testing with an anti-human globulin serum. It is the most sensitive technique for the detection of antibodies.

In Section 3, you learned that the anti-human globulin test is performed in three stages:

Stage 1: Sensitization or coating and incubation

Stage 2: Washing

Stage 3: Addition of anti-human globulin reagent

You also learned that various factors, such as pH and ionic strength, affect antigen–antibody reactions.

Stage 1: Sensitization

In the sensitization phase, the patient's serum and cells are incubated together at 37°C. With red cells suspended in saline (see Technique 9), an incubation time of 45–60 minutes is required. However, if low-ionic strength saline (LISS) is used (see Technique 10.1), the incubation time can be reduced to 15 minutes because the speed with which antibodies bind to red cells is increased.

The ratio of serum to cells is important: 3–4 drops of serum to 1 drop of a 2–3% cell suspension in saline are required to get good results with weakly reacting antibodies. If the red cells are suspended in low ionic strength saline (LISS), you *must* use equal volumes of the LISS suspended cells and serum to get the correct ionic conditions. To 2 drops of serum you should therefore add 2 drops of 2% cells in LISS.

An alternative LISS technique is referred to as 'LISS addition' and uses a commercially available LISS-addition reagent (see Technique 10.2). It is essential to follow the manufacturer's instructions. However, the usual technique is to set up the test with 3 drops of serum and 1 drop of 2–4% cells in saline and then to add 3 drops of the LISS suspension solution. After mixing the contents well, incubate the tube for 15 minutes at 37°C.

After incubation, look for haemolysis and agglutination before washing the cells.

low-ionic strength saline (LISS): Saline that is lowered from its normal strength to 0.32% saline in buffered glycerine. The speed of uptake of most blood group antibodies onto the corresponding antigen is enhanced when the ionic strength of the saline is lowered. Most low ionic strength saline solutions are now produced commercially.

Stage 2: Washing

The washing stage is very important as all traces of serum must be removed from the red cells. Four washes are therefore required. The pH of the saline is also important and must be between 6.5 and 7.5. Outside that range, the antibodies coated onto the cells may be washed off.

Stage 3: Addition of anti-human globulin reagent

Anti-human globulin reagents (AHG) should be used in accordance with the manufacturer's instructions. Most manufacturers recommend the use of 2 drops of reagent followed by immediate centrifugation of the tube. Using less AHG could lead to weak reactions being missed. The reactions should be read and recorded with care, reading one tube at a time as described in Section 7.7 on page 79.

The main cause of false-negative results is a failure to wash the cells properly, with the result that the remaining serum neutralizes the AHG. To ensure that this has not happened, add IgG antibody coated cells to all negative anti-human globulin tests (see Technique 12 in Appendix 1) and then mix the contents and centrifuge again. On reading, there should be agglutination present. This agglutination shows that the AHG is still working and the test can be recorded as negative. If there is no agglutination at this stage, the AHG has been neutralized and the results cannot be relied on. The test must therefore be repeated. With care, practice and attention to detail, failures should not happen very often.

Table 5 shows the main causes of false-positive and false-negative reactions in the indirect anti-human globulin test.

Table 5: Causes of false-positive and false-negative reactions in the indirect anti-human globulin test

Causes of false-positive results in the IAT

- Dust or particles in the tubes
- Cross-contamination from one tube to another
- Centrifuging the tube too fast or for too long

Causes of false-negative results in the IAT

- Failure to wash the cells properly
- Failure to add AHG
- Dirty tubes
- Small clots in the serum or cells
- Using saline at the wrong pH
- Centrifuging the tube at the wrong speed
- Leaving the cells after washing before adding AHG
- Leaving the test after having added the AHG before reading

7.17 THE DIRECT ANTI-HUMAN GLOBULIN TEST

The direct anti-human globulin test (DAT) is used to see whether a patient's red cells have been coated with antibody in vivo (in the body). This happens in rare cases, such as autoimmune haemolytic anaemia,

haemolytic transfusion reactions and haemolytic disease of the newborn. In this test, cells are *not* incubated with serum but are taken from the patient, washed and reacted with AHG; in other words, the patient's cells are tested directly (see Technique 11 in Appendix 1).

Table 6 shows the conditions in which the direct anti-human globulin test is positive.

Table 6: Conditions in which the direct anti-human globulin test is positive

Autoimmune haemolytic anaemia

Red cells being destroyed *in vivo* by an antibody reacting with the patient's own cells

Haemolytic transfusion reactions

Red cells being destroyed *in vivo* by the patient's antibodies destroying transfused cells

Haemolytic disease of the newborn

Red cells being destroyed *in vivo* by the mother's IgG antibody that has crossed the placenta, destroying the fetal red cells

7.18 THE ANTI-HUMAN GLOBULIN TEST USING GELS OR MICROCOLUMNS

As we have seen the major problems encountered with anti-human globulin tests are with the washing but there are now some commercial systems available for the anti-human globulin test that do not need a washing phase. These are sold as a plastic card containing usually six microtubes which contain a matrix of either small glass beads or a gel mixed with the anti-human globulin reagent. The serum and cells are incubated in the chamber above this matrix and when the card is centrifuged, in the special centrifuge supplied, the red cells, but not the serum, are forced into the matrix. If these cells have been sensitised with antibody then they agglutinate by the AHG and are trapped in the gel or glass beads – a positive reaction. Cells not coated with antibody fall to the bottom of the microtube forming a small button of cells – a negative reaction.

These cards can be used for direct anti-human globulin testing.

Gel or microcolumn cards can also be obtained containing grouping sera, for example anti-A and anti-B, in place of the AHG. Further details are available from the suppliers of these systems. When considering using these new technologies the cost of these has to be assessed and compared with the cost of standard tube or microplate techniques.

7.19 THE ALBUMIN ADDITION (LAYERING) TEST

The albumin addition (layering) test is not nearly as sensitive as the IAT. However, it can be used in place of the IAT when AHG is difficult to obtain or with some anti-D reagents for RhD grouping. This technique is performed in two stages:

Stage 1

Serum and cells are incubated at 37°C and the cells are allowed to settle to the bottom of the tube. The red cells will be coated by any antibodies present (both IgG and IgM).

Stage 2

Albumin (20–30% bovine albumin) is then added, allowing it to run down the inside of the tube so it does not disturb the cells. The test is then incubated again for 10–15 minutes. The albumin will cause any coated cells to agglutinate.

The use of albumin in the IAT

Some manufacturers recommend adding albumin to the cells and serum in the IAT to make the test more sensitive. However, if the methods described in this module are followed, there is no need to add albumin because it will not enhance the results.

7.20 ENZYME TECHNIQUES

Enzyme techniques can be used for the detection of immune antibodies but, since the enzyme treatment destroys some blood group antigens, these techniques do not detect all antibodies. They should therefore be used only in addition to the anti-human globulin test, not as a replacement for it, and should be regarded as a reference centre technique.

7.21 COMPATIBILITY TESTS

It is essential to recognize the importance of extreme care and good technique when carrying out compatibility tests to ensure that there are no antibodies present in the patient's serum which will react with the donor cells when transfused. The technical procedure should be kept as simple as possible, without sacrificing any accuracy, since there is an increased risk of error if the test is made too complex.

A number of techniques can be used, some of which are particularly sensitive for certain kinds of antibody. A saline technique at room temperature will detect ABO incompatibilities but not antibodies produced as a result of a previous transfusion or pregnancies. The anti-human globulin test, however, has the advantage of being highly sensitive and capable of detecting all antibodies. It is therefore strongly recommended that this test should form part of every compatibility test, wherever possible.

The cross-match should be performed in tubes at room temperature to detect ABO incompatibility and at 37°C to detect IgG antibodies, using an indirect anti-human globulin test, if possible. If this is not possible, an albumin addition (layering) technique can be used.

Warning: A white tile method is *not* acceptable since it carries a high risk of failing to detect an incompatibility between patient and donor.

The following procedure should be used when performing a compatibility test.

- 1 Check that the blood sample from the patient matches the blood request form for that patient. In all compatibility

testing, the most important factor is to ensure that the donor blood is ABO compatible with the patient.

- 2 Perform an ABO and RhD group using reliable reagents and either tubes or a microplate, following the methods described in Appendix 1.
- 3 Perform a cross-match by testing the patient's serum against some of the red cells taken from the units of blood selected. An indirect anti-human globulin test should be performed to detect immune antibodies. The saline room temperature test can be either an 'immediate spin' test (see Technique 3), followed by incubating that tube for the IAT, or a saline tube technique. If you are unable to perform an IAT, you must perform a saline tube test at room temperature and an albumin addition test at 37°C.
- 4 If no agglutination or haemolysis is detected in the cross-match, the blood can be regarded as compatible. Complete and sign the compatibility testing register and label the units to show all the details of the patient for whom the blood is intended.

If the cross-matching tests show either haemolysis or agglutination, the blood is *incompatible* and cannot be used for that patient.

- 5 In the case of incompatibility, check all your grouping results to see whether a mistake has been made. Repeat the grouping tests on both the patient's sample and the donor unit. If there is no discrepancy, the cross-match should be repeated and further units of blood should also be tested. Any units found to be compatible can be issued, but it is wise to ask the doctor to monitor the patient closely for signs of an incompatible transfusion.

If you are unable to find compatible blood, you should always seek advice and help from a medical officer with knowledge of transfusion practice, possibly at a blood transfusion centre.

There are four possible techniques for cross-matching, which are described in Appendix 1:

- 13.1: *One-tube: immediate spin and IAT*
- 13.2: *One-tube: using LISS addition*
- 13.3: *Using an albumin addition and saline room temperature test*
- 13.4: *Emergency cross-matching.*

ACTIVITY 28

Read Techniques 13.1, 13.2, 13.3 and 13.4 in Appendix 1.

Using one of these techniques, perform a compatibility test using group O serum with a suspension of group A or B cells. Note down your results, using the scoring system described in Section 7.8 on page 79.

Repeat the compatibility test, using an emergency technique. Note down your results, using the scoring system on page 79.

Using the same technique as you used in the first part of this activity, perform a compatibility test with group O serum and group O cells. Note down your results, using the scoring system on page 79.

Repeat the compatibility test, using an emergency technique. Note down your results, using the scoring system on page 79.

Compare your results and ask your supervisor to check them.

SUMMARY

- 1 Errors in blood grouping can seriously harm the patient, and may even cause death.
- 2 High standards of hygiene and laboratory safety must always be maintained.
- 3 Three manual methods can be used when performing blood grouping, using:

- glass slides or white porcelain tile
- test-tubes
- microwell plates or microplates.

The slide or tile technique is insensitive and should be used only to carry out a simple cell blood group before performing a full grouping using either the tube or microplate technique.

- 4 The strength of reaction obtained is scored on a scale of 1+ to 4+, with Negative indicating no agglutination and L indicating lysis.
- 5 The instructions given by the manufacturers for the storage and use of reagents should always be followed carefully.
- 6 Common problems in blood grouping leading to false-positive or false-negative results include:
 - poorly standardized or incorrectly stored antisera
 - rouleaux formation
 - contaminated blood samples
 - Wharton's jelly
 - auto-antibodies and cold reacting antibodies
 - poor technique.
- 7 The indirect anti-human globulin test (IAT) is performed in three stages:

- sensitization or coating
 - washing
 - addition of AHG reagent.
- 8 The direct anti-human globulin test (DAT) is used to detect *in vivo* sensitization by antibody of the patient's red cells.
- 9 The cross-match should be performed in tubes at room temperature to detect ABO incompatibility and at 37°C to detect IgG antibodies, using an indirect anti-human globulin test, if possible.

SELF-ASSESSMENT

- 19 Name two important factors to bear in mind when using a pipette.
- 20 Why is the use of glass slides or white porcelain tiles not recommended?
- 21 Why is a scoring system used to record results?
- 22 What causes the majority of errors in blood grouping?
- 23 What might cause a false-positive reaction in an indirect anti-human globulin test?
- 24 What might cause a false-negative reaction in an indirect anti-human globulin test?
- 25 What conditions can lead to a positive direct anti-human globulin test?
- 26 Describe the stages in an albumin addition technique.

PROGRESS CHECK

Before moving on to Section 8, spend a few minutes thinking about whether you have achieved the learning objectives for Section 7. These were to:

- 1 Accurately perform the main blood grouping techniques and their controls.
- 2 Use an appropriate scoring system to decide the strength of reactions.
- 3 Recognize and avoid the common pitfalls in blood grouping, particularly in relation to poor technique.
- 4 Perform both routine and emergency compatibility tests.

If you feel confident that you have understood everything in this section, turn to Section 8.

If you feel that you need to spend more time on this section, go back to the parts that are most unfamiliar or that you have found difficult. You may find it helpful to talk to other people, such as your supporter or other senior colleagues, about anything you are still not sure about.

8

Action Plan

This final section focuses on the Action List you have been building up as you have worked through this module. You have probably identified a number of improvements that you think could be made in your serology programme and it is now time to identify priorities and begin putting your ideas into action.

LEARNING OBJECTIVES

When you have completed this section, you should be able to:

- 1 Reassess your knowledge and skills in relation to the module objectives now that you have completed Module 3.
 - 2 Review your Action List, identifying improvements that you can implement and those that will require action by others.
 - 3 Prepare and implement a realistic Action Plan to introduce changes that will improve the quality of the serology programme in your laboratory.
-

8.1 REVIEWING YOUR PROGRESS

Before you start making your Action Plan, think carefully about the module objectives and the progress you have made since you started working through this module.

ACTIVITY 29

Complete the table below. You will notice that it is the same as the one you filled in for Activity 2. Use it to review the knowledge you have gained and the skills you have developed as a result of your work on this module. Have you changed your rating in relation to each module objective?

You should have made some identifiable progress in each area covered by this module. If there is anything you still do not feel confident about, however, reread the appropriate section and then discuss any remaining problems with your supporter or trainer before continuing with your Action Plan.

Module objective	Rating (1–4)	Comments
<p><i>Section 2</i> Explain the functions of the main components of blood and their significance in blood transfusion practice.</p> <p><i>Section 3</i> Explain the red cell antigen–antibody reaction and the factors that affect it.</p> <p><i>Section 4</i> Explain the ABO blood group system and use the results of cell and reverse ABO grouping tests to identify the blood groups of donors and patients.</p> <p><i>Section 5</i> Explain the Rh blood group system, and identify when to use Rh D positive or Rh D negative blood and when to test for the weak D (D^u) antigen.</p> <p><i>Section 6</i> Explain the importance of compatibility testing and develop and maintain appropriate procedures and records for the safe request, selection and issue of blood under routine and emergency conditions.</p> <p><i>Section 7</i> Explain the principles of the main techniques used in blood grouping and compatibility testing and perform them safely and accurately.</p>		

8.2 MAKING YOUR ACTION PLAN

The Action Plan provides you with an opportunity to make practical improvements in your own workplace, within any financial or resource constraints that exist. As you worked through this module, you have been noting down your ideas on the Action List on page 93, although you are likely to have fewer items on your Activity List than in the other modules. You should have listed the number of the activity in Column 1 and noted your ideas for improvement in Column 2. You should also have discussed your ideas with your supporter.

You may have tried out some of your ideas already, but some may require more time and effort and so it is important to identify priorities. You may not be in a position to put all your ideas into action yourself and it may be necessary to convince other members of staff to take the actions that you have identified as being both necessary and feasible.

ACTIVITY 30

Look carefully at all the suggestions for improvements that you have written down on your Action List. Mark those where you have not yet been able to take any action. Then divide them into two categories:

- 1 Actions that you can take.** Choose the ones that you think are most important and put them in order of priority. Note them down in Column 1 of the Action Plan on page 96. In Column 2, briefly summarize the action that you plan to take. In Column 3, note down the results that you would expect after taking this action.
- 2 Actions that others could take.** Note them down in Column 1 of the Action Plan on page 96. In Column 2, write down the name of the person who would be responsible for making the changes that you are recommending and, in Column 3, summarize the results that you would expect.

Then show your plan to your supervisor and supporter and discuss it with them. Your ideas for improvement may need to be modified as a result of these discussions. Other senior staff may also need to be consulted before your Action Plan can be agreed. You should also discuss it with your trainer about it at this stage.

When you have reached agreement about the actions you are going to take, set a date when you hope to complete each of them and note this in Column 4. Also use Column 4 to set a date by which you expect the completion of any actions taken by others.

Your Action Plan is now ready.

8.3 IMPLEMENTING YOUR ACTION PLAN

You should now begin to implement your Action Plan along the lines agreed with your supervisor and supporter. It will probably take you

ACTION LIST

Activity number	Ideas for improvement

ACTION PLAN

<i>Ideas for improvement</i>	<i>Planned action</i>	<i>Expected results</i>	<i>Planned completion</i>	<i>Actual completion</i>	<i>Actual results</i>

several weeks or months to put your all plans into action and you may need more time than you expected. In fact, you will probably start the next module in the programme before you are able to complete everything. You may also find that some of your ideas for improvement are more difficult to put into action than you expected and you may need to revise some of your plans if they are too ambitious or are not working as well as you hoped. However, if you have thought carefully about how you could apply what you have learned from this module and have discussed your ideas with the appropriate people, you should be able to put most of them into practice. You may even find that there are some unexpected benefits. If you have any problems during this time, talk to your supporter or supervisor and ask them for any assistance you need. You should also give them regular reports on your progress.

ACTIVITY 31

Once you have completed each action you included in your Action Plan, note down the date in Column 5 and the final results in Column 6. Then review the implementation of your Action Plan by comparing the actual results with the results that you expected. Also compare the planned completion dates with the actual completion dates.

Discuss the outcomes with your supporter and supervisor.

Identify any further actions required to ensure the implementation of the improvements you have identified as being necessary.

Over the next few months, monitor the effectiveness of any changes you have been able to introduce and be prepared to make further changes or take any follow-up action needed to ensure that they continue to lead to improved quality in your transfusion service or blood bank.

PROGRESS CHECK

Now that you have completed this module, spend some time thinking about whether you have achieved the learning objectives for this section. These were to:

- 1 Reassess your knowledge and skills in relation to the module objectives now that you have completed Module 3.
 - 2 Review your Action List, identifying improvements that you can implement and those that will require action by others.
 - 3 Prepare and implement a realistic Action Plan to introduce changes that will improve the quality of the serology programme in your laboratory.
-

Activity Checklists and Answers

SECTION 1

Activity 1

Purpose

To identify a personal 'supporter' for your work on Module 3.

Checklist

You should have:

- Identified senior colleagues in your workplace who could provide assistance to you as you work through this module
- Selected one particular person, ideally your supervisor, to be your supporter for Module 3 and checked that they are willing to assist you
- Explained how the learning programme operates and what the role of the supporter involves
- Agreed how frequently you will meet to discuss your work on this module
- Showed Module 3 to your supporter
- Informed your trainer about who your supporter is
- Asked your trainer for assistance if you have any difficulty in finding a supporter in your workplace.

Activity 2

Purpose

To assess your knowledge, skills and experience in relation to the module objectives before you start work on Module 3.

Checklist

You should have:

- Looked carefully at the module objectives and, for each one, assessed your current knowledge, skills and experience, using the rating 1, 2, 3 or 4

- Completed the table on page 6
- Noted any additional comments you wish to make, such as any module objectives that are not relevant to your own work.

Activity 3

Purpose

To make a realistic Study Plan for your work on Module 3.

Checklist

You should have:

- Quickly looked at the other sections of the module to get an idea of its content, level and approach and assess how much of the material is likely to be new to you
- Estimated the amount of time you think you will need to study each section, including completing the activities and answering the self-assessment questions
- Discussed with your supervisor how much time can be allocated for study on a regular basis
- Completed the Study Plan on page 8, adding the dates by which you plan to complete each section and the dates of meetings with your trainer and supporter.

SECTION 2

Activity 4

Purpose

To find out the minimum haemoglobin levels accepted in your donor clinic.

Checklist

You should have:

- Noted down the minimum haemoglobin levels for men and for women that are accepted in your donor clinic
- Briefly described how haemoglobin levels are measured in your donor clinic.

Activity 5

Purpose

To investigate the main uses of blood issued for transfusion by your blood bank.

Checklist

You should have:

- Checked your records or consulted with colleagues to identify the reasons for the transfusion of the last 20 units of blood that your blood bank issued
- Identified the most common reasons for transfusion
- Noted the reasons for transfusion on the next 20 occasions that blood was issued, if you were unable to obtain the information about previous issues.

SECTION 4

Activity 6

Purpose

To identify whether agglutination will occur in reactions of:

- anti-A and anti-B with the red cells of group A, group B, group AB and group O
- serum of groups A, B, AB and O with A, B and O cells.

Answers

1

Group	Anti-A	Anti-B
A	+	–
B	–	+
AB	+	+
O	–	–

2

Group	A cells	B cells	O cells
A	–	+	–
B	+	–	–
AB	–	–	–
O	+	+	–

Activity 7

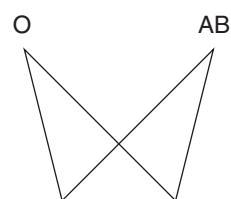
Purpose

To use a family tree to identify genotypes and phenotypes.

Answers

1

Genotype:



Genotype:

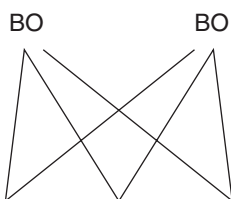
AO BO

Phenotype:

A B

2

Genotype:



Genotype:

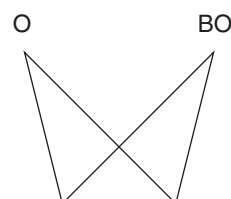
BB BO OO

Phenotype:

B B O

3

Genotype:



Genotype:

BO OO

Phenotype:

B O

Activity 8

Purpose

To use the results of cell and reverse ABO grouping tests to identify blood groups.

Answers

	Group
1	A
2	O
3	AB
4	O
5	B
6	O
7	AB
8	A
9	B
10	A

Activity 9**Purpose**

To calculate the percentage frequency of ABO blood groups in your donor population.

Checklist

You should have:

- Looked at the results of 100 blood grouping tests in your laboratory and noted the percentage that were group A, group B, group AB and group O
- Compared your results with the percentage frequency in Table 4 on page 30
- Checked your records or consulted your colleagues to identify whether your results are typical of the proportion of the different blood groups in your locality.

Activity 10**Purpose**

To identify the possible reasons for selected examples of ABO grouping results.

Answers

- 1 Group A with another antibody present, possibly anti-A₁
- 2 Group O with another antibody present
- 3 Group B with another antibody present
- 4 Group AB with another antibody present, possibly anti-A₁
- 5 Group AB, possibly A₂B.

Activity 11**Purpose**

To review your procedures for identifying high-titre antibodies.

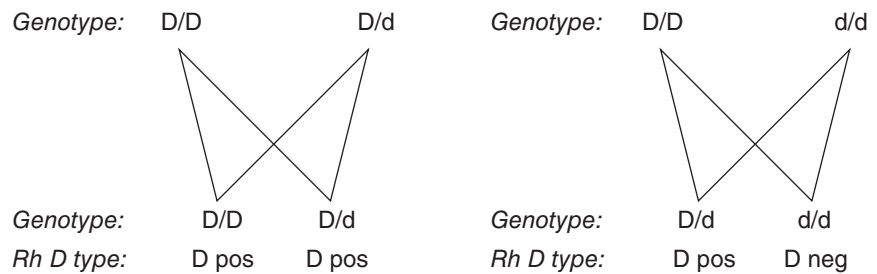
Checklist

You should have:

- Noted down the procedures used in your laboratory for grouping samples from the umbilical cord or from newborn babies
- Noted down whether high-titre antibodies are recorded when testing donors
- Identified any action that needs to be taken if high-titre antibodies are not recorded
- Discussed your ideas with colleagues and noted down your recommendations on your Action List.

SECTION 5**Activity 12****Purpose**

To use a family tree to identify possible genotypes and their Rh D types.

Answers**Activity 13****Purpose**

To identify the percentage frequency of Rh D positive and Rh D negative patients and donors.

Checklist

You should have:

- Noted the results for 100 Rh blood grouping tests for patients and donors
- Identified the percentage of Rh D positive and Rh D negative results
- Compared your results with the percentage frequencies given on page 38.

SECTION 6**Activity 14****Purpose**

To review the procedures followed in your hospital for the request of blood.

Checklist

You should have:

- Compared the example of a blood request form given in Figure 13 with the form used in your hospital
- Identified any improvements that could be made to your form or the way that it is used

- Noted down your recommendations on your Action List
- Talked to your supervisor and medical colleagues about the importance of introducing a blood request form, if one is not yet used in your hospital
- Identified what the form should contain and how it should be used and noted your recommendations on your Action List.

Activity 15

Purpose

To review the procedures used in your blood bank for selecting blood for patients.

Checklist

You should have:

- Compared the procedures used in your blood bank for selecting blood with those outlined in Section 6.3
- Identified any ways in which you think that these procedures could be improved
- Noted down your recommendations on your Action List and discussed them with your supervisor and other senior colleagues.

Activity 16

Purpose

To review the compatibility labels used in your blood bank.

Checklist

You should have:

- Compared the compatibility labels used in your blood bank with the example given in Figure 14
- Identified any ways in which you think that your compatibility labels could be improved
- Noted down your recommendations on your Action List
- Talked to your supervisor about the importance of introducing compatibility labels, if they are not yet used in your blood bank
- Noted down on your Action List your recommendations on what they should contain.

Activity 17

Purpose

To review the procedure used in your blood bank for issuing blood.

Checklist

You should have:

- Compared the procedure used in your blood bank for issuing blood with that outlined in the checklist in Figure 17
- Identified any ways in which you think that this procedure could be improved
- Noted down your recommendations on your Action List
- Developed your own checklist for the issue of blood, taking any local policy guidelines into account, or used the checklist given in Figure 17
- Displayed the checklist in your blood bank and ensured that all members of staff have read it and follow the procedure
- Showed the checklist to your hospital transfusion committee, if there is one in your hospital, to ensure that everyone involved in blood transfusion knows the procedure for the safe issue of blood from your blood bank.

Activity 18**Purpose**

To review the procedure used in your hospital for giving blood.

Checklist

You should have:

- Found out the procedure used in your hospital for giving blood and compared it with that outlined in the checklist in Figure 18
- Identified any ways in which you think that this procedure could be improved
- Noted down your recommendations on your Action List
- Discussed your recommendations with your supervisor and members of the medical staff
- Developed your own checklist for giving blood, taking any local policy guidelines into account, or used the checklist given in Figure 18
- Developed a system for giving out a copy of this checklist each time that blood or plasma is issued from the blood bank or displayed a copy in each place where blood is transfused
- Tried to ensure that all staff involved in giving blood have read your checklist and follow the procedure.

Activity 19**Purpose**

To review the procedures used in your blood bank for investigating transfusion reactions.

Checklist

You should have:

- Compared the procedures used in your blood bank for investigating transfusion reactions with those outlined on pages 57–59
- Identified any ways in which you think that these procedures could be improved
- Noted down your recommendations on your Action List
- Talked to your supervisor about developing an appropriate system, if there are no established procedures for investigating transfusion reactions
- Noted down your recommendations on your Action List.

Activity 20**Purpose**

To review the test registers used in your blood bank.

Checklist

You should have:

- Compared the donor blood grouping register and compatibility register (patient testing register) used in your blood bank with the examples given in Figures 19 and 20
- Identified any ways in which you think that your donor blood grouping register and compatibility register could be improved
- Noted down your recommendations on your Action List
- Talked to your supervisor about the importance of introducing test registers of this kind if they are not already kept in your blood bank
- Noted down your recommendations on your Action List.

Activity 21**Purpose**

To use records of the annual usage of blood and plasma to calculate the average weekly usage for different blood groups.

Answers

<i>Number of units</i>	<i>Blood group</i>
11.5	A Rh positive
0.4	A Rh negative
13.5	O Rh positive
1.0	O Rh negative
9.6	B Rh positive
0.4	B Rh negative
1.9	AB Rh positive
0.2	AB Rh negative

Activity 22**Purpose**

To use records of the average weekly usage of blood and plasma to calculate the number of units for each blood group that need to be added to the stock for the following week.

Answers

<i>Number of units</i>	<i>Blood group</i>
6	A Rh positive
0	A Rh negative
9	O Rh positive
1	O Rh negative
4	B Rh positive
1	B Rh negative
1	AB Rh positive
0	AB Rh negative

Activity 23**Purpose**

To review the method used to calculate the weekly average usage of blood and plasma in your blood bank.

Checklist

You should have:

- Noted down the method currently used to calculate the amount of blood and plasma needed each week in your blood bank
- Calculated the amount of blood and plasma needed each week in your blood bank by:
 - counting the number of units of blood and plasma used each week for the last year or at least during the last six months
 - dividing these figures into ABO and Rh groups and entering this on a table
 - taking away the highest week's figure for each blood group
 - adding up the total number used in each group (leaving out the highest week's figure in each group)
 - dividing each total by the number of weeks minus one week
- Compared the figures obtained using this method with those from any other method currently used to calculate your blood requirements
- Identified any ways of improving the accuracy of the method currently used to calculate your blood requirements
- Noted down your recommendations on your Action List.

Activity 24**Purpose**

To review your blood stock management system.

Checklist

You should have:

- Noted which of the following records are kept in your blood bank:
 - the number of units collected or received
 - the number of units transfused
 - the number of patients transfused
 - the outdate rate
 - the number of times an operation or transfusion is cancelled because of a lack of suitable blood
- Identified any ways in which this information could be used more effectively in planning and managing stocks of blood and plasma
- Noted your recommendations on your Action List
- Developed a simple system to record this information, if any of these records are not kept in your blood bank at present
- Noted down your recommendations on your Action List
- Discussed them with your supervisor and senior colleagues.

SECTION 7**Activity 25****Purpose**

To review the equipment available in your laboratory for routine serological testing.

Checklist

You should have:

- Drawn up a list of the equipment available in your laboratory for routine serological tests
- Compared it with the equipment listed in Figure 23
- Identified any additional equipment required in your laboratory for routine serological testing
- Noted down your recommendations on your Action List
- Discussed your recommendations with your supervisor.

Activity 26**Purpose**

To prepare 3% cell suspensions accurately.

Checklist

You should have:

- Read Technique 1 in Appendix 1
- Collected five previously typed blood samples
- Produced a 3% cell suspension of each, using Technique 1
- Asked your supervisor to check that you have prepared the cell suspensions correctly.

Activity 27**Purpose**

To carry out ABO and Rh D grouping tests accurately.

Checklist

You should have:

- Read Techniques 2–8 in Appendix 1
- Using either tubes (Technique 6) or a microplate (Technique 7), performed an ABO grouping followed by a Rh D grouping test on each sample that you prepared in Activity 26
- Recorded the strength of each reaction, using the scoring system described in Section 7.8 on page 79
- Compared your results with the original grouping test results
- Explained the reason for any differences between these results
- Asked your supervisor to check your results.

Activity 28**Purpose**

To carry out a compatibility test accurately.

Checklist

You should have:

- Read Techniques 13.1–13.4 in Appendix 1
- Selected one of these techniques and performed a compatibility test, using group O serum with a suspension of group A or B cells
- Repeated the test, using an emergency technique

- Used the scoring system on page 79 correctly to record the results
- Used the same techniques to perform a compatibility test with group O cells and group O serum
- Asked your supervisor to check your results.

SECTION 8

Activity 29

Purpose

To assess the progress you have made through your work on this module.

Checklist

You should have:

- Assessed your knowledge, skills and experience in relation to each of the module objectives now that you have reached the end of the module
- Completed the table on page 94
- Identified any areas in which you still do not feel fully confident about your knowledge or skills
- Reread the appropriate sections of the module and discussed any remaining problems with your supporter or trainer.

Activity 30

Purpose

To plan how to implement the improvements that you have identified as being necessary to ensure quality in your blood transfusion service or blood bank.

Checklist

You should have:

- Looked at all the suggestions for improvements that you have included on your Action List and marked those where you have not yet been able to take any action
- Divided them into two categories:
 - Actions that you can take
 - Actions that others could take
- Identified priorities for action
- Filled in your Action Plan, as follows:
 - Column 1: the improvements you have identified as being necessary

- Column 2: the action you plan to take or the name of the person who would be responsible for taking action
- Column 3: the results you would expect as a result of implementing your planned actions
- Discussed your plan with your supervisor, supporter, trainer and any other appropriate senior staff
- Modified your plan, where necessary
- Filled in Column 4 with the dates by which you hope the planned actions will be completed.

Activity 31

Purpose

To review the implementation of your Action Plan and identify any follow-up action required.

Checklist

You should have:

- Filled in Column 5 with the dates on which you completed each planned action and compared them with the planned completion dates
- Summarized the results of your planned action in Column 6 and compared them with the results you had expected
- Discussed the outcomes with your supporter
- Discussed the outcomes with your supervisor
- Identified any further actions required to ensure the implementation of the improvements you have identified as being necessary
- Monitored the effectiveness of the changes you have been able to introduce
- Identified any further changes or follow-up action required.

Answers to Self-assessment Questions

SECTION 2

- 1 The average lifespan of the red cells within the circulatory system is 120 days.
- 2 The main function of haemoglobin is to transport oxygen to the tissues to provide the body with energy and heat, and to remove impurities such as carbon dioxide.
- 3 The function of lymphocytes is to produce antibodies against foreign antigens and to fight viral infections.
- 4 The three main reasons for transfusing blood are:
 - to correct anaemia (a low haemoglobin level)
 - to replace blood lost by bleeding, either during an operation or because of an accident
 - to replace other constituents of blood, such as clotting factors.

SECTION 3

- 5 The primary response occurs when a specific antigen invades the body for the first time. This response develops slowly and is often associated with small amounts of IgM antibody.

The secondary response occurs when the body meets the same foreign antigen for the second time. This response is much greater than the primary response and produces much larger amounts of antibody, mainly IgG, often in a short period of time.

- 6 There are no ABO antibodies in the serum of cord or newborn babies or, at the most, very small amounts. The appearance of these antibodies around 12 weeks

later is due to so-called 'naturally-occurring' antibodies that are the result of bacteria invading the body and stimulating the appropriate antibody, which is usually IgM.

- 7 The two stages in producing agglutination are:

Stage 1

The antibody binds to its red cell antigen, coating or sensitizing the cell.

Stage 2

A lattice forms, producing the clump or agglutination.

- 8 The anti-human globulin test has three stages:

Stage 1: Sensitization or coating

Cells and serum are incubated to allow any antibodies present in the serum to bind to the antigens in the red cells.

Stage 2: Washing

These cells are then washed several times in a large volume of saline to remove any protein or immunoglobulins not coated onto the cells.

Stage 3: Addition of anti-human globulin reagent

Anti-human globulin reagent (AHG) is added to the washed cells. If the cells are coated with IgG antibodies (or C3 component of complement) they will be agglutinated by the anti-human globulin reagent binding to the IgG antibodies coating the cells. If there are no antibodies on the cells, there will not be any agglutination.

SECTION 4

- 9 The four main blood groups have the following antigens on the red cells and antibodies in the serum:

	<i>Antigen on cells</i>	<i>Antibodies in serum</i>
Group A	A	anti-B
Group B	B	anti-A
Group AB	A and B	none
Group O	none	anti-A and anti-B

- 10 Anti-AB is used as part of the standard blood grouping test to ensure that the weaker group A and group B antigens are not missed in routine grouping tests.

SECTION 5

- 11 The RhD antigen is well-developed in fetal life. Cord and newborn babies' red cells therefore type as strongly as normal adult blood.

- 12 It is particularly important that RhD negative females are correctly RhD typed because if an RhD negative child or woman is wrongly grouped as RhD positive, she would be transfused with RhD positive blood. As a result, she would produce anti-RhD, which is an IgG antibody. If she later carried a D positive fetus, the antibody would cross the placenta and destroy the fetal red cells, resulting in haemolytic disease of the newborn.
- 13 You would test *patients* for the D^u antigen when:
- there is a high incidence of D^u in the population and it is local policy to test for D^u
 - the result of the RhD grouping test is different from previous results
 - the reactions between two reagents are different: that is, one is positive and one is negative.
- You would test *donors* for D^u when:
- samples give a negative result with the test anti-D.
- 14 A D^u donor is regarded as RhD positive. As D^u people have the D antigen, although only weakly, they cannot produce anti-D. They can therefore be transfused with D positive blood.

SECTION 6

- 15 The following blood should be selected:
- the freshest whole blood available, preferably less than seven days old
 - red cell concentrates less than 7 days old
 - red cell concentrates of any age
 - the freshest whole blood available
 - red cell concentrates less than 7 days old, if available.
- 16 It is necessary to perform a compatibility test because it is possible for a patient who is both ABO and RhD compatible with their donor to react severely against the blood following transfusion. This is because there are very many blood group systems other than ABO and Rh. A patient who is ABO and Rh compatible with the donor may have other antibodies such as anti-Kell or anti-Duffy. If this is the case, ABO and Rh compatible blood could be grossly incompatible if the donor's red cells have either of these antigens.
- 17 It is necessary to investigate every reported transfusion reaction to identify the cause and ensure the safety of the patient by preventing it from recurring if further transfusions are necessary.

- 18 To calculate the outdate rate it is necessary to know:
- the number of units collected or received in a year
 - the number of units that have gone out of date during the same period of time.

SECTION 7

- 19 Two important factors to remember when using a pipette are to:
- hold the pipette at the same angle each time to ensure that you always use the same size drops
 - use a clean pipette for each new sample or wash the pipette thoroughly between each test to ensure that all traces of foreign material are washed out of it.
- 20 The use of glass slides or white porcelain tiles is not recommended because this technique is insensitive and results in too many errors.
- 21 A scoring system is used to record the strength of the reaction obtained.
- 22 The majority of errors in blood grouping are caused by poor technique.
- 23 A false-positive reaction in an indirect anti-human globulin test could be caused by:
- the presence of dust or particles in the tubes
 - cross-contamination from one tube to another
 - centrifuging the tube too fast or for too long.
- 24 A false-negative reaction in an indirect anti-human globulin test could be caused by:
- failure to wash the cells properly
 - failure to add AHG
 - dirty tubes
 - the presence of small clots in the serum or cells
 - using saline at the wrong pH
 - centrifuging the tube at the wrong speed
 - leaving the cells after washing before adding AHG
 - leaving the test after having added the AHG before reading.
- 25 The conditions that can lead to a positive direct anti-human globulin test are:
- autoimmune haemolytic anaemia
 - haemolytic transfusion reactions
 - haemolytic disease of the newborn.

26 The albumin addition test is performed in two stages:

Stage 1

Serum and cells are incubated at 37°C and the cells are allowed to settle to the bottom of the tube. The red cells will be coated by any antibodies present (both IgG and IgM).

Stage 2

Albumin is then added, allowing it to run down the inside of the tube so it does not disturb the cells. The test is re-incubated for 10–15 minutes. The albumin will cause any coated cells to agglutinate.

Glossary

Agglutination

The clumping together of red cells.

Allelic gene

An alternative form of gene occupying a single locus on either of a pair of homologous chromosomes.

Antibody

A protective protein produced by the immune response of an individual to stimulation by a foreign protein. It recognizes antigen on foreign red cells and may cause in vivo agglutination and subsequent haemolysis.

Antigen

Any substance recognized as foreign by the body which stimulates the immune system to mount a response against it.

Anti-human globulin reagent (AHG)

A blood grouping reagent that reacts specifically with human globulin.

Anti-human globulin test

A test using anti-human globulin reagent to detect the presence of human globulin (antibodies) on sensitized red cells.

Basophil

A member of the family of white cells involved in fighting infection.

Blood ordering schedule

An agreed list of the number of units of blood to be cross-matched before the listed surgical procedures or operations take place.

Chromosome

A thread-like structure that carries genes and is present in the nucleus of cells.

Coagulation

Clotting of blood which takes place when blood is collected into a dry container or reaches an open wound.

Coated cells

See sensitized cells.

Complement

A series of proteins present in normal human serum often involved in antigen–antibody reactions and immunological disorders.

Cross-matching

A term used when testing the patient's serum against the donor's red cells and the donor's serum against the patient's red cells, before transfusion.

Direct anti-human globulin test (DAT)

A test used for detecting the presence of human globulin (antibodies) on the surface of sensitized cells.

Enzyme

In blood group serology, used to define a substance that has the ability to remove some of the protein and chemical surrounding the red cells, thereby lowering the forces of attraction around the cells (zeta potential). This causes the cells to become more sensitive to agglutination and allows an IgG antibody to agglutinate red cells suspended in saline.

Eosinophil

A member of the family of white cells involved in fighting infection.

Erythrocyte

The red blood cell (the most numerous blood cell), which contains the red pigment haemoglobin and is responsible for transporting oxygen to the body tissues.

Fibrin

Fine protein strands produced when soluble fibrinogen is acted upon by thrombin in the process of blood coagulation.

Fibrinogen

A substance involved in the process of blood coagulation.

Gammaglobulin

The class of serum proteins that includes antibody molecules.

Gene

The basic unit of inheritance which is carried on the chromosome.

Genotype

The genes inherited from each parent which are present on the chromosomes.

Globulin

A serum protein from which antibodies are made.

Granulocyte

A white blood cell (leukocyte) containing neutrophil, eosinophil and basophil granules in the cytoplasm.

Haemoglobin

A red fluid found in the red blood cells which is made up of iron (haem) and polypeptide chains (globin).

Haemoglobinaemia

Free haemoglobin in the bloodstream (plasma).

Haemolysin

An antibody that combines with complement causing it to destroy (lyse) the red cells carrying its specific antigen.

Haemolysis

The breaking down (lysis) of the red cell membrane which liberates its contents: haem and globin. Haemolysis results from the reaction between a haemolytic antibody and its specific red cell antigen in the presence of complement.

Haemolytic disease of the newborn

A disease in which maternal antibody crosses the placenta and destroys the fetal red cells that possess the corresponding antigen, leading to anaemia.

Haplotype

A number of genes that reside together on a chromosome and are usually inherited as if they were a single gene, sometimes called a 'gene complex'.

Heterozygous

A condition in which non-identical allelic genes are carried on homologous chromosomes.

Homozygous

A condition in which two identical allelic genes are carried on homologous chromosomes.

Immunoglobulin

An antibody molecule synthesized by specialized lymphocytes in response to an antigen.

Indirect anti-human globulin test (IAT)

A tube haemagglutination method, commonly referred to as the Coombs test, in which antibodies incapable of causing direct agglutination can be shown to have combined with their specific red cell antigens by testing with an anti-human globulin reagent.

In vitro

A reaction occurring outside the body: that is, a test-tube reaction.

In vivo

A reaction occurring within the body as, for example, in autoimmune haemolytic anaemia.

Laryngeal oedema

Swelling of the larynx creating difficulty in breathing.

Leukocyte

A family of nucleated white cells involved in fighting infection and making antibodies.

LISS

See low ionic strength saline

Low ionic strength saline (LISS)

Saline that is lowered from its normal strength to 0.32% saline in buffered glycerine. The speed of uptake of most blood group antibodies onto the corresponding antigen is enhanced when the ionic strength of the saline is lowered. Most low ionic strength saline solutions are now produced commercially.

Lymphocyte

A type of white blood cell formed in the lymph nodes. There are two kinds of lymphocyte: B lymphocytes which produce circulating antibodies and T lymphocytes which are responsible for the cellular immune response.

Macrophage

A phagocytic cell type found in the bloodstream as well as tissues. Macrophages ingest bacteria and cell debris.

Monocyte

A large leukocyte that ingests bacteria and other foreign bodies.

Naturally-occurring or naturally-acquired antibody

An antibody that appears in the bloodstream without any known antigenic stimulus.

Neutrophil

A member of the family of white cells involved in fighting infection.

Phagocytosis

The process by which certain white cells ingest bacteria and other foreign bodies.

Phenotype

The observable effect of the inherited genes: the blood group itself.

Plasma

The fluid part of blood which carries the cells and other substances such as proteins, clotting factors and chemicals.

Primary antibody response

The response that the body makes when meeting a foreign antigen for the first time.

Pseudo-agglutination

False agglutination, which is usually due to an upset in the albumin/globulin ratio.

Reticuloendothelial system

A collection of endothelial cells that produce macrophages or large mononuclear cells. They are found in the bone marrow, liver, spleen and lymph glands.

Reverse grouping test

A test to detect ABO antibodies in serum or plasma.

Rouleaux formation

A type of reaction where the red cells form together, appearing like agglutination. It is therefore a false agglutination. See pseudo-agglutination.

Secondary antibody response

The increase in titre of an antibody when meeting its antigen for the second time.

Secretor

A person who possesses the dominant secretor gene and produces blood group specific substance in some body fluids, such as saliva and serum.

Sensitized cell

A cell coated with antibody, but not agglutinated.

Serum

The fluid surrounding red cells that have been allowed to clot.

Thrombocyte

A blood platelet, which plays a major role in the blood clotting mechanism.

Urticaria

The appearance of weals on the skin.

Voluntary non-remunerated blood donor

A donor who gives blood, plasma or other blood components freely and voluntarily, without receiving any payment in the form of money or a substitute for money.

Appendices

Techniques for Blood Grouping and Compatibility Testing

SALINE

It is very important to ensure that the saline used in red cell work has a pH of 6.5–7.5. If it is outside this range, some antibodies will not combine with their antigen and false negative results will result.

To make up saline, dissolve 8.5 g sodium chloride in 1 litre of fresh distilled or deionized water. Check the pH. No buffer will be required if it is within the pH 6.5–7.5 range and it is to be used on that day.

However, most saline is used over a 2–3 day period and the pH falls on storage. It is therefore necessary to add some buffer salts, either by using buffer tablets pH 7 or by adding buffer solutions, as detailed below.

Phosphate buffer solutions

Solution A: acidic

0.15 mol/L sodium dihydrogen phosphate
Sodium dihydrogen phosphate dihydrate 23.4 g
(Anhydrous 18.0 g)
Deionized water, up to 1000 ml

Solution B: alkaline

0.15 mol/L disodium hydrogen phosphate
Disodium hydrogen phosphate dodecahydrate 53.7 g
(Dihydrate 26.7 g)
(Anhydrous 21.3 g)
Deionized water, up to 1000 ml
40 ml solution A plus 60 ml solution B gives pH 7.0.

Add 10 ml of this buffer to each litre of saline. Check the pH. If it is outside the range 6.5–7.5, add more buffer to bring it into range.

See Appendix 1 of the Introductory Module for a detailed standard operating procedure for the preparation of saline solutions, including phosphate buffer solutions.

TECHNIQUE 1

WASHING CELLS AND MAKING RED CELL SUSPENSIONS

Materials

- 75 x 12 mm tubes
- Sample of blood
- Saline

Method

- 1 Centrifuge the sample so that the serum or plasma is separated from the red cells. The serum or plasma can then be removed to a clean, labelled tube.
- 2 With a Pasteur pipette, place 0.2–0.5 ml of red cells into each tube.
- 3 Fill the tube to within 1 cm of the top with saline.
- 4 Centrifuge for 1–2 minutes, until the cells are packed.
- 5 Take off the saline.
- 6 Tap the tube to resuspend the cells. This constitutes one wash.
- 7 Repeat steps 3–6 if required. The last wash should always have clear saline left, with no signs of haemolysis.
- 8 To make a 3% suspension, add 1 volume packed cells to 30 volumes of saline.

TECHNIQUE 2

SLIDE (TILE) TECHNIQUE

This is a quick technique, but weak acting antibodies will not agglutinate red cells by this method and it is not recommended as a routine

APPENDIX 1

technique. If it is used for a rapid group, the group must be performed using a full tube or microplate technique to confirm the results.

Materials

- Opal glass tile or microscope slides
- Reagent or test serum
- Reagent or test red cells
- Wooden applicator sticks

Method

- 1 Use a single slide for each test or a ruled 3 cm section on a white tile.
- 2 Label each section of the tile to identify the cells and serum used.
- 3 To 1 drop of serum (25 μ l), add 1 drop of a 10–20% cell suspension.
- 4 Mix reactants with a clean applicator stick to an area with a diameter of 2 cm.
- 5 Rock the slide or tile gently and look for agglutination.
- 6 Record the results within 2 minutes.

TECHNIQUE 3

IMMEDIATE SPIN (IS) TECHNIQUE

This is a good method for rapid ABO grouping and RhD typing.

Materials

- 75 x 12 mm tubes
- Reagent serum
- Test red cells

Method

- 1 To 1 drop of a 2–3% cell suspension, add 2 drops of serum. Mix by shaking the tube gently.
- 2 Centrifuge for 15–20 seconds.
- 3 Look for haemolysis and agglutination.
- 4 Record lysis and/or agglutination.

Reagents prepared for use by an IS technique need to be warmed to room temperature before use.

TECHNIQUE 4

SALINE ROOM TEMPERATURE TECHNIQUE

Materials

- 50 x 7 mm precipitin tubes
- Reagent or test serum
- Reagent or test red cells

Method

- 1 To 1 drop of serum or antiserum, add one drop of 2–3% red cell suspension. Mix gently.
- 2 Incubate at room temperature for 60 minutes.
- 3 Look for lysis and agglutination.
- 4 Record the reaction.

TECHNIQUE 5

ALBUMIN ADDITION TECHNIQUE FOR RhD TYPING

Materials

- 50 x 7 mm tubes
- Suitable anti-D reagent
- Test red cells
- Bovine albumin (20–30%)

Method

- 1 To 1 drop of anti-D, add 1 drop of a 2–3% suspension of red cells.
- 2 Mix and incubate at 37°C for 45–60 minutes.
- 3 Add 1 drop of albumin to each tube, allowing it to run down the inside of the tube so it does not disturb the cell button.
- 4 Incubate the tubes again at 37°C for 15 minutes.
- 5 Read and record the results.

- 2 Add 1 drop of a 2–3% suspension of A, B or O cells.
- 3 Incubate at room temperature (20–23°C) for 60 minutes.
- 4 Mix the plate gently to resuspend the unagglutinated cells and examine the plate over a white surface.
- 5 Record the reactions.

Saline RhD

If your anti-D is suitable for use in a microplate at room temperature, the same plate can be used.

Method

- 1 In row G, add 1 drop of anti-D to each well plus 1 drop of the patient's red cells and the A, B and O cells as controls. Row H can be used for a second anti-D or the reagent control, if one is supplied with the anti-D.
- 2 Mix the plate gently and cover it with a lid or plastic film and leave undisturbed on the bench for 60 minutes.
- 3 Examine all the wells for lysis and record any that you see.
- 4 Mix the plate gently to resuspend the unagglutinated cells and examine the plate over a white surface.
- 5 Record the reactions.

TECHNIQUE 8

TESTING FOR WEAK D (THE D^u TEST)

If a D^u test needs to be performed, an anti-D reagent that can be used by an indirect anti-human globulin test (see Technique 9) must be used.

Materials

- 75 x 12 mm tube
- Suitable anti-D reagent
- Test red cells
- Anti-human globulin (AHG) reagent

Method

- 1 To 1 drop of a 2–3% suspension of test red cells, add 2 drops of anti-D.
- 2 Incubate at 37°C for 30 minutes.
- 3 Look for agglutination.
- 4 If positive, record the sample as being D positive.
- 5 If there is no agglutination, wash the cells four times.
- 6 Add 2 drops of AHG and spin.
- 7 Read the reactions.
- 8 If there is a positive reaction, perform a direct anti-human globulin test (DAT) on the cells (see Technique 11).
- 9 If the DAT is negative, the results obtained in the D^u test are correct and the patient or donor can be called Rh positive (D^u).

If the DAT is positive, the results cannot be relied upon.

If a reagent control is supplied or recommended with the anti-D reagent, this must be used when D^u testing in the same way as the anti-D. This should be negative. If positive, the results cannot be relied upon.

If the results are originally negative, add 1 drop of control IgG-coated cells. Then spin and record the reactions.

TECHNIQUE 9

INDIRECT ANTI-HUMAN GLOBULIN TEST (IAT)

Materials

- 75 x 12 mm glass tubes (glass tubes are preferable to plastic)
- Reagent or test serum
- Reagent or test cells
- Anti-human globulin (AHG) reagent
- Control IgG-coated cells (see Technique 12)

Method

- 1 In a tube, mix 3–4 drops of serum with 1 drop of a 2–3% suspension of cells.

- 2 Incubate at 37°C for 45–60 minutes.
- 3 Look for haemolysis and agglutination. If either is observed, record as positive.
- 4 If no haemolysis or agglutination is seen, wash the cells four times in saline.
- 5 To the washed cells, which have been shaken from the bottom of the tube, add 2 drops of AHG and mix.
- 6 Centrifuge the tubes at 1000g for 15–20 seconds (the speed and time for each centrifuge varies).
- 7 Remove the tubes and read over a light box or white tile.
- 8 Record the results.
- 9 If the test is still negative, add 1 drop of control IgG-coated cells.
- 10 Repeat steps 6 and 7.

A positive reaction indicates that a negative result in step 7 is valid, but if the control IgG-coated cells fail to agglutinate, the test must be repeated.

TECHNIQUE 10

LOW IONIC STRENGTH SALINE SOLUTION INDIRECT ANTI-HUMAN GLOBULIN TEST (LISS/IAT)

10.1 LISS-suspension method

Materials

- 75 x 12 mm glass tubes
- Low ionic strength saline (LISS)
- Reagent or test serum
- Reagent or test red cells
- Anti-human globulin (AHG) reagent
- Control IgG-coated cells (see Technique 12)

LISS, LISS-suspended cells and serum should be brought to room temperature before use.

Method

- 1 Wash test cells twice in saline and then once in LISS.

- 2 Resuspend test cells in LISS to a 2–3% suspension.
- 3 In a tube, mix *equal* volumes of test serum and LISS-suspended cells (2 or 3 drops from a Pasteur pipette or 100 μ l measured volume).
- 4 Incubate tubes at 37°C for 15 minutes (in a water-bath, if possible).
- 5 Remove tubes and look for haemolysis and/or agglutination. If present, record as positive.
- 6 Wash cells at least three times in saline.
- 7 To the washed cells, which have been shaken from the bottom of the tube, add 2 drops of AHG and mix.
- 8 Centrifuge the tubes at 1000g for 15–20 seconds (the speed and time for each centrifuge varies).
- 9 Remove the tubes and read over a light box or white tile.
- 10 Record the results.
- 11 If the test is still negative, add 1 drop of control IgG-coated cells.
- 12 Repeat steps 8 and 9.

10.2 LISS-addition method

Materials

- 75 x 12 mm glass tubes
- LISS-addition solution
- Reagent or test serum
- Reagent or test red cells
- Anti-human globulin (AHG) reagent
- Control IgG-coated cells (see Technique 12)

Method

- 1 In a tube, place 3 drops of serum and one drop of a 2–3% saline suspension of red blood cells.
- 2 Add 3 drops of LISS-addition solution and mix contents (this volume may vary with different makes of reagent).
- 3 Incubate the tubes at 37°C for 15 minutes.

- 4 Look for haemolysis and agglutination. If either is observed, record as positive.
- 5 If no haemolysis or agglutination is seen, wash the cells four times in saline.
- 6 To the washed cells, which have been shaken from the bottom of the tube, add 2 drops of AHG and mix.
- 7 Centrifuge the tubes at 1000g for 15–20 seconds (the speed and time for each centrifuge varies).
- 8 Remove the tubes and read over a light box or white tile.
- 9 Record the results.
- 10 If the test is still negative, add 1 drop of control IgG-coated cells.
- 11 Repeat steps 6 and 7.

TECHNIQUE 11

DIRECT ANTI-HUMAN GLOBULIN TEST (DAT)

Materials

- 75 x 12 mm glass tubes (glass tubes are preferable to plastic)
- Test red cells
- Anti-human globulin (AHG) reagent
- Control IgG-coated cells (see Technique 12)

Method

- 1 Wash 1 volume of a 2–3% red cell suspension at least three times.
- 2 To the washed cells, which have been shaken from the bottom of the tube, add 2 volumes of AHG and mix.
- 3 Centrifuge the tubes at 1000g for 15–20 seconds (the speed and time for each centrifuge varies).
- 4 Remove the tubes and read over a light box or white tile.
- 5 Record the results.

- 6 If the test is still negative, add 1 drop of control IgG-coated cells.
- 7 Repeat steps 3 and 4.

A positive reaction indicates that a negative result in step 4 is valid, but if the control IgG-coated cells fail to agglutinate, the test must be repeated.

TECHNIQUE 12

PREPARATION OF CONTROL IgG-COATED CELLS FOR THE ANTI-HUMAN GLOBULIN TEST

Materials

- 75 x 12 mm tubes
- Group O Rh positive red cells
- Anti-D reagent
- Saline
- Anti-human globulin (AHG) reagent

Method

- 1 Wash the cells three times in saline.
- 2 Add an equal volume of anti-D to the packed cells.
- 3 Incubate at 37°C for 30 minutes.
- 4 Wash the cells four times.
- 5 Suspend in saline to a 5% suspension.
- 6 Take 1 volume of the 5% suspension, add 2 volumes of the routine AHG, mix gently and centrifuge the tube. The reaction should be +++. If this reaction is too strong or too weak, these cells will not properly control the anti-human globulin test.
- 7 These sensitized cells can be stored in suspension at 4°C for 48 hours.

TECHNIQUE 13**CROSS-MATCHING****13.1 One-tube: immediate spin and IAT****Materials**

- 75 x 12 mm tubes
- Patient's serum
- Donor's red cells
- Anti-human globulin (AHG) reagent
- Control IgG-coated cells

One tube is required for each donation being cross-matched and must be carefully labelled to ensure that the correct blood is being tested.

Method

- 1 Wash some red cells from each donation to be cross-matched.
- 2 Into a labelled tube, place 3 drops of the patient's serum.
- 3 Add 1 drop of a 2–3% suspension of the donor's red cells.
- 4 Mix.
- 5 Centrifuge the tube lightly to sediment the cells.
- 6 Examine the tube for lysis and agglutination. If either is present, the blood is *incompatible*.
- 7 If negative at this stage (the immediate spin), mix the contents and incubate the tube at 37°C for 45–60 minutes.
- 8 Examine the tube for lysis and agglutination. If either is present, the blood is *incompatible*.
- 9 If negative, wash the cells four times.
- 10 To the washed cells, which have been shaken from the bottom of the tube, add 2 drops of AHG and mix.
- 11 Centrifuge the tubes at 1000g for 15–20 seconds (the speed and time for each centrifuge varies).
- 12 Remove the tubes and read over a light box or white tile.
- 13 Record the results.
- 14 If the test is still negative, add 1 drop of control IgG-coated cells.
- 15 Repeat steps 11 and 12.
- 16 If the IAT is confirmed as *negative*, the blood is *compatible* and can be issued for the patient when the correct documentation and labelling has been completed.

13.2 One-tube, using LISS-addition**Materials**

- 75 x 12 mm tubes
- Patient's serum
- Donor's red cells
- LISS-addition solution
- Anti-human globulin (AHG) reagent
- Control IgG-coated cells

One tube is required for each donation being cross-matched and must be carefully labelled to ensure that the correct blood is being tested.

Method

- 1 Wash some red cells from each donation to be cross-matched.
- 2 Into a labelled tube, place 3 drops of the patient's serum.
- 3 Add 1 drop of a 2–3% suspension of the donor's red cells.
- 4 Mix.
- 5 Centrifuge the tube lightly to sediment the cells.
- 6 Examine the tube for lysis and agglutination. If either is present, the blood is *incompatible*.
- 7 If the immediate spin is negative, add 3 drops of the LISS-addition solution, mix the tube's contents and incubate at 37°C for 15 minutes.
- 8 Examine the tube for lysis and agglutination. If either is present, the blood is *incompatible*.

- 9 If negative, wash the cells four times.
- 10 To the washed cells, which have been shaken from the bottom of the tube, add 2 drops of AHG and mix.
- 11 Centrifuge the tubes at 1000g for 15–20 seconds (the speed and time for each centrifuge varies).
- 12 Remove the tubes and read over a light box or white tile.
- 13 Record the results.
- 14 If the test is still negative, add 1 drop of control IgG-coated cells.
- 15 Repeat steps 11 and 12.
- 16 If the IAT is confirmed as *negative*, the blood is *compatible* and can be issued for the patient when the correct documentation and labelling has been completed.
- 2 For each donation, 2 tubes (50 x 7 mm) are needed. Place one drop of patient's serum into each tube.
- 3 Into each tube, place one drop of 2–3% suspension of donor's cells.
- 4 Incubate *one* of each set of tubes at 37°C for 45–60 minutes.
- 5 Add 1 drop of albumin to each of these tubes, allowing it to run down the inside of the tube. Ensure that the drop reaches the cells and serum. *Do not mix*.
- 6 Incubate the tubes again for 10–15 minutes.
- 7 Leave the other set of tubes at room temperature.
- 8 After the incubation period for the albumin test, read both sets of tubes, looking for haemolysis and agglutination.
- 9 If both tubes are free from lysis and agglutination, the blood is *compatible*.

The LISS-addition material is added after the immediate spin stage to prevent false positive reactions due to cold reacting antibodies.

The donor's red cells can be suspended in LISS. Two drops of this cell suspension and two drops of serum are then used. To prevent false-positive reactions due to cold reacting antibodies, the LISS and serum should be at room temperature before use. A LISS IAT requires 15 minutes incubation at 37°C.

13.3 Cross-matching using an albumin addition and saline room temperature test

Materials

- 50 x 7 mm tubes
- Patient's serum
- Donor's red cells
- Bovine albumin (20–30%)

Method

- 1 Wash some red cells from each donation to be cross-matched.

13.4 Emergency cross-matching

In an emergency, the cross-match should be set up by your routine method. Blood can be issued as *cross-matched by an emergency technique* after reading the immediate spin stage if no agglutination is found. The cross-match should then be completed.

If any incompatibility is found, inform the patient's doctor immediately.

Reducing incubation times can be dangerous. If saline suspended cells are used in the IAT, the minimum incubation time is 30 minutes. For LISS techniques, it is 10 minutes at 37°C.

In the albumin plus saline room temperature technique, the saline tube can be centrifuged for the immediate spin phase. If a shorter incubation is required, the tube at 37°C can be lightly centrifuged after 30 minutes, the albumin added and reincubated for 10 minutes and read.

Preparing Red Cell Concentrates

When red cell concentrates are requested, for example for a patient with anaemia, you may need to remove the plasma from a unit of whole blood. This should not be necessary if red cell concentrates are provided by your blood transfusion service. However, if this is not possible or you have no red cell concentrate of a particular group, you can use whole blood collected into a single bag as long as the aseptic techniques described in the method below are used.

If you do not have a plasma expressor, you cannot prepare red cell concentrates.

Equipment

To prepare red cell concentrate, you will need:

- a plasma expressor
- metal or plastic forceps
- a bowl.

A hand-held tube sealer with metal clips is useful, but not essential.

Method

- 1 Allow the blood to settle out until there is a distinct line between the red cells and the plasma (see Figure 1).

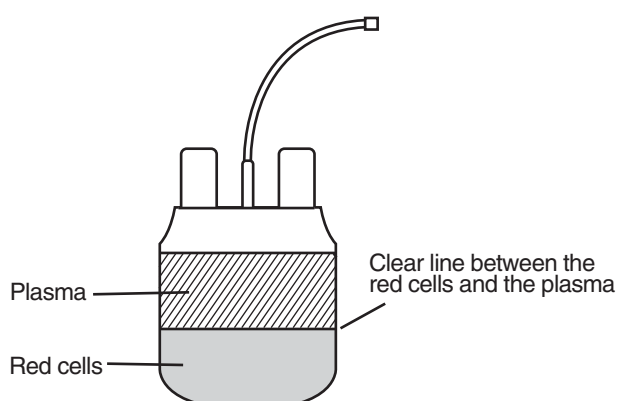


Figure 1

- 2 Place the bag containing the whole blood into the plasma expressor (see Figure 2).

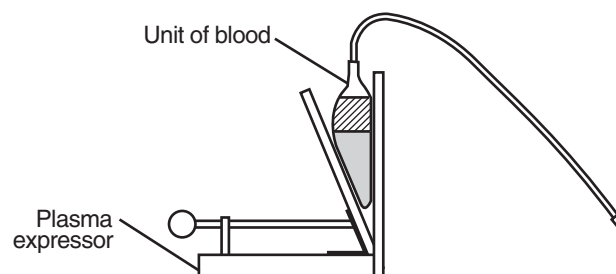


Figure 2

- 3 Pinch the tubing using the forceps, then break the seal between the bag and the tubing (see Figure 3).

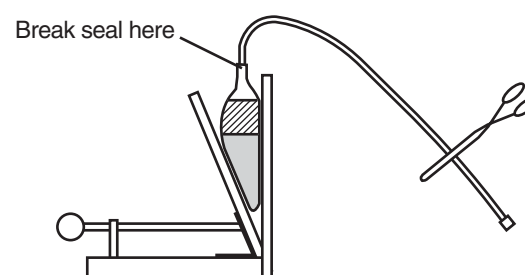


Figure 3

- 4 Place the bowl so that the plasma will run into it and cut the end of the tubing. Then release the forceps (see Figure 4).

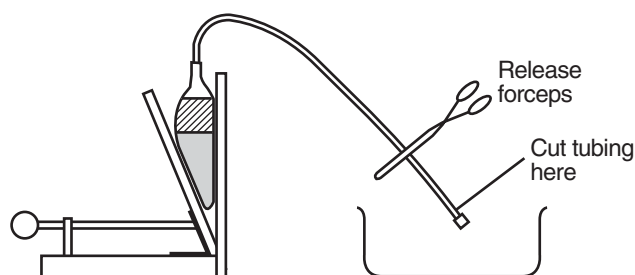


Figure 4

- 5 Allow the plasma to flow into the bowl until there is approximately 2–3 cm (1 inch) of plasma left on the red cells (see Figure 5). Then stop the flow, using the forceps again (see Figure 6).

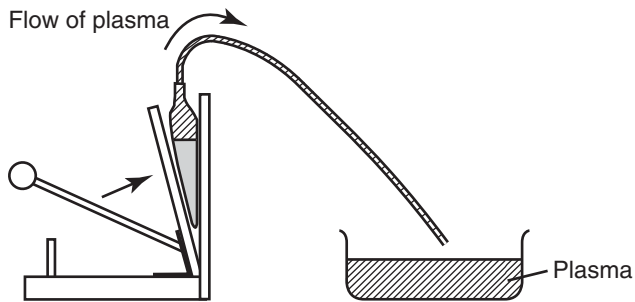


Figure 5

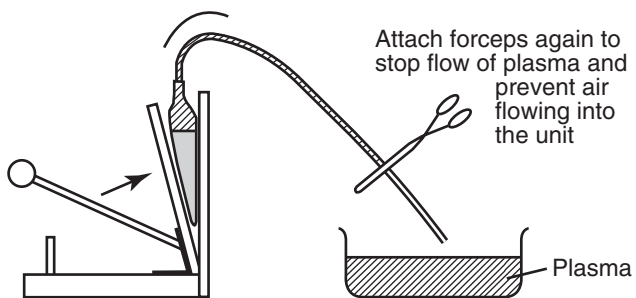


Figure 6

- 6 Seal the tubing using metal clips and a sealer or by tying a knot very tightly (see Figure 7).

It is extremely important that the seal is airtight in order to prevent contamination. You can check this by gently squeezing the bag.

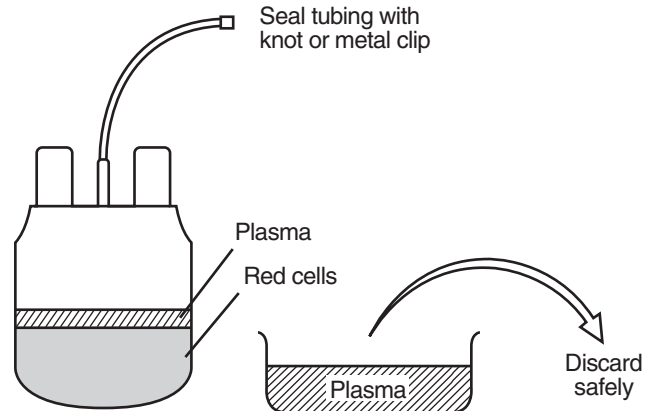


Figure 7

- 7 Release the bag from the plasma expressor.
- 8 Store the red cell concentrate at between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$. It must be transfused within 24 hours, in case there is any contamination. For this reason, you should never prepare red cell concentrate from a single bag of whole blood unless you are certain it will be transfused that day.
- 9 Discard the plasma safely in the same way as other potentially infectious materials. See Section 3 of the Introductory Module for further information on the safe disposal of waste.