Safe Blood and Blood Products

Module 2

Screening for HIV and Other Infectious Agents

World Health Organization

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Conversion of electronic files for the website edition was supported by Cooperative Agreement Number PS001426 from the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC.

Contents

Preface

Safe Blood and Blood Products is a series of interactive learning materials developed by the World Health Organization (WHO). They 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 learning 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*
- *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
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Introduction to Module 2

The purpose of this section is to introduce you to Module 2: *Screening for HIV and Other Infectious Agents,* which focuses on developing an effective screening programme to detect infectious agents in donated blood.

LEARNING OBJECTIVES

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

1 Explain the purpose of Module 2.

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 2: *Screening for HIV and Other Infectious Agents* 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 3: *Blood Group Serology*.

You should already be familiar with the use of these distance learning materials from your work on the Introductory Module. If you have not yet read it, 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 2

You should find this module useful if you work in a blood transfusion service laboratory, hospital blood bank or public health laboratory and are involved in any aspect of screening blood for transfusion-transmissible infections (TTIs).

You may also find Module 2 of interest if you are a member of the medical or technical laboratory staff, such as a medical superintendent or a senior technologist, and are responsible for training or supervising staff who are involved in any aspect of screening blood. 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 2 contains some material which is quite complex, so you may find it hard to understand everything at first. Don't worry about this. 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 can understand them. If you still find them complicated or are unable to complete any 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 as a means of revision and to make sure that your knowledge is completely up to date. There have been many recent developments in laboratory practice, particularly in relation to screening for transfusion-transmissible infections, and it is important that you are fully aware of them. However, some sections, particularly Sections 3, 4 and 8, contain a great deal of information which is primarily provided as reference material. It is not necessary to memorize all the details.

There may also be some parts of the module that are not relevant to your own work. For example, Chagas disease, which is described in Section 8, does not occur in many parts of the world. Even if you do not need to know about it in detail, you may still find it interesting to read about it and compare it with the diseases that are prevalent in your country.

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 2, 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". Hopefully, you were 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 specialist experience in screening for transfusion-transmissible infections.

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 2. 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 he or she becomes 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 involved in your laboratory's screening programme.

1.3 MODULE 2: SCREENING FOR HIV AND OTHER INFECTIOUS AGENTS

With the rapid spread of the AIDS pandemic, there is an urgent need to ensure the safety of all blood and blood products. Module 1: *Safe Blood Donation* addresses the first step in reducing the risk of transmission of infectious agents through blood, which is to select low-risk donors and screen them thoroughly before they donate blood. Voluntary non-remunerated donors who give blood on a regular basis are more likely to be free from transfusion-transmissible infections (TTIs) than family or replacement donors, or commercial donors.

Even with the most careful screening of donors, however, some donors will prove to be seropositive for TTIs and rigorous screening of all donated blood is required to ensure the safety of the blood supply. This module sets out to strengthen your own knowledge and skills in screening blood for infectious agents.

Section 1: *Introduction to Module 2* outlines the contents of the module and contains activities that are designed to help you to prepare for your work on it.

Section 2: *Infection and Infectious Agents* looks at four types of infectious agent and the transmission of these agents by blood transfusion. It also provides an introduction to the basic immunology of infection and to screening for infectious agents, such as HIV.

Section 3: *The Human Immunodeficiency Viruses* examines HIV infection and the consequences of infection. It describes the structural features of HIV and the basic stages of infection and considers routes of transmission and the prevention of its spread. It will provide you with background information which you can use for reference purposes.

Section 4: *Principles of Screening Assays for Transfusion-Transmissible Infections* focuses on the possible approaches to screening for TTIs and explains the principles behind the different types of assay.

Section 5: *Selecting Screening Assays for Transfusion-Transmissible Infections* will help you to select the most suitable type of screening assay for your particular circumstances. It explains the importance of sensitivity and specificity and considers a number of factors to take into account when developing a screening programme.

Section 6: *Using Screening Assays for Transfusion-Transmissible Infections* deals with the performance of screening assays, including handling screening results, confirmatory testing, recording and storing test results and safety procedures in handling positive donations, assay components and waste.

Section 7: *Quality in Screening for Transfusion-Transmissible Infections* emphasizes the importance of quality assurance in the maintenance of an effective screening programme, and covers quality systems, standard operating procedures, laboratory worksheets and audit trails.

Section 8: *Screening for Other Transfusion-Transmissible Infections* describes the basic features of infection with hepatitis B virus, hepatitis C virus, HTLV-I and II, syphilis, malaria and Chagas disease, and explains their significance for blood transfusion practice.

Section 9: *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 2 on page 132.

1.4 MODULE OBJECTIVES

There are seven 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 Module 2, you should be able to achieve the following objectives:

Section 2
Explain the role of microorganisms as infectious agents in human disease and their significance for blood transfusion.

Section 3
Describe HIV infection and the significance of infection for blood transfusion practice.

Section 4
Outline the principles of the diagnostic assays most commonly used to detect transfusion-transmissible infections and the differences between them.

Section 5
Select the most suitable type of screening assay for transfusion-transmissible infections for use in your own laboratory.

Section 6
Develop an effective screening programme for transfusion-transmissible infections and maintain accurate records of the screening results.

Section 7
Help to develop an appropriate quality system for your laboratory to maintain an effective screening programme.

Section 8
Recognize the basic features of other infectious agents and their significance for blood transfusion practice.

Some sections of the module may not be directly relevant to your work. Before starting work on this module, therefore, you should identify any sections that cover tasks that you do not currently perform. Discuss them with your trainer before you draw up your Study Plan. You may decide to work through every section and attempt all the activities; alternatively, you may agree that you should complete only the sections that relate directly to your work and simply read the remainder.

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 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:

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 on page 7. 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 undertake.

You have now identified the areas which will be mostly revision for you and the areas to which you need to pay particular attention. The 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.

Module objective	*Rating (1 to 4)*	*Comments*
Section 2 Explain the role of microorganisms as infectious agents in human disease and their significance for blood transfusion. *Section 3* Describe HIV infection and the significance of infection for blood transfusion. *Section 4* Outline the principles of the diagnostic assays most commonly used to detect transfusion-transmissible infections and the differences between them. *Section 5* Select the most suitable type of screening assay for transfusion-transmissible infections for use in your own laboratory. *Section 6* Develop an effective screening programme for transfusion-transmissible infections and maintain accurate records of the screening results. *Section 7* Help to develop an appropriate quality system for your laboratory to maintain an effective screening programme. *Section 8* Recognize the basic features of other infectious agents and their significance for blood transfusion practice.		

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 2. The activities are likely to 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.

STUDY PLAN

Section	*Rating (1–4)*	*Planned completion dates*	*Meeting dates*	
			with trainer	*with supporter*
Section 2 Infection and Infectious Agents				
Section 3 The Human Immunodeficiency Viruses				
Section 4 Principles of Screening Assays for Transfusion-Transmissible Infections				
Section 5 Selecting Screening Assays for Transfusion-Transmissible Infections				
Section 6 Using Screening Assays for Transfusion-Transmissible Infections				
Section 7 Quality in Screening for Transfusion-Transmissible Infections				
Section 8 Screening for Other Transfusion-Transmissible Infections				
Section 9 Action Plan				

Notes

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 2.

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 2 focuses on the screening of donated blood for anti-HIV and other transfusion-transmissible infections.

2 You should identify a personal supporter to provide ongoing support while you work through this module.

3 Before starting work on Module 2, 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.

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 2.

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 2 or the learning programme as a whole, contact your trainer to discuss anything you are unsure about or talk to your supporter.

2

Infection and Infectious Agents

The purpose of this section is:

- to review the various types of infectious agent
- to examine the features of certain infectious agents that enable transmission by blood transfusion
- to help you to understand the role of transfusion-transmitted infections in modern blood banking
- to review the basic immunology of infection.

pathogenic microorganism: Any disease-causing microorganism.

Simple descriptions of the four main types of **pathogenic microorganism** are given but, if you are already familiar with these infectious agents, simply use this part of the section to revise your knowledge.

LEARNING OBJECTIVES

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

1 Identify examples of the four main types of infectious agent.

2 Understand the term "latency", as applied to viral infections, and its significance in blood transfusion practice.

3 Identify infectious agents which can be transmitted by blood transfusion.

4 Use your knowledge of immunology to predict likely markers of infection at different stages following infection.

2.1 INFECTIOUS AGENTS

There are four main types of infectious agent known. They are:

- viruses
- bacteria
- protozoa
- fungi.

Only the first three types of infectious agent – viruses, bacteria and protozoa – have been reported to be transmitted by blood transfusion; the extent of the transmission of each varies from country to country. Serious fungal infections usually make people too ill to be accepted as blood donors.

prion: A small proteinaceous infectious particle which resists inactivation by procedures that modify nucleic acids.

nucleic acid: A complex organic compound found in living cells that consists of chains of nucleotides. There are two types, DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), which make up cells' genetic information.

nucleotide: A compound formed from a nitrogen-containing base, either a purine or pyrimidine, phosphoric acid and a pentose sugar. DNA and RNA are formed from long chains of nucleotides.

There is however, a fifth type of infectious agent, the **prion,** which has been known about for some while but which has unusual properties and is still not fully understood. The term "prion" was first used to describe the then unknown infectious agent responsible for a number of neurodegenerative diseases found in mammals. Prions are unique as infectious agents because they do not contain any **nucleic acid**, but appear to consist only of a protein which is found in the membranes of normal cells; the protein however, has an altered shape or conformation. The current hypothesis is that this protein is able to bind to other proteins of the same type with a normal structure and cause them to change their conformation. This seems to cause a chain reaction that causes the disease process. At present, it seems that all the infections thought to be due to prions affect the central nervous system. At this time, there is no conclusive evidence to suggest that they are transmitted by blood transfusion.

Viruses

Viruses are the simplest known forms of life (see Figure 1). They infect all forms of life. Viruses are not cellular. They lack certain components needed to live and grow on their own and depend on the host cell that they infect to provide these missing components.

Following infection of a suitable host cell, the virus alters the normal functions in the cell. The viral nucleic acid instructs the cell to make new virus particles, called **virions**. These are then released to infect other

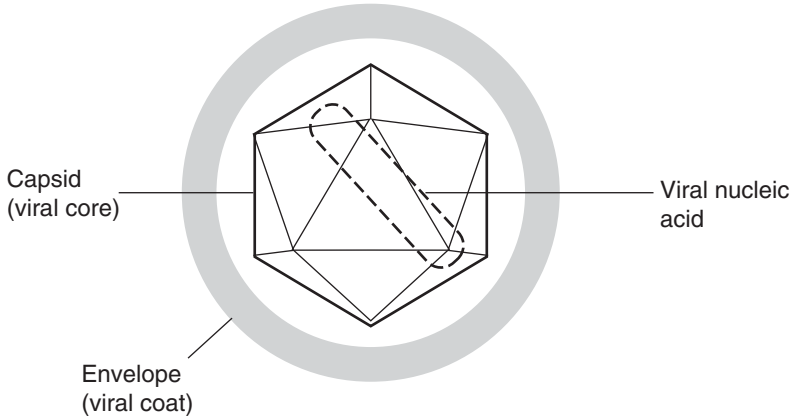


Figure 1: Diagrammatic representation of a virus particle

virion: A virus particle.

latency: The property of infectious agents, generally viruses, to remain hidden in a previously-infected individual and in an inactive state for an extended period of time – often the lifetime of the individual. Although the individual may be immune to the agent, specific antibody can be detected and reactivation of the agent may occur at any time.

nucleus: Part of the cell that contains the cell's DNA. The nucleus functions as the control centre of the cell.

cells. Proteins present in the viral coat and the viral core are recognized by the immune response of the organism.

Some examples of common viruses are:

- hepatitis A virus
- hepatitis B virus
- human immunodeficiency virus (HIV)
- measles virus
- hepatitis C virus
- *Varicella zoster* (chickenpox) virus.

Some viruses have the property of **latency**. This is the ability of a virus to join its own nucleic acid with the nucleic acid of the host cell without taking complete control of the cell as a virus would normally do. Latency usually occurs after an active infection when the individual has recovered and immunity is building up. The viral nucleic acid exists in an inactive form that does not seem to harm the host cell. When the host cell divides, the cell nucleic acid is copied, together with the viral nucleic acid. In this way, the viral nucleic acid becomes part of the cell nucleic acid and is copied every time the cell divides.

Latency is usually indefinite and without any harmful effects on the host cell. However, at any time, the latent nucleic acid could become active and take over the cell functions, resulting in an active infection.

Bacteria

Bacteria are individual distinct cells which possess cell walls, but which have a very simple structure and lack a true **nucleus** (see Figure 2).

Many bacteria are coated in a capsule formed from simple sugars bound together in long complex chains. These capsules are often important in the immune response against the bacterium because they carry the antigens against which the response is mainly directed.

Examples of common bacteria and bacterial infections are:

- *Treponema pallidum*: syphilis
- *Vibrio cholerae*: cholera
- *Clostridium tetanii*: tetanus.

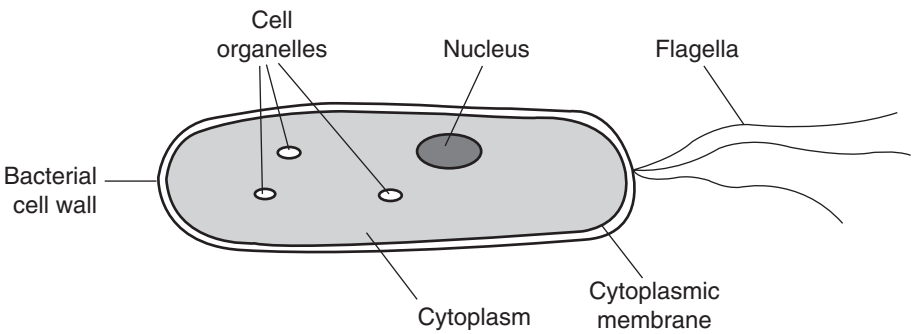


Figure 2: Diagrammatic representation of a bacterium

toxin: Any poisonous compound, usually produced by living organisms.

unicellular: Consisting of a single cell.

eukaryote: An organism in which the genetic material of the cell is contained within a distinct nucleus.

organelle: A permanent structure within a cell with characteristic morphology which is specialized to perform a specific function in the cell activities.

Most bacteria are not of medical importance. Few bacteria can actually survive or grow in animals or humans. Often the bacteria are already present on the external surfaces of the body without causing any adverse effects. Damage to the tissues can subsequently lead to infection. Following infection, the disease process is often caused by the production and release of **toxins** by the bacteria.

Protozoa

Protozoa are **unicellular** organisms. They are classified as **eukaryotes**. They have a well-defined cellular structure with a clear nucleus and other **organelles** (see Figure 3). Proteins present in the cell membrane are recognized by the immune response against the organism.

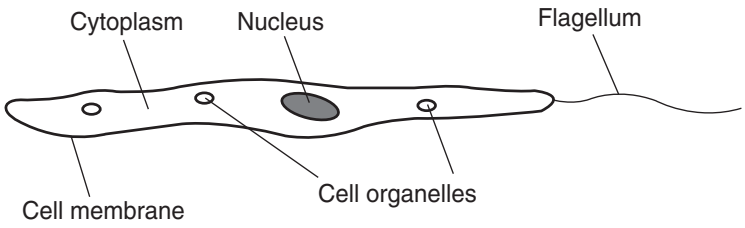


Figure 3: Diagrammatic representation of a protozoan

cytoplasmic: Referring to the cytoplasm, the material surrounding the nucleus of a cell.

spore: A minute reproductive cell of fungi and some plants. A protective state which some bacteria are able to assume in adverse conditions.

Examples of common protozoal infections are:

- *Plasmodium* species: malaria
- *Trypanosoma* species: sleeping sickness and Chagas disease.

They have various mechanisms that allow free movement. They exist in a wide variety of shapes and sizes, ranging from 5 μm to 2 mm in diameter. Some may even change shape as they move.

Protozoa are mainly aquatic, living in soil, ponds, rivers and lakes, although some live only as parasites in animals. They can cause disease in humans, especially in tropical areas where conditions are best suited to their survival. The infection of humans with protozoa can occur in a number of ways. Often an intermediary is involved, usually through injection by an insect vector. However, a number of other protozoa are transmitted by ingestion of contaminated food or water, or by direct contact through the skin.

Fungi

Fungi are organisms that usually grow in the form of branched filaments which are called mycelia. These forms, known as moulds, often produce both sexual and asexual **spores**. Fungi that do not form mycelia, but remain as single cells and reproduce by budding, are known as yeasts (see Figure 4 on page 14).

Most fungi break down organic materials and wastes. Only a few of the many different species of fungi cause disease in humans. Common examples of fungi and fungal infections are:

- *Tinea* species: ringworm infections of the skin
- *Candida* species: candidiasis
- *Histoplasma* species: histoplasmosis.

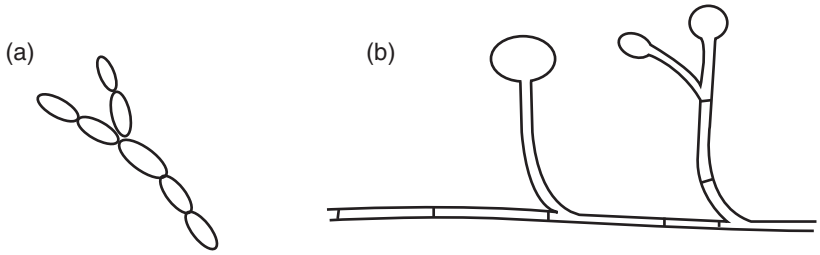


Figure 4: Diagrammatic representation of fungi

ACTIVITY 4

What types of infectious agent are prevalent in your particular locality? List as many as possible, including examples from each of the following four groups:

- *viruses*
- *bacteria*
- *protozoa*
- *fungi.*

Identify the normal routes of transmission of each of these agents.

Indicate the agents you think are likely to be transmitted by blood transfusion.

Keep this list as you will need it again for Activity 5.

2.2 TRANSMISSION OF INFECTIOUS AGENTS BY BLOOD TRANSFUSION

transfusion-transmissible infection: An infection that is capable of being transmitted by blood transfusion.

transfusion-transmitted infection: An infection that has been transmitted by blood transfusion.

In order to be transmitted by blood transfusion, an infectious agent must be present, in some form, in the donated blood. Each blood transfusion service or blood bank laboratory should therefore screen for evidence of infection by the specific infectious agents that are present in the population from which blood donors are drawn.

There are three basic conditions that will determine whether an infectious agent is likely to be transmitted by transfusion.

1 The agent must be capable of using the bloodstream as a means of entry into its host, the patient.

2 The infected donor must be essentially free of any noticeable signs and symptoms of disease – otherwise they would have been identified during donor screening and the donor would have been excluded or deferred.

3 The agent must exist naturally for a period of time, either free in the plasma or present in a cellular component in the bloodstream of an infected donor.

Any infectious agent meeting all these conditions can be transmitted by blood transfusion. However, whether transmission actually occurs or not

depends on a number of other factors, particularly on the immune status of the patient and the amount of infectious agent transfused.

Although blood transfusion can be an efficient route of transmission of an infectious agent, it is important to remember that, under normal circumstances, it is not the *primary* route of infection of any infectious agent. This is because, no matter how infectious any individual agent may be, most people do not have a blood transfusion during their lifetime. Any agent that depends solely on transfusion for transmission will therefore not persist in the population.

It is known that the transmission of certain infectious agents through blood transfusion can, and does, occur, however, and that it can be an important route of infection. The key point to remember here is that transmission can be avoided in most cases by the design and use of suitable selection and screening programmes.

ACTIVITY 5

Look back at your answers to Activity 4 where you identified the infectious agents that you think could be transmitted by transfusion.

From the information that you now have, would you make any changes to your list of agents that can be transmitted by transfusion? If so, amend your list and check it with your supervisor or another senior member of staff.

2.3 THE BASIC IMMUNOLOGY OF INFECTION

Human immunology is concerned with the study of how the body responds to foreign proteins and the mechanisms by which the body protects itself from attack by infectious agents.

The main function of the immune system is to protect our bodies from attack by the many infectious agents that we all meet during our lives. It consists of two parts:

- a detection system that recognizes foreign proteins in the body
- a number of different mechanisms that prevent the growth of the infectious agent in the body.

antigen: Any substance recognized as foreign by the body and 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 substance. It plays a role in the defence against pathogens, often by neutralization or by identifying the pathogen as foreign and to be eliminated by the immune system.

Importantly, the immune response is specific.

Unfortunately, however, these systems are not perfect and they may fail or take some time to respond. When this happens, it can sometimes result in infection by an infectious agent and can also lead to damage to the body which is caused by components of the immune system itself.

Foreign substances that enter the body and induce the immune response are called **antigens** (Ag). One possible response to these antigens is the production of a protein called an **antibody** (Ab). Another possible

lymphoid cell: A cell of the lymphatic system.

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

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

response is the activation of cells that kill infected cells by direct contact or by activating other cell-killing mechanisms, or by both means.

Antibody response

Antibodies are molecules that are made up of proteins and carbohydrates. They are produced by **lymphoid cells** in response to stimulation by antigen. They are specific to a particular antigen and they bind to the antigen to enable the organism to eliminate it. They belong to a group of human serum proteins known as **gammaglobulins**, and are known as **immunoglobulins**.

The basic unit structure of an antibody molecule consists of two types of peptide chains. One type has an approximate molecular weight of 50 000 and is termed the "heavy chain". The other type has an approximate molecular weight of 25 000 and is termed the "light chain". Each antibody molecule consists of two heavy chains in a "Y" shape and two light chains attached to the arms of the "Y", as shown in Figure 5, although different immunoglobulins may consist of multiples of the basic unit. Chemical bonds hold the molecule together.

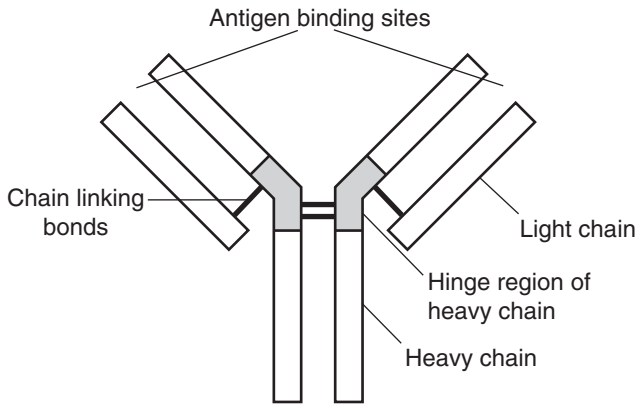


Figure 5: Diagrammatic representation of an IgG antibody molecule

There are five types of immunoglobulin (Ig): IgG, IgM, IgA, IgD and IgE. These immunoglobulins differ in the composition and structure of the heavy chains.

IgG accounts for approximately 73% of the total human immunoglobulin. It is the major antibody produced in the immune response and usually persists for long periods after the initial antibody response. IgG comprises a single basic unit (see Figure 5).

IgM accounts for about 8% of the total human immunoglobulin and is the first antibody produced by the immune response, although it persists for only a short time until the IgG level increases. IgM comprises five basic units linked via the ends of the heavy chains (see Figure 6 on page 17).

IgA accounts for about 18% of the total human immunoglobulin. It is the major immunoglobulin in secretions and at mucous membranes where it protects the external body surfaces. IgA comprises two basic units linked via the ends of the heavy chains and a specific protein called the "J" chain (see Figure 7 on page 17).

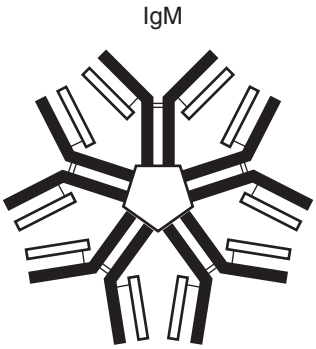


Figure 6: Diagrammatic representation of an IgM antibody molecule

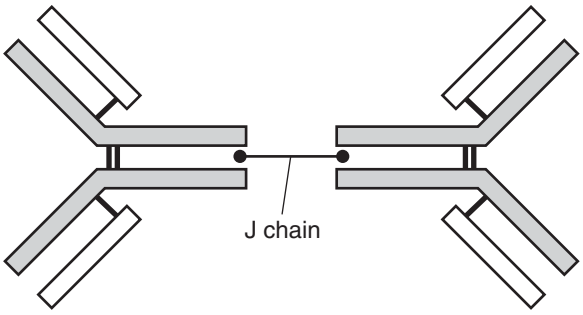


Figure 7: Diagrammatic representation of an IgA antibody molecule

lymphocyte: A type of circulating mononuclear white blood cell. It plays a role in both the cellular and humoral immune responses.

mast cell: A cell found in the loose connective tissue running alongside blood vessels that produces a number of bioactive substances, e.g. histamine, heparin.

basophil: A type of white blood cell that contains many cytoplasmic granules which contain bioactive substances.

bioactive: Biologically active.

histamine: A substance found in many cell types, especially mast cells and basophils, that is released when vessels are injured.

hypersensitivity: Overreaction to an allergen that results in pathological changes in tissues.

IgD and IgE together account for only about 1% of the total human immunoglobulin and are very specialized. IgD is found on the surface of **lymphocytes** where it acts as a surface receptor. IgE is associated with allergic reactions and is found on the surface of certain types of cells known as **mast cells** and **basophils** where it can trigger the release of certain **bioactive** substances such as **histamine**. In this module, we shall concentrate on IgG and IgM.

Cellular response

The cellular immune response is a separate, but not alternative, part of the immune system that responds to the same antigens that induce an antibody response. The cellular response basically involves two groups of lymphocytes:

- T cells
- large granular lymphocytes (LGL).

The T cells include:

- T helper cells (Th)
- T cytotoxic cells (Tc).

These can be recognized by specific proteins on their surface: CD4 on T helper lymphocytes and CD8 on T cytotoxic lymphocytes.

There are two sub-sets of T helper lymphocytes:

- Th1 which are involved in cellular immune responses (delayed **hypersensitivity**)
- Th2 which collaborate in antibody production by B lymphocytes.

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

phagocytosis: The process by which cells ingest solid matter, especially cell debris and pathogens.

T cytotoxic cells (Tc) are the effectors in the destruction of cells infected with intracellular micro-organisms, such as virus.

Large granular lymphocytes comprise 5–10% of the lymphocyte population. They include natural killer cells (NK cells) which are capable of lysing a variety of tumour and virus-infected cells without overt antigenic stimulation. In response to high levels of Interleukin 2, NK cells differentiate into lymphokine-activated killer cells (LAK cells) which kill target cells in a relatively indiscriminate way. There is a further subset called killer cells (K cells) which lyse infected cells but only recognize those cells coated with IgG. Both of these cell types act in a similar manner to Tc cells.

One cell type, **macrophages**, plays a central role in the immune response, both in the antibody response and the cellular response. Macrophages **phagocytose** infectious material and infected cells. They destroy the infectious agent and also present it to other cells of the immune system, both T cells for the cellular response and B cells for the antibody response. The results of the cellular response are not used for blood screening purposes.

2.4 INTRODUCTION TO SCREENING FOR INFECTIOUS AGENTS

The extent and range of the screening performed on the blood supply vary greatly from country to country. Sometimes this is simply because of differences in the countries' needs, but it is sometimes due to financial constraints. As a result, the effectiveness of screening programmes also varies.

Whatever level of service is provided, however, the main purpose of screening blood is *to ensure that the available blood supply is as free as possible from infectious agents by detecting any that may be present before the blood is issued for transfusion*.

Blood transfusion is an ideal route for the transmission of certain infectious agents from the donor to the recipient of the blood. The risk can, however, be reduced in the following ways.

1. The careful selection of donors to ensure that, wherever possible, blood is not collected from people who are likely to be carriers of infectious agents. Module 1: *Safe Blood Donation* shows that building a panel of regular, voluntary non-remunerated donors is the first step towards ensuring a safe and adequate supply of blood. In countries where much of the blood is collected from family or family replacement donors or from commercial or professional donors, the risk of transfusion-transmitted infection is higher.

2. The direct screening of the donated blood for evidence of the presence of infectious agents.

3. The removal of specific components of blood thought to harbour infectious agents: for example, by the filtration of blood to remove white blood cells.

4 The physical inactivation of any contaminating agents that may be present: for example, by heat treatment of Factor VIII concentrates (used in the treatment of haemophilia A) during production.

Not all infectious agents can be detected directly in donated blood. Blood is most often screened for evidence of previous infection by looking for the presence of specific antibody raised against the infectious agent.

As we have already mentioned, some organisms possess the property of latency. In such cases, while antibody has been produced and acute infection has resolved, the organism remains in a **dormant** state within host cells. Although dormant, the agent is capable of reactivating and producing an acute infection at any time. The transfusion of blood cells containing latent organism could, therefore, lead to the transmission of infection.

dormant: An inactive period in the life-cycle of organisms in which growth slows or ceases.

markers of infection: The detectable signs of infection, including the body's own response to the infectious agent, appearing in the bloodstream during, or following, infection.

immunity: The state of being resistant to infection by an infectious agent due to previous exposure to the agent, with resultant production of a protecting immune response.

Markers of infection

Markers of infection are the detectable signs of infection appearing in the bloodstream during, or following, infection. These can be detected by the presence of the agent itself but, more commonly, by the presence of specific antibodies against the infectious agent.

Clearly, it is only by understanding which markers of infection are produced by an infectious agent that screening for the correct marker can be introduced.

ACTIVITY 6

Think about the following situation. A donor is infected with an infectious agent that can be transmitted by blood transfusion. The donor has an acute infection, but with no symptoms, that lasts for one month. After this period, ***immunity*** *to the agent develops and the agent is destroyed and cleared from the body. The natural immunity lasts for at least one year.*

What markers of infection are most likely to be found in donations taken at the following periods after the donor was first infected?

- *2 weeks*
- *4 weeks*
- *6 weeks*
- *8 weeks*
- *18 months.*

If this infectious agent was likely to be transmitted only during the period of acute infection, would it be better to screen donors for the agent itself or for antibody produced against the agent?

Write down your answers and then check them with the answers given in the Activity Checklists and Answers on page 139.

You should recognize that it would be better to screen donors for the agent itself, rather than for antibody. In this case, the presence of antibody only indicates immunity – not an infectious state.

In the case of viral hepatitis B, screening for the protein known as surface antigen (HBsAg) (see page 106) is used to identify infected individuals. In the cases of the human immunodeficinecy virus (HIV) and hepatitis C virus (HCV), the presence of the antibody to the virus is used to screen donated blood for infection.

With HBV infection, the virus causes the shedding of large amounts of surface antigen into the bloodstream during acute infection. This can persist for long periods. The detection of this HBsAg is therefore used to identify infected donors. The presence of antibody to HBsAg (HBsAb) indicates immunity following infection and protects against further infections.

With HIV or HCV infection, although there is a period during which only viral antigen can be detected, this period is very short, possibly no more than a few days in some cases, as the antigen is very quickly complexed with circulating antibody as antibody levels rise. Detection of viral antigen is therefore generally not a suitable approach. The presence of specific antibody is the primary approach used to identify HIV or HCV infected donors. The virus is still present in the unit of blood, in the white cells for HIV and free in the plasma for HCV, and the antibody does not generally protect against reinfection. The presence of antibody therefore identifies donors carrying the virus.

SUMMARY

1 There are four main types of infectious agent:
   - viruses
   - bacteria
   - protozoa
   - fungi.

   Only the first three types are known to be transmitted by blood transfusion.

2 Prions are an unusual type of infectious agent whose transmission by blood transfusion has not yet been conclusively demonstrated.

3 Infectious agents can be transmitted by blood transfusion only if they are present in the donated blood. A screening programme should therefore focus on the infectious agents present in the donor population.

4 Blood transfusion can be a significant, but not the primary, route of transmission of infectious agents.

5 The immune response to infectious agents consists of two parts:
   - the antibody response
   - the cellular response.

6 Screening tests must detect the different markers of infection that different infectious agents produce.

SELF-ASSESSMENT

1 What does the term “latency” mean?

2 What are the three conditions required for transmission of infectious agents by blood transfusion?

3 Why do you think that blood transfusion cannot be the primary route of infection of an infectious agent?

4 What are antigens and antibodies?

5 Name the five types of immunoglobulin.

6 What is the main reason for screening blood for the presence of infectious agents?

7 What are the four main ways of minimizing the risk of transmission of infectious agents by blood transfusion?

8 In some cases, the presence of antibody as a result of a previous infection may indicate that the blood is still infectious. Why is this the case?

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 Recognize examples of the four types of infectious agent.

2 Understand the term “latency”, as applied to viral infections, and its significance in blood transfusion practice.

3 Identify infectious agents which can be transmitted by blood transfusion.

4 Use your knowledge of immunology to predict likely markers of infection at different stages following infection.

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 find 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

The Human Immunodeficiency Viruses

The purpose of this section is to help you to understand HIV and the consequences of infection. You will study various aspects of the human immunodeficiency viruses, including:

- history
- physical characteristics
- epidemiology
- clinical infection
- prevention of transmission
- the role of HIV as the causative agent of AIDS.

LEARNING OBJECTIVES

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

1 Identify the structural features of HIV.

2 Describe the basic stages of HIV infection and the entry of the virus into susceptible cells.

3 Describe the most common clinical course of HIV infection and its progression to AIDS in your country.

4 Describe the measures that are being taken to reduce the transmission of HIV infection in your country.

morphology: The study of the shape or form of organisms.

cross-reactivity: When an antibody recognizes not only its corresponding specific antigen, but also other antigens that may have certain similarities.

envelope (viral): An external protein coat that surrounds the viral capsid. Not all viruses are enveloped.

RNA (ribonucleic acid): A complex chemical found in the cytoplasm and concerned with protein synthesis. In some viruses, it is the hereditary material.

DNA (deoxyribonucleic acid): The genetic material of most living organisms that determines hereditary characteristics by the control of protein synthesis.

reverse transcriptase: A naturally-occurring enzyme which translates RNA into DNA.

retrovirus: A virus family that is characterized by RNA as the nucleic acid, a unique morphology, the presence of a unique enzyme (reverse transcriptase) and latency.

3.1 THE BACKGROUND TO HIV INFECTION

HIV is the primary cause of acquired immunodeficiency syndrome (AIDS). Although the way in which HIV infection causes AIDS is not fully understood, it is clear that it damages part of the immune system. This can lead to serious infections by agents that would normally be easily overcome by the immune system.

HIV was first isolated from the cells of an infected patient in 1983 (HIV-1). The virus was subsequently identified as the causative agent of AIDS. In 1986 a second type of HIV, HIV-2, was identified in certain areas of West Africa. HIV-2 appears to cause the same diseases as HIV-1, but may be less pathogenic. It is **morphologically** similar to HIV-1. The two types can be distinguished by the presence or absence of an antibody that is specific to a protein found only on HIV-2. Although **cross-reactivity** occurs between the core protein of both viruses, the **envelope** proteins are different.

Cross-reactivity

Cross-reactivity is the situation that occurs when an antibody recognizes not only its own antigen, but also other unrelated antigens that have certain similarities. In the case of HIV-1 and HIV-2, this means that an individual infected with HIV-1 would produce antibodies that recognize both core and envelope proteins of HIV-1 and core proteins of HIV-2. Similarly, an individual infected with HIV-2 would produce antibodies that recognize both core and envelope proteins of HIV-2 and core proteins of HIV-1. However, the cross-reactivity commonly seen in anti-HIV and other TTI screening assays is usually between specific antibody and unrelated human proteins that are non-specifically bound during the sample incubation phase of the assay.

3.2 THE STRUCTURE OF HIV

There are two types of nucleic acid:

- ribonucleic acid (**RNA**)
- deoxyribonucleic acid (**DNA**).

DNA is usually double-stranded. It is the genetic material passed to daughter cells when a cell divides. It is DNA that is responsible for the transmission of hereditary characteristics from parents to children.

The nucleic acid in HIV is RNA. There is no DNA present. Instead, the virus uses the machinery of the human cells that it enters to convert its RNA to DNA so that the virus can replicate or integrate itself in the cell's DNA.

The viral RNA is condensed in a cylindrical core together with two closely-associated structural proteins and an important enzyme called RNA-dependent DNA polymerase. This is more commonly known as **reverse transcriptase**. This enzyme is found in all **retroviruses** as it is needed to copy the viral RNA into DNA.

The way that viral and other such proteins are described is based on their molecular weight (measured in daltons) and on whether they are proteins or glycoproteins. The two proteins associated with the RNA of HIV are

glycoprotein: A protein molecule with a sugar molecule attached. Glycoproteins are common constituents of cell membranes.

capsid: The inner protein core of a virus particle which contains the nucleic acid. It is made up of identical protein subunits.

7000 daltons (7 kDa) and 9000 daltons (9 kDa). These are abbreviated to p7 and p9 respectively. The reverse transcriptase enzyme is a 66 kDa protein, which is abbreviated to p66. **Glycoproteins** are similarly abbreviated to "gp". We shall now use this notation throughout this module.

The core is totally enclosed in a cone-shaped shell of p24 protein. This is called the major core protein and appears to be the same in both HIV-1 and HIV-2. The whole unit is called the viral **capsid**.

The capsid is itself covered by two layers. The first of these is a shell of p17 matrix protein to which proteins that project from the surface of the virus particle are attached. This is covered by a lipid bilayer. Projecting through the lipid are many transmembrane proteins. These proteins, gp41, are attached to the p17 matrix and themselves attach the gp120 envelope proteins. These appear as small projections on the surface of the virus particle. It is the structure of these small projections and their attaching proteins that appears to be the major difference between HIV-1 and HIV-2. The corresponding HIV-2 proteins are gp110/130 and gp36 respectively. Antibodies to these two specific sets of proteins do not cross-react.

The entire virus particle is called the virion. This is the infectious particle that is secreted and transmitted between individuals. The complete virion is 100–120 μm in diameter.

Look at Figures 8 and 9. Figure 8 shows an electron microscope image of HIV-1, grown in lymphocyte cell culture. The virus particles with their

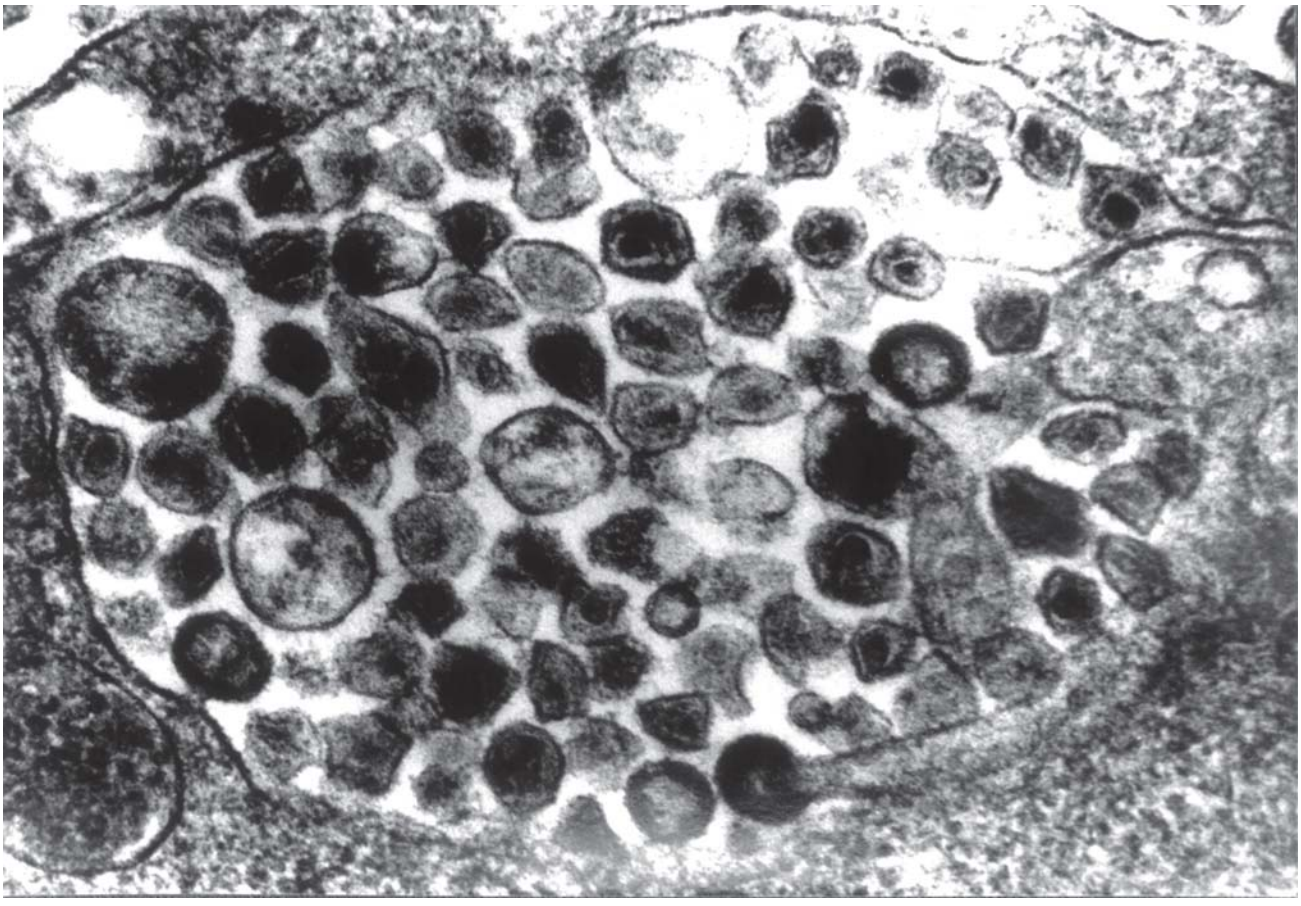


Figure 8: Electron micrograph of HIV infection of CD4 lymphocytes

cone-shaped core can be clearly seen although, in this example, the cells were producing so much virus that there are many defective forms with no nucleic acid. Figure 9 is a detailed diagram of the virus, showing the main structural features.

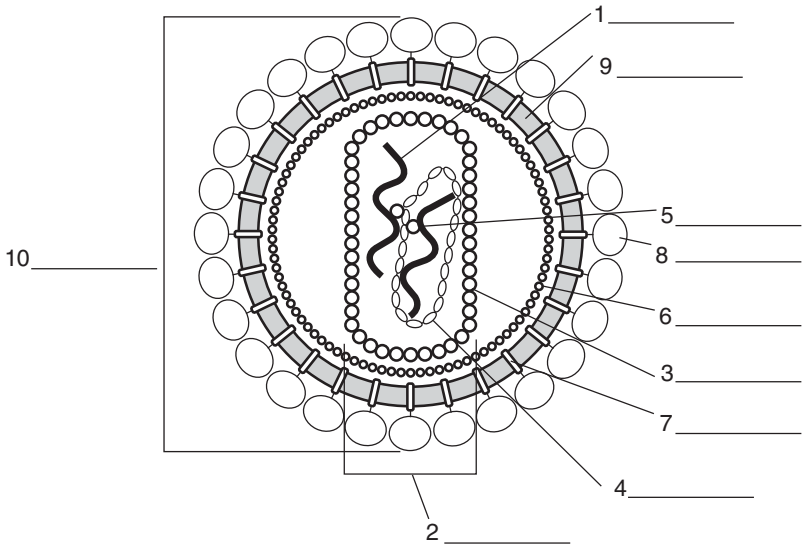


Figure 9: The structure of HIV

ACTIVITY 7

You will see that, in Figure 9, the structural features labelled 1–10 are not named. From what you have just read, try to identify them and fill in the appropriate spaces. Check your answers with those given in the Activity Checklists and Answers on pages 139–140.

3.3 ENTRY OF HIV INTO CELLS

HIV enters susceptible cells by binding to a receptor – a protein called CD^4 – on the cell surface. CD^4 is found on the surface of a number of different cells within the immune system:

- the T cells that help to stimulate the immune response
- the T helper cells (Th cells)
- the macrophages that engulf virus particles in many parts of the body.

Following fusion of the virion to the cell membrane, the uncovered capsid passes into the cytoplasm of the cell. Within the cytoplasm, the RNA is copied to double-stranded DNA by the reverse transcriptase enzyme present in the capsid using raw materials from within the cytoplasm. The DNA then passes into the nucleus of the cell and **integrates** into the cellular DNA. Once in the cell, the DNA remains latent.

integration: The joining of foreign nucleic acid into the genome of an organism.

The final stage of infection occurs when the virus starts to replicate. Large quantities of infectious virus (virions) are produced. As the virus emerges (buds) from the cell, it is packaged in the cell membrane to produce the completed virus particles. These virions are then released and can infect other cells. Figure 10 on page 26 shows the entry of HIV into a susceptible cell. Note that the labels on Figure 10 have not yet been completed.

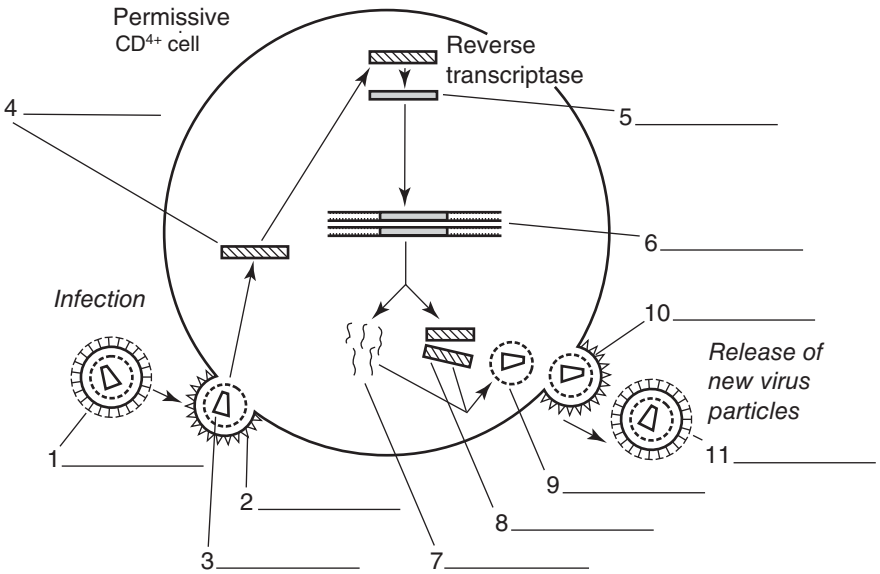


Figure 10: Diagram of HIV entry into a susceptible cell

genome: The complete genetic structure of an organism.

opportunistic infection: Uncontrolled infection by a normally present, but controllable, infectious agent.

To summarize, HIV is a retrovirus and integrates into the host cell **genome**. The virus consists of a number of structural proteins, but p24, gp41 and gp120/160 are generally considered to be the most immunogenic and are therefore the most important in virus serology. HIV primarily infects and destroys cells of the immune system, mainly infecting CD^{4+} cells.

ACTIVITY 8

Look at Figure 10. From reading the text, complete the diagram by naming each stage that occurs when HIV infects a cell. Check your answers with those given in the Activity Checklists and Answers on page 140.

3.4 THE CLINICAL PRESENTATION OF HIV INFECTION AND AIDS

Initially it was thought that infection with HIV led only to AIDS. It then became clear that HIV infection could lead to a number of different conditions of varying severity, although it usually (and finally) results in AIDS.

In individuals suffering from AIDS, the main cause of illness is the occurrence of secondary infectious diseases, the **opportunistic infections**. These opportunistic infections are uncontrolled infections of normally present, but controllable, infectious agents. Common infections include:

- pneumonia caused by *Pneumocystis carinii*
- tuberculosis caused by *Mycobacterium tuberculosis* or *Mycobacterium avium/intracellularis*
- chronic cryptosporidiosis caused by *Cryptosporidium parvum*
- toxoplasmosis caused by *Toxoplasma gondii*
- viral infections, such as cytomegalovirus.

Secondary cancers such as Kaposi's sarcoma and non-Hodgkins lymphoma are other conditions commonly found in AIDS patients. These cancers are usually aggressive and do not respond very well to standard chemotherapy. Kaposi's sarcoma, as originally described, was a benign malignancy found in elderly men that had no adverse affect on the individual. However, the Kaposi's sarcoma found in AIDS patients is a fast-growing, and usually fatal, malignancy now known to be associated with the more recently identified human herpes virus 8 (HHV8). Most HIV-infected individuals with Kaposi sarcoma are also infected with HHV8. In many parts of the world, patients with developing ARC (AIDS-related complex) or AIDS often present simply with severe diarrhoea. The presence of opportunistic infections or secondary cancers is only then determined following clinical and laboratory investigation.

ACTIVITY 9

Try to find out the following information about HIV infection and AIDS in your country:

- *the **prevalence** of HIV infection and the number of AIDS cases*
- *the most common clinical course of HIV infection and its progression to AIDS*
- *the average time taken for AIDS to manifest following the initial infection*
- *the main opportunistic infections found in AIDS patients, apart from* Pneumocystis carinii*. Are these opportunistic infections found in all areas of your country or are there local variations?*
- *the incidence of Kaposi's sarcoma in your region or country about 25 years ago and today. Compare the two figures and relate them to the current prevalence of AIDS in your population.*

prevalence: The proportion of a specific population that is infected with the infectious agent at any particular time.

You may need to consult your Ministry of Health, a larger public health laboratory or a specialized AIDS counsellor in order to obtain this information.

This information should give you an approximate picture of the situation in your country. It is important to find out this information so that you can understand the extent of the problem.

3.5 LABORATORY TESTING FOR HIV INFECTION IN BLOOD DONATIONS

Before HIV was identified, AIDS was diagnosed by its clinical appearance. Any laboratory tests that were used were surrogate tests; they measured the results of HIV infection rather than specifically detecting either viral antigen or specific antibody against the virus.

surrogate testing: Testing for an indicator of infection which is thought to indicate the presence of an infectious agent, but which is not a specific marker of infection by that agent.

seroconversion: A change in serostatus of an individual from seronegative to seropositive.

serostatus: The serological findings in an individual following infection.

"**window period**": The period between infection and the first appearance of circulating detectable marker of that infection.

Surrogate testing is testing for a specific marker which is not a part of the agent under investigation, but which is thought to indicate the presence of the infectious agent. For example, there is now a specific assay to detect antibody to the hepatitis C virus (anti-HCV) the major cause of the previously termed non-A, non-B hepatitis (NANB). However, prior to the development of this assay, some countries used testing for anti-HBc (hepatitis B core antibody) and measuring levels of ALT (alanine aminotransferase, a measure of liver damage) to try to identify those donors who may have been more likely to transmit NANB hepatitis.

The production of specific tests for HIV has helped us to understand both HIV infection and AIDS. The finding of anti-HIV in patients with previously unexplained immunodeficiency is diagnostic of AIDS.

The confirmed presence of anti-HIV in an asymptomatic individual (an individual without symptoms) means that the individual has been exposed to the virus. It is accepted that, in almost all cases, the virus will still be present in the individual. **Seroconversion** (a change in **serostatus** from negative to positive) in sequential samples means that the infection is recent. The continued absence of antibody, lack of seroconversion following possible exposure to the virus, means that it is highly unlikely that the individual has been infected.

Following infection and before the appearance of circulating markers of infection, there is a "**window period**" of varying length, during which the infection becomes established. The window period is an important issue in blood screening as it is the period during which screening tests will be unable to detect a potentially infectious donation. Following an initial period during which no markers can be detected, viral RNA is the first marker to appear. Following this, viral antigen (p24) appears and finally antibodies appear. Figure 11 shows the relative time intervals between the point of infection and the appearance of the different markers in HIV infection. The time intervals are approximate and represent average times.

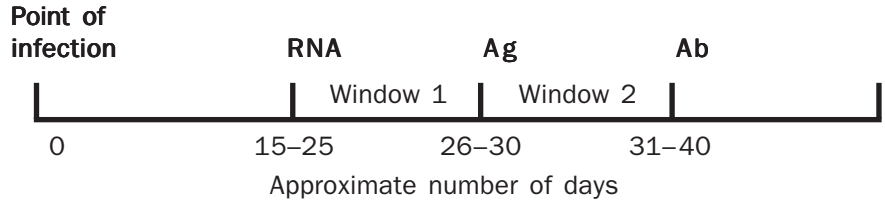


Figure 11: Appearance of circulating markers of HIV infection

Figure 12 shows the laboratory events following infection by HIV in more detail. Viral RNA, the first marker of infection to appear, reflects the replication of virus and its release into the bloodstream. The detection of viral RNA is still a relatively complex procedure and significant investment and infrastructure is required to be able to perform it reliably and consistently and with the sensitivity required. In addition, RNA levels fluctuate and testing cannot replace conventional virus serology.

After the appearance of RNA, viral antigen (p24) appears around the same time as viral DNA. The DNA is generally not found free in the plasma, but is cell-associated. The viral antigen levels rise rapidly but then peak and start to fall off again as antibody is produced and complexes with it; production does not cease but free antibody falls to

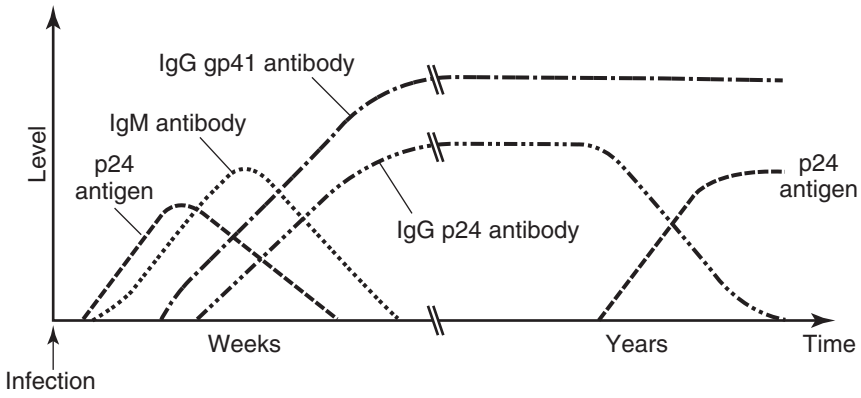


Figure 12: Serological profile following HIV infection

below detectable levels. Infected donations can be identified earlier with the detection of viral antigen than antibody, but the significance of this varies depending on the prevalence and incidence of HIV in the donor population. Again, antigen testing cannot replace antibody screening. Until recently, HIV antigen tests were separate assays and their sensitivity was variable. Some countries with a particularly high incidence of HIV have found the introduction of antigen testing to be beneficial, although this has not been the case in all countries.

Soon after the appearance of antigen, antibody emerges, with a corresponding decrease in free antigen. The levels of antibodies peak and remain constant throughout the stages of persistent asymptomatic infection and persistent generalized lymphodenopathy (PGL). Although p24 antigen production never ceases, free circulating p24 antigen is usually not detectable at this time as it is usually completely bound in circulating antigen–antibody complexes.

Later, as ARC develops, the level of anti-p24 falls and detectable p24 antigen reappears. This is the prelude to the development of full-blown AIDS.

3.6 EPIDEMIOLOGY OF HIV INFECTION

epidemiology: The study of the occurrence, distribution and spread of infection and disease in the population.

The first population groups identified as having HIV infection and AIDS were homosexual men in the USA and, shortly after, injecting drug users. HIV infection and AIDS were subsequently found in heterosexual and bisexual individuals who had sexual contact with individuals in the first two groups. At the same time, infection was found in certain ethnic groups and individuals from Haiti and Central Africa.

The next group found to be infected with HIV and to be developing AIDS were patients with haemophilia and other transfused patients. Finally, infants born to infected mothers were found to be infected and also to be developing AIDS.

While the number of HIV-infected individuals and AIDS sufferers has increased dramatically, the risk factors for HIV infection have remained the same. However, the proportions, or risks, vary from country to country.

In sub-Saharan Africa, the main route of transmission is heterosexual; HIV prevalence is similar in males and females. Mother-to-child transmission and, to a lesser extent, ritual scarification contribute to the HIV epidemic in highly affected countries.

Blood transfusion is a potentially significant route of transmission in all countries, although the risk may be reduced by the selection of donors before donation and the screening of the donated blood.

An important factor to be taken into account when considering the prevalence of both HIV infection and AIDS is that, for many reasons, there is still considerable under-reporting in many parts of the world.

ACTIVITY 10

Make a list of any reasons for possible under-reporting of HIV infection and AIDS in your country. Consider the following reasons:

- *economic: your country may be unable to afford to test*
- *political: your country may be unwilling to test because any action subsequently required may overload its health care system*
- *practical: it may not be possible to set up a suitable sample collection programme that is sufficiently representative of the population*
- *technical: the expertise may not exist in your country for wide-scale testing to be performed with any reliability*
- *cultural: individuals may be unwilling to be tested.*

Note that all these reasons are interrelated.

3.7 TRANSMISSION OF HIV INFECTION

The modes of transmission of HIV infection are now well established. While virus can be isolated from many body secretions, infection is transmitted in only a limited number of ways.

There are three principal modes of transmission of HIV infection.

1. Unprotected penetrative sexual contact with an infected person, either between men or between men and women.
2. Inoculation of infected blood, either by blood transfusion or as the result of the use of contaminated needles, syringes or knives used, for example, in injecting drug use, ritual scarification or tattooing.
3. From an infected mother to her child, in the uterus, during birth or by breastfeeding.

ACTIVITY 11

Which of these three routes is the main route of transmission in your country and why?

The transmission of HIV by sexual contact is an extremely important route. In Africa, the effects of heterosexual transmission can be clearly seen and are increasing as women who become infected pass on their infection to any children they have. Thus, infection of the population increases from two sides:

- in adults (horizontal transmission)
- in infants (vertical transmission).

In this way, HIV can spread very quickly in a population.

As you will remember from Section 2, blood transfusion can be a significant route of infection. The efficiency of the transmission of HIV through blood transfusion is estimated to be virtually 100%. WHO reports that the viral dose in HIV transmission through blood is so large that one HIV-positive transfusion leads to death, on the average, after two years in children and after three to five years in adults. Nevertheless, the extent to which blood transfusion is an actual route of transmission depends on the prevalence of infected individuals in the population and on the effectiveness of the screening programme used. In a population with a low prevalence of infected individuals and a good screening programme, transmission by blood transfusion should be extremely rare; transfusion would therefore not be a significant route of transmission. In a population with a high prevalence of infected individuals and a poor or nonexistent screening programme, transmission by blood transfusion is likely to be relatively common and would be an important route of infection in the population.

The following activity should help you to understand the potential significance of transfusion-transmitted HIV infection.

ACTIVITY 12

A recently-married, previously healthy, HIV-negative young man has an accident and is taken to hospital. There he receives a transfusion of two units of whole blood and recovers normally. HIV testing is not performed on donated blood at the hospital and one of the units was anti-HIV positive. What are the likely consequences of this situation, both short-term and long-term?

Check your answer with that given in the Activity Checklists and Answers on pages 141–142.

This activity highlights the importance of testing as it is clear that blood transfusion can spread HIV infection very widely if blood is not systematically screened.

While we are looking at the routes by which infection is transmitted, we should clarify that there are a number of potential infection routes that are *not* implicated in the transmission of HIV or AIDS.

1 There is no evidence that nonsexual, social or domestic contact leads to the transmission of infection. Indeed, the virus itself is not very stable and can easily be destroyed by environmental conditions.

2 There is no evidence that infection is spread by any insect vector in Africa or any other part of the world. Importantly, there is no evidence that HIV replicates in any arthropods; in fact, the evidence appears to be against any such transmission.

   If insect transmission did occur, a higher incidence of HIV infection in otherwise low-risk individuals should be seen in areas with a high incidence of insect-borne infection, such as malaria. This is not the case.

3 There is no evidence that health care workers have a greater risk of infection than any other individual with no other specific risk factors. If the correct safety procedures for handling pathological material are applied at all times, and good laboratory practice is followed, any risks are minimized.

3.8 PREVENTING THE SPREAD OF HIV INFECTION

The transmission of infectious diseases has been a problem for mankind for hundreds, if not thousands, of years; epidemics of different kinds have swept across the world at various times in history. In order to find effective ways of preventing the further spread of an infection, it is crucial to identify the routes of transmission.

The prevention of the spread of HIV infection essentially relies on two approaches that are closely interlinked and that are both related to the provision of a safe blood supply:

- education to enable people to avoid situations in which there is a risk of transmission occurring
- the physical prevention of infection.

Let us now look at some ways in which the transmission of HIV infection can be prevented or reduced.

Sexual transmission

The sexual transmission of HIV infection can be dramatically reduced by the avoidance of unsafe sexual practices, the use of condoms and a reduction in the number of sexual partners. These measures have been shown to be very effective in slowing the spread of infection among homosexual men. Among heterosexual contacts, the simple use of condoms has helped to reduce infection rates.

Transmission by injecting drug use

Transmission among injecting drug users can be reduced by the use of disposable syringes and needles and through schemes where used syringes are exchanged for new ones. Unfortunately, this approach is rarely feasible or practical. Whilst disposable syringes can be made freely available in some countries, in others they cannot even be provided for clinical use because of their cost. Furthermore, as the use of

injectable drugs is illegal in most countries, injecting drug users are generally reluctant to come forward to obtain clean equipment.

Transmission by blood transfusion

Transmission by blood transfusion, or the infusion of blood products, can also be avoided. The first approach to the prevention of transmission by transfusion is the selection of donors who are at low risk for transfusion-transmissible infections. Remember that *a safe donor provides a safer donation*.

Module 1 focuses in detail on safe blood donation and, in particular, on the importance of:

- identifying low-risk donor groups
- avoiding unsuitable blood donors
- recruiting voluntary non-remunerated blood donors
- promoting self-exclusion by individuals at risk through an effective donor education programme
- effective donor screening through:
  - predonation counselling, including an assessment of risk factors and an opportunity for self-exclusion or confidential unit exclusion
  - a brief medical history, including possible signs and symptoms related to transfusion-transmissible infections
  - a basic health check, including a brief examination of the arm for needle marks
- promoting regular, voluntary non-remunerated blood donation.

Self-exclusion is probably the most effective approach in preventing transmission, but is dependent on the education of potential donors about risk behaviour. It is particularly important to encourage self-exclusion by people such as sex workers, homosexual and bisexual men, injecting drug users, those who have any unprotected sexual contact other than with a regular partner, and the sexual contacts of any of these people.

Ultimately, however, screening tests for anti-HIV are needed to enable infected donors to be identified and the donated blood to be discarded. We shall look at this in more detail in Section 4.

ACTIVITY 13

Briefly list the various approaches that have been taken in your country to reduce the spread of HIV infection and its transmission by blood transfusion.

Find out what advice is given in your country about the transmission of HIV by sexual routes. Are condoms widely available? If so, have they been effective or are people generally reluctant to use them?

Paediatric infection

The transmission of HIV from mother to child is a significant route of infection. Transmission may occur by infection of the developing fetus during pregnancy, by infection of the infant at birth through virus present in cervical secretions and via breastfeeding.

The prevention of mother-to-child transmission is a very complex area and it is not appropriate to include it in this module.

SUMMARY

1 HIV is the primary cause of AIDS. HIV damages part of the immune system, which can lead to serious infections by agents that would normally be easily overcome by the immune system.

2 HIV is a retrovirus and integrates into the host cell genome. The virus consists of a number of structural proteins, but p24, gp41 and gp120/160 are in general the most immunogenic and are therefore among the most important in virus serology. HIV primarily infects and destroys cells of the immune system, mainly infecting CD^{4+} positive cells.

3 In individuals suffering from AIDS, the main cause of illness is the occurrence of secondary infectious diseases.

4 The spread of HIV infection has followed different patterns in different countries.

5 There is considerable under-reporting of the prevalence of HIV infection and AIDS in some countries.

6 The transmission of HIV through blood transfusion is more than 90% efficient.

7 The prevention of the spread of HIV infection essentially relies on two approaches:

- public education to reduce risk behaviour
- the physical prevention of infection.

SELF-ASSESSMENT

9 How does HIV enter susceptible cells?

10 What are the two HIV-specific antibodies that have been found to be the best confirmation of HIV infection?

11 How long after infection will antibodies first appear?

12 What are the three main routes of transmission for HIV infection?

13 What are the three other routes of transmission of infection that are not implicated in HIV transmission?

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 Identify the structural features of HIV.

2 Identify the basic stages of HIV infection and the entry of the virus into susceptible cells.

3 Describe the most common clinical course of HIV infection and its progression to AIDS in your country.

4 Explain the measures that are being taken to reduce the transmission of HIV infection in your country.

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

Principles of Screening Assays for Transfusion-Transmissible Infections

The purpose of this section is to examine the possible approaches to screening for transfusion-transmissible infections (TTIs) and to explain the principles behind the different types of assay available.

The section contains a considerable amount of information which you may wish to use as reference material in the future. At this stage, however, we suggest that you read through the text, concentrating on the assays to which you have access but completing all the activities.

LEARNING OBJECTIVES

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

1 Outline the principles of the diagnostic assays most commonly used to detect the specific TTI markers used to identify infected donations.

2 Explain the differences between the main types of screening assay.

4.1 SCREENING BLOOD DONATIONS FOR TTIs

In considering the screening of blood donations and the specific screening tests that are available to detect TTIs, it is important to remember that a safe donor provides a safer donation. As Module 1 emphasizes, the safest donors are regular, voluntary and non-remunerated. Clearly, donors specifically at risk of any blood-borne infection (or any other infectious diseases) should be encouraged not to donate blood.

There are a number of other specific points to remember.

1 There is a risk of transmission of infection if donated blood is not tested before the blood is transfused.

2 The collection of a unit of blood from an infected donor wastes precious resources in terms of consumables and staff time.

3 If many TTI-positive donations are found, the number of repeat tests and confirmatory tests needed increases. This raises the total cost of testing.

An effective donor education and selection programme that promotes self-exclusion by donors at risk of transfusion-transmissible infections therefore makes your job a great deal easier, saves time and money and also results in a safer donor population.

4.2 TYPES OF SCREENING ASSAY

The choice of specific markers to screen for depends on the infectious agent that you are screening for. Screening for specific antibody is usually most appropriate for HIV, HCV and syphilis. Screening for specific antigen (HBsAg) is necessary for HBV. This section is concerned with the principles of the assays most commonly used for the screening of blood donations: for both antibody and antigen.

Three main kinds of primary screening assay are available to detect infectious disease markers in donated blood:

1 Enzyme Linked ImmunoSorbent Assays (ELISA)/Enzyme ImmunoAssay (EIA).

2 Particle agglutination assays.

3 Simple rapid assays.

Note that the abbreviation ELISA is often replaced with the abbreviation EIA. This is a simpler description which we shall use throughout the remainder of the module.

There are a number of features that need to be taken into account when selecting the most appropriate assay to use:

- scientific principle of the assay
- complexity of the assay
- incubation times
- sensitivity
- specificity
- suitability for different situations

- availability
- cost.

ACTIVITY 14

List the assays used in your laboratory when screening donated blood for TTIs. Note whether they are EIA, particle agglutination or simple rapid assays.

All three types of assay are based on the same biological principle and involve the same two basic elements.

1 The presence of specific antigen or antibody in a sample is demonstrated in an immunological reaction involving antigen/antibody (immune) complex formation, with one of the components bound to a solid phase.

2 The formation of the immune complex is subsequently detected by an indicator system.

Test samples

Most of the assays in use today can be used with either serum or plasma. However, there are still some that work with serum only. The instructions with the assay must be followed.

Terminology

Before we consider the assays in detail, it is important to be clear about the use of the following terms in classifying the actual screening results:

- positive/negative
- reactive/non-reactive
- equivocal/indeterminate.

Although the terms "positive" and "negative" are the main terminology used in this module, this is for reasons of simplicity. Many people use these words to report screening results, but it is not really correct to do so. "Positive" and "negative" should really be used only to describe the final status of the donation or the donor. This is really of minor importance in the case of "non-reactive" screening results as long as it is clearly understood that only when all testing is completed and no evidence of infection is found should the overall donation status be described as "negative". In the case of "positive" screening test results, however, the results should be classified as "reactive" until they have been confirmed. The reason for this is that frequently a high percentage of the screen "reactive" results are nonspecific and are finally confirmed as "negative". There are no real fixed rules concerning the use of these words, but you should be aware of the differences in meaning and significance.

equivocal/indeterminate: A result that cannot be classified as clearly positive or negative.

The WHO terminology for a result that cannot be classified as clearly positive or negative (usually around the cut-off value) is **equivocal.**

ACTIVITY 15

List the words that are used in your laboratory to classify screening results. Check the instructions from the assay manufacturers to see what words they use. If you find that different terms are used, you need to decide on the terminology to be used in your laboratory in order to establish uniformity and avoid confusion. Discuss this with your supervisor and colleagues. Note down your recommendations on your Action List.

4.3 ENZYME IMMUNOASSAYS

Although EIA is the most complex of the three types of assay, we shall consider it first since the principles involved are basically the same as in the other two types. Once you understand the principles of EIA, therefore, you should quickly be able to understand the principles of particle agglutination and simple rapid assays.

EIAs exist in many forms and can be used to detect both antigen and antibody. In general, the simplest and most commonly used antibody detection assays are based on the use of immobilized antigen which captures any specific antibody present in the test sample. The most commonly used antigen detection assays are based on the use of immobilized antibody.

There are a number of different presentations of EIA using different solid phases to immobilize the antigen or antibody. Most commonly the solid phases currently in use are:

1 The bottom and sides of the inside of a polystyrene microwell.

2 The surface of polystyrene, or other material, microparticles.

3 The surfaces of specific, dedicated, disposable devices used in automated, self-contained assay systems. These vary according to the manufacturer, but are usually polystyrene and generally work on the same basic principles.

4 Strips of nylon or nitrocellulose membrane. These are specifically used in western blots and line assays.

Four main types of EIA are commonly used in blood screening: three specifically to detect antibody and one that can be used to detect antibody or antigen, depending on the specific design format of the assay.

To help you understand the basic principles of EIAs, we shall consider the four basic types of EIA in the microwell format. However, these types can just as easily be presented on microparticles, dedicated systems or strip formats. Indeed, today, these four types may be found in a wide range of commercial assays using a variety of different solid phases and in

different formats. In addition, apart from assays specifically manufactured to work on a specific, dedicated, automated system (closed system assays), assays may be performed manually or on non-dedicated, automated assay processing systems (open system assays).

1 Antiglobulin EIA: this is the simplest form of EIA, in which any specific antibody present in a test sample is bound to immobilized specific antigen and is detected by enzyme-labelled anti-human antibody.

2 Competitive EIA: this is a slightly more complex assay, in which specific antibody that may be present in a test sample competes with enzyme-labelled specific antibody for binding sites on immobilized specific antigen.

3 Sandwich EIA: this is a highly specific type of EIA which can be used in either antibody or antigen sandwich format. Specific antibody (or antigen) in the test sample is bound to immobilized specific antigen (or antibody) and then detected by enzyme-labelled specific antigen (or antibody).

4 Antibody capture EIA: this is a type of EIA in which any antibody in the test sample, irrespective of specificity, is captured by immobilized anti-human immunoglobulins (IgG) and any captured specific antibody is detected by enzyme-labelled specific antigen.

The descriptions that follow explain the principles of the assays. You may find, however, that they differ from the manufacturers' instructions. You should always follow the instructions supplied with the assays.

Type 1: Antiglobulin EIA (screening for specific antibody: e.g. anti-HIV)

Methodology

1 The basis of the simplest form of antibody immunoassay is an immobilized antigen (see Figure 13) which is bound to the surface of the microwell.

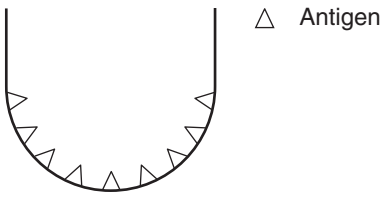


Figure 13

2 This binding can be achieved simply by adding a solution of antigen in bicarbonate buffer to each well and incubating at 4°C for 12–16 hours. The plastic used to produce the microwells, polystyrene, is designed to bind proteins. After incubation, the antigen solution is removed, the wells are washed with distilled water or a specific buffer and are immediately dried. They are then stored at 4°C, often for long periods, without any loss of antigen. These plates are provided by the manufacturer, ready-coated and standardized for use.

3 Sample or diluted sample is added to the wells, or sample is added to wells already containing diluent, and the wells are incubated for the defined period of time and at the correct temperature, according to the manufacturer's instructions. The time can vary from as little as 30 minutes to as long as 2 hours, and the temperatures can range from 18°C–45°C. Positive and negative controls are also added to a number of wells on each plate. Sometimes a low-level positive control is also used. During this time, any specific antibody present in the test sample binds to the viral antigen (see Figure 14).

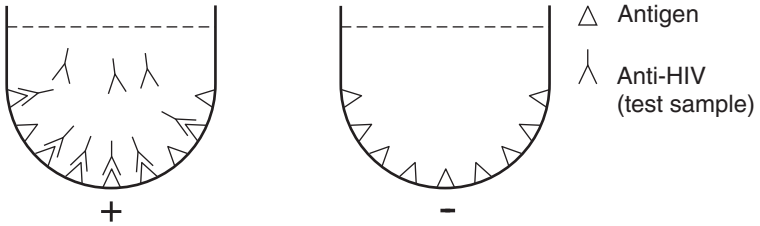


Figure 14

4 At the end of the incubation period, the wells are washed to remove the sample and to prepare them for the next stage of the assay (see Figure 15).

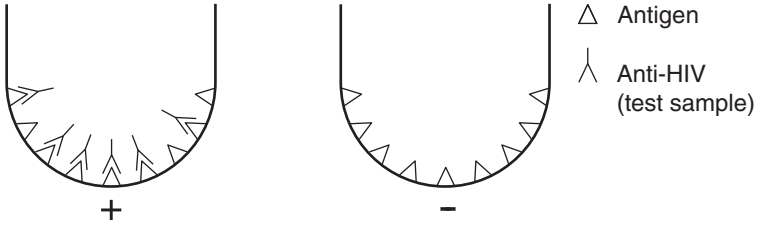


Figure 15

The method of washing and the wash fluid used differ according to the assay. However, mechanical washing using an automated plate washer is the best way to wash the wells. If you have a mechanical washer, consult the manufacturer's instructions before use and make sure that it is maintained properly.

Manual washing is an acceptable method for small numbers of assays, but it is time-consuming when performing larger numbers of assays and can be subject to operator variability. Manual washing can be performed with a hand-held wash head, either connected to an electric vacuum and pressure pump or gravity-fed with wash fluid from a reservoir and the suction achieved using a water pump. Alternatively, manual washing can be performed using a multi-channel pipette to fill and then empty the wells with wash fluid. The wash fluids used include distilled water, saline, buffered saline and phosphate buffer. Some buffers can be very complex solutions which have a specific concentration and pH value.

The wash process removes the excess sample from the well without dislodging and removing any bound, specific antibody. The first wash removes the sample and the well

is then filled with wash fluid and emptied again. This process is repeated a number of times, according to the manufacturer's instructions. Sometimes the wash fluid is left in the wells for up to 1 minute before it is removed and replaced with fresh fluid. After the final wash, the wash fluid is removed and the wells are left empty. It is very important that the wells are as dry as possible before the next stage of the assay. The plate can be turned upside-down and gently tapped dry on some absorbent tissue if the wells are still wet.

5 Conjugate solution is added to all the wells and they are incubated at the defined temperature and for the correct period of time. The conjugate solution contains an anti-human immunoglobulin antibody which has been chemically linked to an enzyme. The antibody is usually anti-human IgG and the enzyme label is usually horseradish peroxidase. Horseradish peroxidase is an oxidizing enzyme which oxidizes the substrate, causing the colourless, inactivated substrate to develop a specific colour on activation. The conjugate binds only to human antibody (IgG) that has bound to the antigen immobilized on the wells (see Figure 16). Conjugate will therefore not be bound in those wells that did not initially contain sample with specific antibody present.

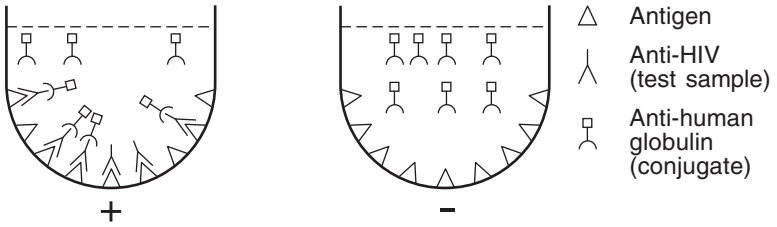


Figure 16

6 At the end of the incubation period, the wells are washed to remove the excess, unbound conjugate and are prepared for the next stage of the assay (see Figure 17). The washing is performed in the same way as described in point 4 above.

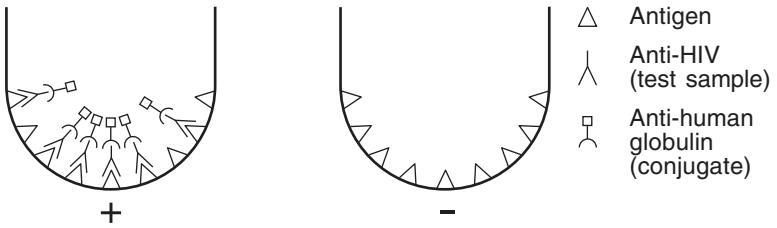


Figure 17

chromogen: A synthetic soluble compound that changes colour following oxidation, reduction or other chemical modification by an enzyme.

7 Substrate solution is immediately added to all the wells and they are incubated at the defined temperature, usually 18°C–25°C, and for the correct period of time.

The substrate solution contains a chemical called a **chromogen**. Chromogens are synthetic soluble compounds

that change colour following oxidation, reduction or other chemical modification by the enzyme label. Sometimes the activated chromogen becomes insoluble and a coloured precipitate is formed. EIAs performed in microwells can work only if the substrate remains soluble at all times. In this case, the chromogen is colourless in the inactive state, but forms a coloured soluble product when activated. Western blots and line assays use a chromogen that becomes insoluble and precipitates on activation.

When the substrate solution is added to those wells containing bound conjugate, the enzyme that is present activates the substrate and so causes the formation of colour in the well. Wells not containing any bound conjugate do not change the colour of the substrate added to the well (see Figure 18). Thus, reactive wells (those initially containing sample with specific antibody present) are coloured, while non-reactive wells (those containing sample from uninfected individuals) are colourless.

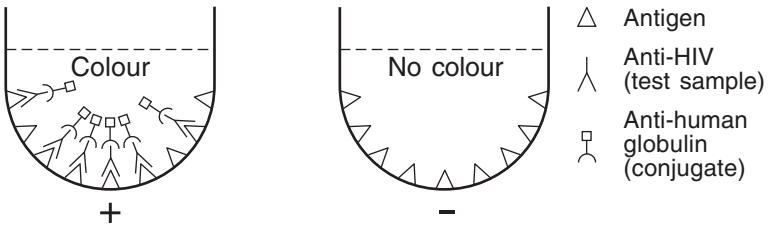


Figure 18

8 At the end of the incubation period, dilute acid solution is added to all the wells to stop the reaction. The acid inactivates the enzyme and fixes the colour. With some substrates, the acid also intensifies the colour produced.

9 The optical densities (OD values) of the solutions in the microwells are read and the results of the assay are determined.

Type 2: Competitive EIA (screening for specific antibody: e.g. anti-HIV)

The basic principles of the competitive EIA are the same as the antiglobulin type EIA: specific antibody binds to immobilized antigen and the presence of the antibody is then detected.

The assay differs in the way in which the specific antibody is detected. Antigen is bound to the surface of the microwell and test sample is added. At the same time, the conjugate is added and the sample and conjugate are incubated together. However, the conjugate is an enzyme-labelled antibody of the same specificity rather than a labelled, nonspecific anti-human-antibody. The conjugated specific antibody competes with any natural specific antibody for the antigen binding sites. The concentration of the conjugated antibody is set at a level so that the smallest amount of natural specific antibody present in the test sample is in sufficient excess to ensure that it binds to the antigen in preference to the conjugated antibody.

The basic assay stages are outlined below. Various aspects of general EIA technology that have already been discussed are not repeated here.

Methodology

1 Antigen is bound to the surface of the microwell, as described above (see Figure 13 on page 40).

2 Test sample is added to the wells. Competitive assays use undiluted serum.

3 Conjugate is added to all the wells. The conjugate consists of enzyme-labelled antibody of the same specificity as the infectious agent being tested for and the antigen on the wells. The enzyme is usually horseradish peroxidase.

The wells are incubated for the defined period of time and at the correct temperature. During this time, any specific natural antibody present in the test serum competes with the conjugated antibody for binding sites on the antigen (see Figure 19).

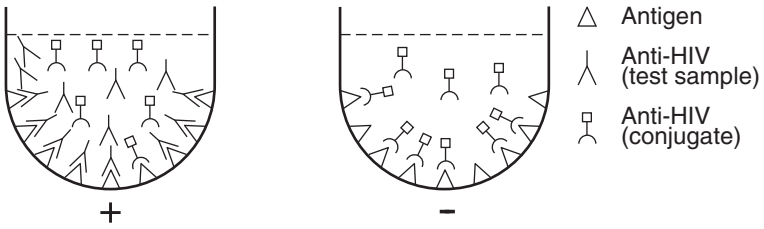


Figure 19

4 At the end of the incubation period, the wells are washed to remove the sample and conjugate (see Figure 20).

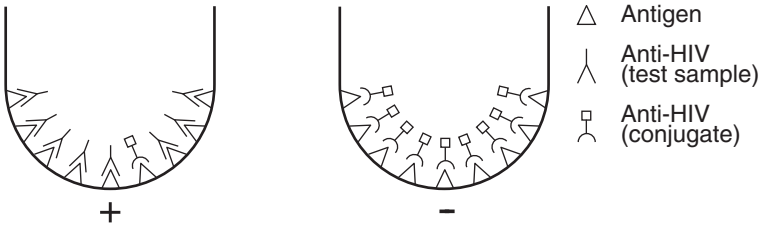


Figure 20

5 Substrate solution is immediately added to all the wells (see Figure 21) and they are incubated at the defined temperature, usually 18°C–25°C, and for the correct period of time.

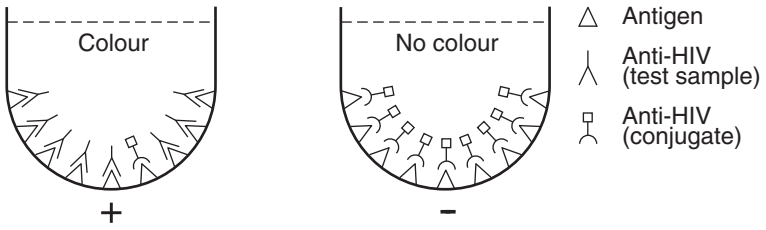


Figure 21

6 At the end of the incubation period, dilute acid solution is added to all the wells to stop the reaction.

7 The OD values of the solutions in the microwells are read and the results of the assay are determined.

In the absence of natural specific antibody in the test sample, conjugated antibody is bound and the enzyme present activates the substrate to produce colour. In those wells where natural specific antibody is present in the test sample, its presence blocks the binding of the conjugated antibody and there is little or no enzyme present. There is therefore little or no substrate activation and no colour is produced.

Type 3: Sandwich EIA (screening for antibody: e.g. anti-HIV or antigen: e.g. HBsAg)

Most modern anti-HIV and HBsAg assays from the major international diagnostic manufacturers are of this type.

a) Antibody detection (anti-HIV)

The basic principle of the antibody sandwich EIA is again the same as the antiglobulin EIA: specific antibody binds to immobilized antigen and the presence of the antibody is then detected.

The assay differs in the way in which the specific antibody is detected. Antigen (usually recombinant proteins and/or synthetic peptides) is bound to the surface of the microwell. Test sample is added to the microwell and incubated. At the end of the incubation period, the sample is washed off and conjugate is added and incubated. Rather than being enzyme-labelled anti-human IgG (as in the antiglobulin EIA), the conjugate is enzyme-labelled antigen (recombinant and/or peptide). During the incubation period, the conjugated antigen binds any specific antibody bound to the antigen immobilized on the microwell. A "sandwich" is built up of antigen – antibody – antigen. The excess conjugate is washed away and chromogen is added in the same way as in the antiglobulin assay. The main advantage of this type of assay is its specificity; the number of false-positive reactions is reduced.

The basic assay stages are outlined below. Various aspects of general EIA technology that have already been discussed are not repeated here.

Methodology

1 Antigen is bound to the surface of the microwell, as described above (see Figure 13 on page 40).

2 Test sample or diluted sample is added to the wells. The wells are incubated for the defined time and at the correct temperature. During this time any specific antibody present binds to the antigen (see Figure 22).

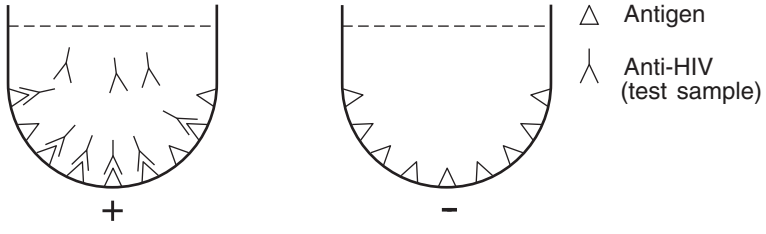


Figure 22

3 At the end of the incubation period, the wells are washed to remove the sample.

4 Conjugate is added to the wells. The wells are incubated for the defined time and at the correct temperature.

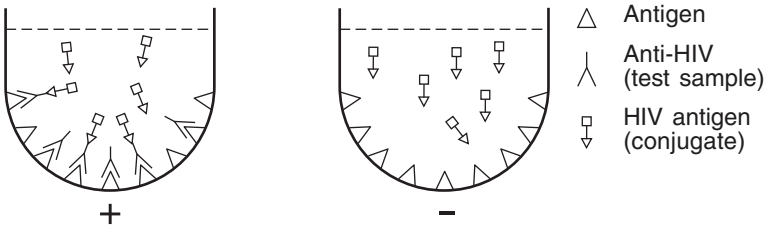


Figure 23

During this time, the conjugate binds to any specific antibody bound to the wells (see Figure 23).

5 At the end of the incubation period, the wells are washed to remove the unbound conjugate.

6 Substrate solution is immediately added to all the wells (see Figure 24) and they are incubated at the defined temperature, usually 18–25°C, and for the correct period of time.

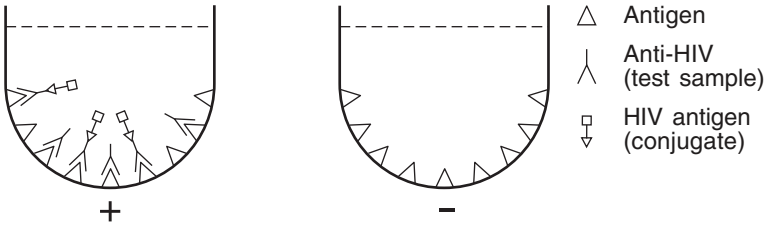


Figure 24

7 At the end of the incubation period, dilute acid solution is added to all the wells to stop the reaction.

8 The OD values of the solutions in the microwells are read and the results of the assay are determined.

In those wells where specific antibody is present in the test sample, conjugate is bound to the captured antibody and activates the substrate to produce colour.

b) Antigen detection (HBsAg)

The basic principle of the antigen sandwich EIA is the same as the antibody sandwich EIA, but the antigen and antibody roles are reversed: specific antigen binds to immobilized antibody and the presence of the antigen is then detected.

Antibody (monoclonal, polyclonal or a mixture of both) is bound to the surface of the microwell. Test sample is added to the microwell and incubated. At the end of the incubation period, the sample is washed off and conjugate is added and incubated. Rather than being enzyme-labelled antigen (as in the antibody sandwich EIA), the conjugate is enzyme-labelled specific antibody (monoclonal, polyclonal or a mixture of both). During the incubation period, the conjugated antibody binds any

specific antigen bound to the antibody immobilized on the microwell. A "sandwich" is built up of antibody – antigen – antibody. The excess conjugate is washed away and chromogen is added in the same way as in the antibody sandwich assay.

The basic assay stages are outlined below. Various aspects of general EIA technology that have already been discussed are not repeated here.

Methodology

1 Antibody is bound to the surface of the microwell in a similar manner to antigen (see Figure 25).

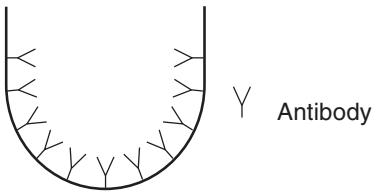


Figure 25

2 Test sample or diluted sample is added to the wells. The wells are incubated for the defined time and at the correct temperature. During this time any specific antigen present binds to the antibody (see Figure 26).

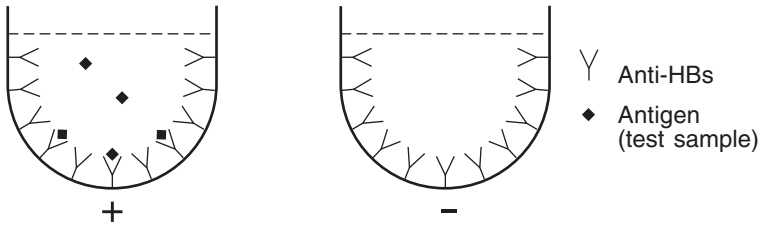


Figure 26

3 At the end of the incubation period, the wells are washed to remove the sample.

4 Conjugate is added to the wells. The wells are incubated for the defined time and at the correct temperature. During this time, the conjugate binds to any specific antigen bound to the wells (see Figure 27).

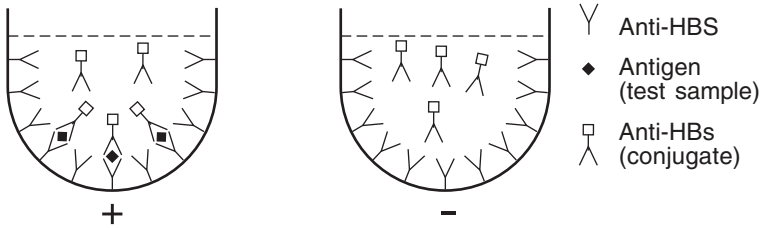


Figure 27

5 At the end of the incubation period, the wells are washed to remove the unbound conjugate.

6 Substrate solution is immediately added to all the wells (see Figure 28 on page 48) and they are incubated at the defined temperature, usually 18–25°C, and for the correct period of time.

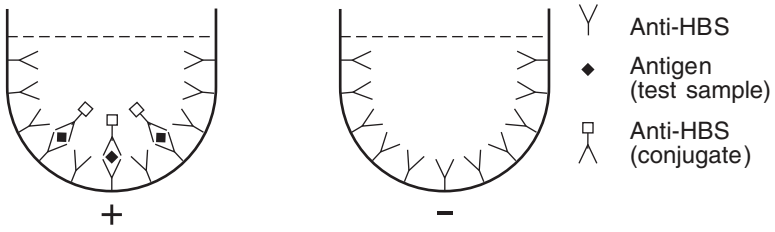


Figure 28

7 At the end of the incubation period, dilute acid solution is added to all the wells to stop the reaction.

8 The OD values of the solutions in the microwells are read and the results of the assay are determined.

In those wells where specific antigen is present in the test sample, conjugate is bound to the captured antigen and activates the substrate to produce colour.

Type 4: Antibody capture EIA (screening for antibody: e.g. anti-HIV)

The basic principle of the antibody capture EIA is quite different from the other three types; any antibodies in a test sample, both specific and nonspecific, are captured by immobilized anti-human globulins and the presence of the specific antibody of interest is then detected.

The assay differs in the way in which the antibody is both captured and detected. Anti-human globulins (anti-IgG, -IgM, -IgA) are bound to the solid phase, either separately or in combination depending on the assay. Test sample is added and incubated and any antibody in the sample, specific and nonspecific, is captured by the anti-human globulins. At the end of the incubation period, the test sample is washed off and conjugate is added and incubated.

Like the sandwich EIA, the conjugate is enzyme-labelled synthetic antigen. During the incubation period, the conjugated antigen binds to any captured specific antibody. The excess conjugate is washed away and chromogen is added in the same way as in the antiglobulin assay. The main advantage of this type of assay is its flexibility. The antibody capture stage captures all specific antibodies in the sample and these can be detected by using different specific conjugated antigens. Combination assays (assays detecting markers of more than one specific agent in a single test) can be developed using this type of EIA.

Methodology

1 Anti-human globulin is bound to the surface of the microwell in a similar way to specific antibody, as described above (see Figure 25 on page 47).

2 Test sample or diluted sample, is added to the wells. The wells are incubated for the defined time and at the correct temperature. During this time a proportion of all of the antibodies in the test sample are captured by the anti-human globulin (see Figure 29 on page 49).

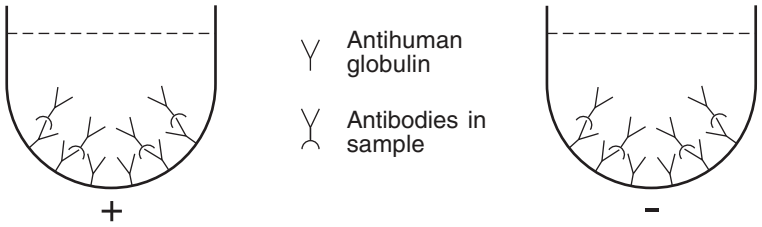


Figure 29

3 At the end of the incubation period, the wells are washed to remove the sample.

4 Conjugate is added to the wells. The wells are incubated for the defined time and at the correct temperature. During this time, the conjugate binds to any specific antibodies captured by the anti-human globulin (see Figure 30).

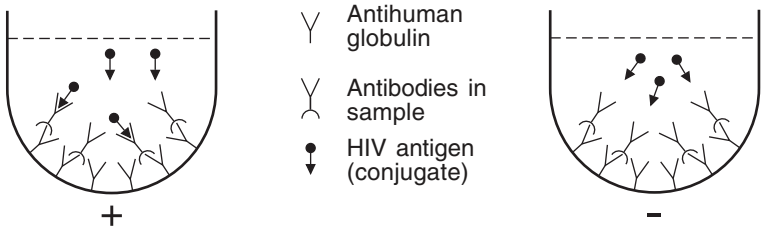


Figure 30

5 At the end of the incubation period, the wells are washed to remove the excess conjugate.

6 Substrate solution is added immediately to the wells (see Figure 31) and they are incubated for the defined time and at the correct temperature.

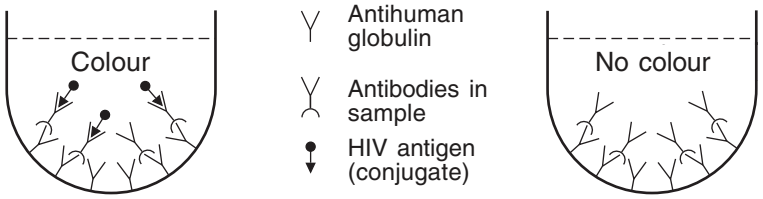


Figure 31

7 At the end of the incubation period dilute acid is added to the wells to stop the colour reaction and fix any colour already formed.

8 The OD values of the solutions in the microwells are read and analysed, and the results of the assay determined. In those wells where specific antibody is present in the test sample, conjugate is bound to the antibody which activates the substrate to produce colour.

Microparticle assays

Microparticle based assays differ in principle from microwell assays only in that the solid phase is the whole of the surface of a gelatin or latex microparticle, approximately 1 mm in diameter, in suspension in suitable buffer. Each assay utilizes a set volume of the suspension, which

contains millions of these particles. This provides a large overall surface on which the assay reactions take place. Most microparticle assays are designed for dedicated systems where the microparticles are contained in small wells, which simply act as "test-tubes", in which the reaction takes place. The assay principles and methodology are broadly the same as microwell EIAs. The Abbott PRISM and AxSYM are examples of systems that use microparticles.

Dedicated device/system assays

In addition to the PRISM and AxSYM dedicated assay systems mentioned above, there are other systems that use basic EIA principles, but in different ways as part of dedicated systems. The bioMerieux VIDAS system is an example of such a system. The solid phase is the inside surface of a polystyrene tip and the sample and other solutions are sucked inside the tip and incubated there. All the reagents used in this system are provided in a sealed strip of wells, similar to microwells, which is loaded onto the machine at the start of the assay procedure.

Chemiluminescent assays

Although the substrate used in most microwell EIAs is a chromogen that turns from a colourless to a coloured solution in the presence of the specific enzyme catalyst, many microparticle assays and dedicated assay systems now use a chemiluminescent substrate. Chemiluminescence is the chemical generation of light at a certain wavelength and is an enzyme-catalysed reaction that works in broadly the same way as a chromogenic substrate. In this case, however, the conjugated enzyme catalyses the generation of small quantities of light rather than a coloured solution. This type of assay may be more sensitive in some cases as the reaction does not need to be stopped at a certain time, the measurement of light generated can be continuous and the readers can detect very low levels of emitted light. However, chemiluminescent assays need more specialized and expensive equipment (microplate format luminometers) to detect the light generated and the overall benefits of such assays are not significant in most situations. While a number of commercial dedicated assay systems use this technology with success, most of these systems are not ideal nor designed for blood screening.

Blots/line assays

Western blots and line assays are yet other presentations of the same basic EIA principles, but in a different format. Blots and line assays are primarily confirmatory assays which are used as part of appropriate confirmatory algorithms to confirm reactivity: for example, in samples found reactive in screening assays. Most blots or line assays are for the detection of antibody and the specific antigens are immobilized on a strip of nitrocellulose or nylon membrane. The assays are performed in small individual troughs which contain the strip and into which the sample/reagents/wash fluid are added at the appropriate time. A major difference between blots and other EIAs is that the chromogen used is not soluble when activated by the enzyme conjugate. On activation, the chromogen forms a coloured precipitate at each position along the strip where the conjugate is bound.

Western blots

In western blots, the antigen is presented as a range of discreet specific bands of proteins that are bound at different positions along the strip according to weight. Western blots usually use native antigen prepared directly from the specific infectious agent. The different antigen components are separated out in order of their molecular weight by gel electrophoresis and are then transferred (blotted) onto the membrane.

Line assays

In line assays, the antigen is presented as a selected range of discreet, specific bands of proteins that are bound at different positions along the strip. In this case, however, the antigens used are specifically selected purified antigens, usually recombinants and/or peptides, which are of most value in the detection of antibodies to the particular agent and which are applied directly onto the strip in specific predetermined positions.

4.4 COMBINATION ANTIGEN/ANTIBODY ASSAYS

Recently a number of HIV antigen/antibody combination EIAs have been developed and are available in microwell format; they detect both HIV antigen and anti-HIV simultaneously in one test. These assays are an important step forward in HIV screening of blood donors as they offer a way in which the sensitivity of screening can be increased significantly at very little extra cost, with no additional equipment or staff requirements and no significant changes to screening algorithms.

The combination assays currently available are all sandwich EIAs, but they utilize differing methodologies in their performance. The assays utilize a combination of monoclonal antibodies to p24 and/or p26 antigen and recombinant/peptide HIV antigens on the solid phase (see Figure 32). One or two conjugates may be used, depending on the assay.

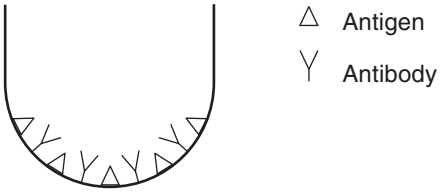


Figure 32

In an example of a one-conjugate assay, the conjugate consists of similar antigens/antibodies as on the solid phase, but all conjugated with peroxidase. During or after sample incubation, depending on the individual design of the assay, the conjugate is added and binds in a "sandwich" fashion to any immobilized specific antibody or antigen (Figure 33 on page 52). Addition of substrate will produce colour if either p24 antigen or anti-HIV, or both are present in a sample.

In an example of a two-conjugate assay, one conjugate, biotin-labelled anti-p24, is added immediately prior to the addition of sample. In this case, any p24 antigen present in the sample will bind to both the immobilized and free (conjugated) anti-p24 (Figure 34 on page 52).

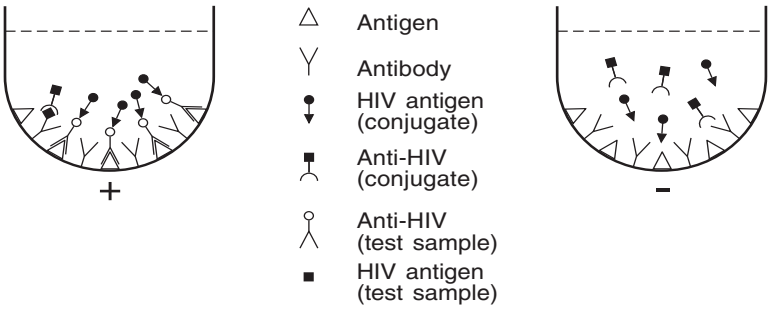


Figure 33

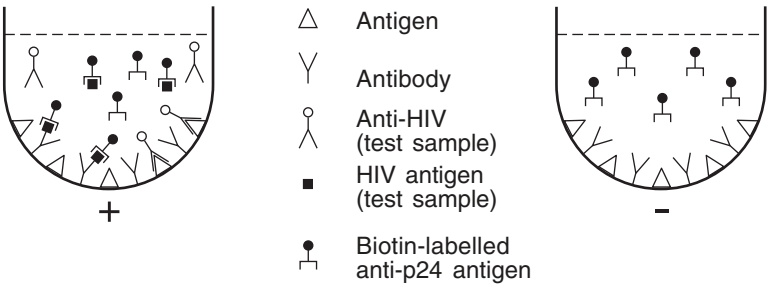


Figure 34

The second conjugate is added after the sample incubation and consists of peroxidase-labelled specific antigen which binds to any bound antibody present and peroxidase-labelled avidin which binds to any bound biotin labelled anti-p24 present (Figure 35). The addition of substrate will produce colour if either p24 antigen or anti-HIV, or both are present in a sample.

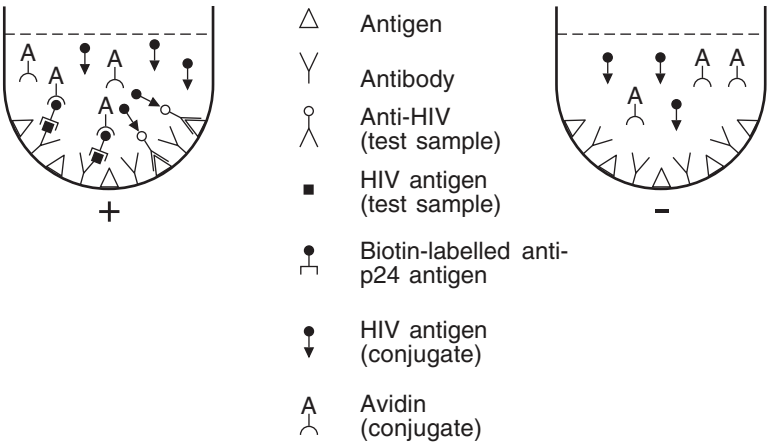


Figure 35

4.5 DETERMINING THE RESULTS OF EIAs

Optical densities and cut-off values

The optical densities (OD values) of the individual wells are a measurement of the amount of colour produced by bound conjugate as a result of any specific binding of antigen or antibody. They are determined by passing a beam of light of a certain wavelength up through the bottom of the wells and measuring the amount of light that is absorbed by the solution. This is performed on a specialized piece of equipment called a plate reader, a multi-channel spectrophotometer, usually referred to as an EIA (plate)

reader. Plate readers are designed to read the OD values of microplates by sequentially reading the OD values of the individual wells. The results are either directly printed out by the plate reader or sent to a microcomputer for analysis and presentation in a predefined format.

The end result of an EIA is a set of numbers – the OD values – which then have to be converted to positive and negative results. Figure 36 shows results obtained from an anti-HIV assay performed on samples from British blood donors. (You will be asked to complete Columns B and C of Figure 36 in Activities 16 and 17.)

Sample ID	A (OD value)	B (result)	C (signal/cut-off ratio)
1	0.141		
2	0.158		
3	0.903		
4	0.161		
5	0.148		
6	1.321		
7	1.201		
8	1.098		
9	0.139		
10	0.173		
11	0.169		
12	0.145		
Neg C	0.156		
Neg C	0.167		
Neg C	0.157		
Pos C	1.352		
Pos C	1.283		

A – The actual OD values of samples and controls
B – The corresponding positive and negative results (not yet determined)
C – The signal/cut-off ratio: sample OD/cut-off OD (not yet determined)

Figure 36: Example of results from an antiglobulin/sandwich type EIA

In order to make use of the OD values, they must be compared with known standard results. These are provided by the controls set up with the test samples. There are many different ways of calculating the results but, as a minimum, three calculations need to be performed for any EIA. Two of them involve finding the mean (average) OD values of the positive and negative controls which, in order to be valid, must be within the values for the assay run set by the manufacturer. Finally, to determine whether a result is reactive or non-reactive, a value known as the "cut-off value" must be calculated.

In Type 1 EIA (antiglobulin), Type 3 EIA (sandwich) and Type 4 EIA (antibody capture), a high OD value, colour present, is a reactive result; a low OD value, no colour, is a non-reactive result. Therefore, results above the cut-off are reactive results and values below the cut-off are non-reactive results.

In Type 2 EIA (competitive), a high OD value, colour produced, is a non-reactive result; a low OD value, little or no colour, is a reactive result. Therefore, values below the cut-off are reactive results and values above the cut-off are non-reactive results.

Selecting the right cut-off value is very important when developing an EIA. Although there are differences between manufacturers, assay formats and assay specificities, the calculation of the cut-off value of an assay is often based on the negative control OD values. The mean value of the individual negative controls – the negative control mean (Ncm) – is calculated and this is entered into a simple formula to calculate the actual cut-off value for the assay plate. The following formula is typical:

Cut-off value = Ncm + 0.2

In our example in Figure 36 on page 53, the Ncm = 0.16. The cut-off value is therefore 0.36 (0.16 + 0.2). Any OD values above 0.36 are considered to be reactive results and any below 0.36 are considered to be non-reactive results.

Alternatively, specific cut-off controls may be included in an assay, formulae based on positive and negative control OD values or even fixed cut-off values provided by the manufacturer may be used. Whatever method is used to determine the cut-off value, however, the principles applied are the same.

ACTIVITY 16

Look back at the OD values in Figure 36. Using the cut-off value that we have just calculated (0.36), complete Column B in Figure 36 with the final assay results.

A further simple calculation can be made which converts the individual OD values to a standard ratio. This is used to make a direct comparison of a number of different plates of samples tested using the same assay, or the results of testing the same set of samples using different assays. This standard ratio is called the signal/cut-off ratio and is calculated by dividing the individual sample OD value by the calculated cut-off value. A value below 1 indicates a non-reactive result and a value above 1 indicates a reactive result. In the case of competitive EIAs, the ratio is calculated as the cut-off/signal ratio (cut-off value divided by the sample OD value).

ACTIVITY 17

Look back at the OD values in Figure 36. Using the cut-off value that we have just calculated, fill in Column C in Figure 36 with the signal/cut-off ratios.

Figure 37 on page 55 shows the results from Figure 36 plotted on a simple graph. The cut-off point is marked, and the distribution of negative (non-reactive) and positive (reactive) results can be seen.

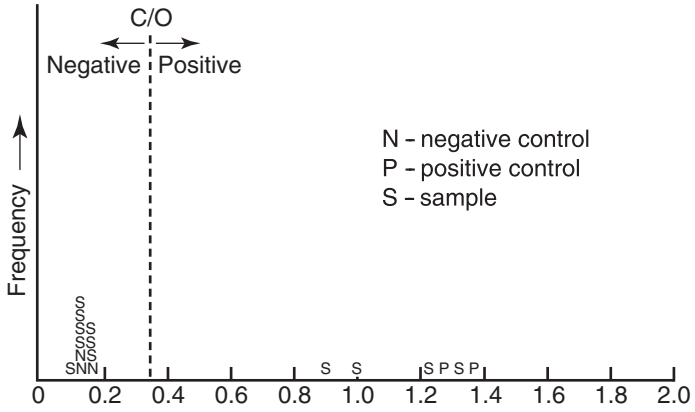


Figure 37

4.6 PARTICLE AGGLUTINATION ASSAYS

Particle agglutination assays detect the presence of specific antibody or antigen in a test sample through the agglutination of particles coated with the complementary specific antigen or antibody respectively.

Originally, the particles used were exclusively red cells – usually sheep, chicken or turkey; these are all nucleated and settle out of solution quickly. The assays were termed "haemagglutination assays". Today, however, many agglutination assays, particularly antibody assays, now use gelatin or latex particles rather than red cells. This has the advantage of reducing non-specific reactivity against cross-reacting red cell antigens.

Whatever the particle used, however, the basic principles are the same for both haemagglutination and particle agglutination assays. The assay is generally performed in microplates, but the microwells are not coated with antigen or antibody; they simply act as miniaturized "test-tubes" in which to perform the test. One major advantage of this type of assay is that no expensive equipment is needed. These assays do not have a lot of different stages, do not need wash equipment and can be read visually.

Methodology

a) Antibody detection

1 The basis of the test is the immobilization of specific antigen on the particle, the equivalent of the microwell previously described for EIAs. The source and the type of antigen is essentially the same as for the EIA (see Figure 38).

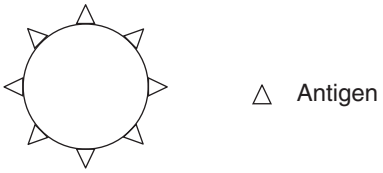


Figure 38

b) Antigen detection

2 The basis of the test is the reverse of the antibody detection format: immobilization of specific antibody on the particle. The source and the type of antibody is essentially the same as for the EIA (see Figure 39).

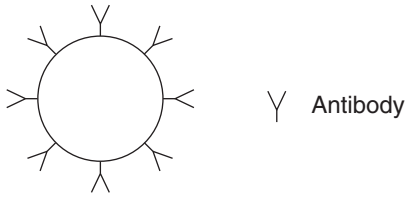


Figure 39

3 Antibody and antigen particle agglutination tests are essentially performed in the same manner. Test samples and controls are diluted with sample diluent. All particle agglutination assays require some predilution of the samples to minimize false positive reactions. The diluent can be very complex in some assays and is always provided as part of the assay kit.

The dilution is normally performed in microwells. The appropriate volume of the final correctly-diluted sample is transferred to a new, unused microplate to perform the actual assay.

4 The particles are added to the diluted sample and are incubated, usually at room temperature (18–25°C), for the set incubation period; this varies considerably, but is generally between 0.5 and 2.5 hours. During the incubation period, the particles are agglutinated, in a similar way to red cells and blood group antibodies, by any antibody or antigen present in the sample (see Figure 40).

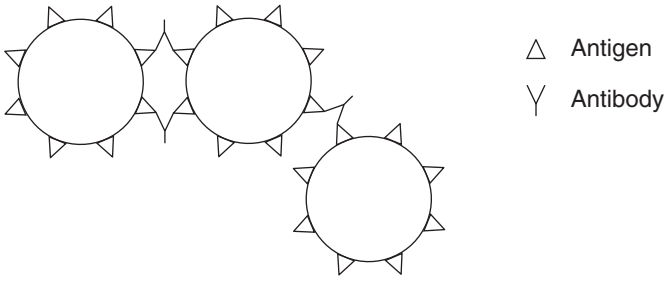


Figure 40

In some assays, the samples are tested in duplicate against coated and uncoated (control) particles. The uncoated particles are used to help identify any nonspecific reactions.

Results

At the end of the incubation period, the tests can be read. Particle agglutination assays are generally read by eye and the results are scored as either positive or negative. Visual reading is suitable for these assays since the agglutination of the particles can be seen clearly by the naked

eye. If coated red cells are used, the agglutination is quite similar to that seen when red cell serology is performed in microplates. If gelatin particles are used, the agglutinates appear a bluish colour. If latex particles are used, the agglutinates appear a white colour which can be seen clearly against a black background.

Settle method

A reactive result appears as an even mat of agglutinated particles across the bottom of the well. A non-reactive result appears as a button or ring of unagglutinated particles that have settled in the centre of the well (see Figure 41). These results are obtained by what is known as the "settle method" of particle agglutination. This is the method most commonly recommended by the manufacturers.

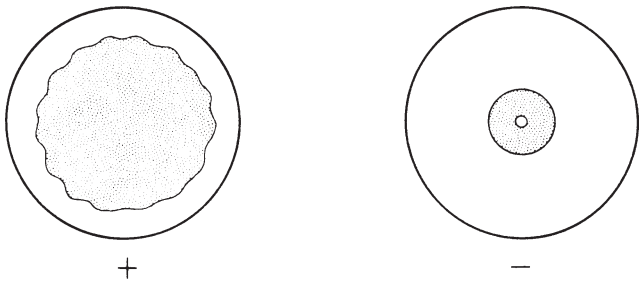


Figure 41

Initially reactive test samples need to be retested to confirm a true reaction. The assay should be repeated, but using both coated and uncoated particles. The results obtained are the same, except that there are two wells to read for each assay. A true-positive result is identified by a reactive result with the coated particles and a non-reactive result with the uncoated particles. A negative result is identified by non-reactive results with both types of particle. Any other combination of results indicates a possible nonspecific reacting sample. Care must be taken to ensure that a nonspecific result does not mask a true-positive result in the same sample.

To ensure that maximum sensitivity is achieved when reading the agglutination patterns, it is important to include a weakly reactive control sample. The sample should give the weakest possible agglutination pattern that can still be read as positive.

It is possible to read particle agglutination assays using an automated reading system. Some modern plate readers used for EIAs can also be used to scan across the bottom of the microwell and detect the reaction patterns of the particles. The reader can then interpret these patterns and identify the results as reactive or non-reactive.

In both cases, the control samples are used only to give the appropriate reaction pattern. True OD values are not used with this type of assay, even if the results are read on an automated system.

4.7 SIMPLE RAPID ASSAYS

The third type of assays to be considered is the simple rapid test device. These assays are single-use disposable devices which give results in

terms of minutes rather than hours and, in most cases, with good sensitivity. Assays of this format are available which detect antibody (e.g. anti-HIV, anti-HCV) and antigen (e.g. HBsAg).

These simple assays exist in a number of different presentations. The basic presentation is that of antigen or antibody (depending on the test), immobilized on a porous or a semiporous membrane, or on a strip. This is provided in the form of a single test module/strip to which the test sample and any other reagents are added. Most simple rapid assays are in the form of a kit containing all the reagents (if any) needed to perform the assay. They can generally utilize serum or plasma and, in some cases, whole blood as the sample type.

These assays can be divided into three main groups according to the underlying principle behind the assay. In general, these principles determine the overall presentation of the assay:

- immunochromatographic
- immunofiltration
- conventional EIA.

There follow examples of the general principles, methodology and applications of the three different groups. There is, however, considerable variation in these simple rapid assays although, in general, the variations reflect minor differences in design and construction by different manufacturers rather than any major differences in the basic principles adopted.

Immunochromatographic assays

Immunochromatographic membrane assays are based upon the principle of flow of sample along a specially designed porous strip containing dissolved reagents, with the subsequent deposition and visualization of any immune complexes at defined positions along the strip. This type of simple rapid assay is the most common format currently commercially available.

Simple immunochromatographic membrane assay (e.g. anti-HIV, anti-HCV)

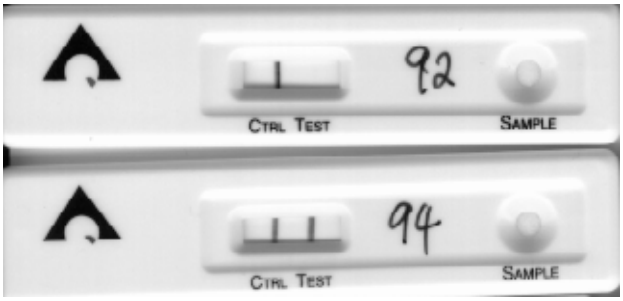
1 Sample is added to an absorbent pad at the start of the strip. The sample starts to migrate along the strip.

2 The strip contains dried conjugate, viral antigens conjugated to colloidal gold or selenium, which dissolves in the sample as it flows along the strip.

Colloidal gold or selenium (other elements may also be used) are preparations of gold or selenium consisting of minute, identical particles that form a suspension in solution and can be chemically attached to larger molecules, such as antigens and antibodies. While the individual particles are too small to be seen by eye, aggregates and agglutinates of the particles can be seen clearly.

3 Any specific antibodies in the sample bind to the reconstituted conjugate and continue to flow down the strip as an immune complex.

4 Viral antigens are immobilized in a line further along the strip. As the sample flows over this line, any specific antibody present binds to the immobilized antigen. As most of this specific antibody already has conjugate bound to it, a visible line forms across the strip as the colloidal gold or selenium builds up at the site (Figure 42).



Negative result

Positive result

Figure 42: Immunochromatographic assay for the detection of anti-HCV

Control test

Immunofiltration assays

Immunofiltration assays are based on the principle of filtration of sample and reagents through a porous membrane onto which specific antigen/antibody is immobilized and into a reservoir containing absorbent material. Any specific antibody/antigen in the sample will be bound on the membrane by immune complex formation. When conjugate is added, it binds to any specific immune complexes present, producing a coloured area on the membrane.

Immunofiltration assay

1 The sample is applied to the assay device and any antibody present binds to the immobilized antigen as the sample passes through the membrane.

2 After sample incubation, the membrane is rinsed by the addition of the rinse solution provided, which passes through the membrane carrying with it any remaining sample. Some assays do not require this step.

3 Conjugate is then added to the module; this is supplied in a prediluted form. The composition of the conjugate varies between assays, but the use of protein A labelled with a colloidal element, such as gold or selenium, is common.

Protein A is a naturally-occurring protein produced by the bacterium *Staphylococcus aureus*. It has the property of being able to bind to the Fc (complement-binding) region of the normal IgG molecule.

When conjugate is added, the protein A will bind to any specific IgG antibodies present. This binding will become apparent by the colour of the colloid that builds up on the membrane where any specific antibody is bound (Figure 43).

Negative result Positive result

Figure 43: Immunofiltration assay for the detection of anti-HCV

Conventional EIA type simple rapid assays

These simple rapid assays are based on conventional EIA methodology and generally consist of a module containing a solid phase onto which the sample and other reagents are pipetted. In most cases, the liquids run slowly over the membrane into a reservoir containing absorbent material or are removed from the device at each stage of the assay. Binding of any specific antibody or antigen in the sample to the immobilized specific antigen or antibody occurs, followed by binding of the conjugate. The addition of a chromogenic substrate is followed by the deposition of coloured precipitate onto the solid phase if the specific marker is present in the test sample.

EIA type assays (e.g. anti-HIV, anti-HCV)

1 The sample is applied to the assay device and any antibody present binds to the immobilized antigen. Predilution of the sample in a separate vial may be required with the diluted sample then being added directly to the device.

2 After sample incubation, the sample is removed and the module is rinsed by the addition of the rinse solution provided. Some assays do not require actual removal of the sample first.

3 Conjugate is then added to the module; this is supplied in a prediluted form. The composition of the conjugate varies between assays. Some assays use an enzyme-conjugated anti-human IgG in a similar way to that used in the microwell antiglobulin type EIA. When this type of conjugate is used, a further wash step and the addition of the chromogen are required to visualize the results. The activated chromogen is insoluble and deposits on the membrane as a coloured precipitate.

4 An alternate approach is to detect the presence of bound specific antibody by using a conjugate of protein A labelled with colloidal gold. When this conjugate is added,

the protein A will bind to any specific IgG antibody present. This binding will become apparent by the red colour of the colloidal gold building up where any specific antibody is bound. This type of conjugate is simpler to use as only one step is involved in the simultaneous detection of bound antibody and the visualization of the reaction; a separate chromogen substrate is not required.

5 The final result is read visually and is compared to the typical results described by the manufacturer. Although the assay is based on EIA principles, no OD values are generated; the result is read visually. No specific controls are required and there are no calculations to be made (Figure 44).

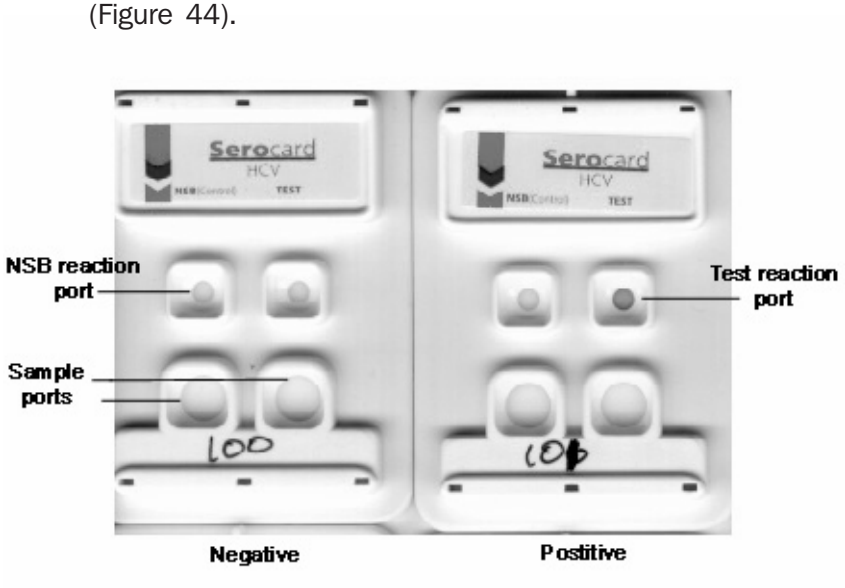


Figure 44: An example of a rapid test for the qualitative detection of anti-HCV based on the EIA principle

General points about the use of simple rapid assays

1 In addition to the specific line or spot (or whatever is the appearance of a positive result), most of simple rapid assays now also have an internal control, usually based on the detection of human proteins or albumin in the sample. This is used to demonstrate that the assay is working correctly.

2 A negative result can be considered when the control line/spot is present, but the test line/spot is not present. A positive result can be considered when both the control and test lines/spots are present. If the control line/spot is absent, the test is considered invalid and must be repeated.

3 Although external controls are not required to be able to perform these tests, it is desirable to set up at least a weak-positive control sample with each batch of tests; ideally, a negative control should also be set up. The use of known control samples can be very useful where there is some difficulty in interpreting a test result: for example because of lower than expected colour formation, where a direct comparison can be made.

4 In general, the whole procedure for these tests, from adding sample to obtaining a usable result, usually takes 10–15 minutes. However, the manufacturer's timings must be adhered to as over-incubation can lead to false positive reactions in some assays.

We have considered the principles of the three main types of TTI screening assay available. Although you may only use one type of assay, it is important that you understand the principles of all three basic types. It is also useful if you can assess for yourself the different types of assays that are available to you.

WHO assesses on a continuing basis the operational characteristics of many commercially available HIV, HCV and HBV antibody and antigen assays. Reports of these WHO evaluations are issued regularly and are also posted on the WHO website. Copies of these reports and additional information can be obtained by visiting the BCT section of the WHO website (http://www.who.int/bct) or by contacting the Department of Blood Safety and Clinical Technology, Geneva, or the WHO Regional Offices.

SUMMARY

1 The time and costs involved in screening donated blood for any TTI can be reduced by an effective donor education and selection programme that promotes self-exclusion by donors at risk of transfusion-transmissible infection.

2 The three principal types of screening assay involve the same two basic steps:

- demonstrating the presence of specific antigen or antibody in a sample in an immunological reaction involving antigen/antibody (immune) complex formation, with one of the components bound to a fixed surface
- detecting the formation of the immune complex in a linked procedure.

SELF-ASSESSMENT

14 What are the three main types of screening assay?

15 What is the difference between the term "positive" and the term "reactive"?

5

Selecting Screening Assays for Transfusion-Transmissible Infections

The purpose of this section is to help you to determine the most suitable screening strategy for your own circumstances and identify the basic equipment required.

LEARNING OBJECTIVES

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

1 Define the terms "sensitivity" and "specificity" and explain their relevance to TTI screening assays.

2 Identify the most suitable type of primary screening assay for use in your own laboratory.

3 Explain the factors that have to be considered when choosing a screening assay.

4 Assess the factors that contribute to the overall cost of screening in your laboratory.

5.1 SENSITIVITY AND SPECIFICITY

When considering different assays, you need to be aware of the data concerning the sensitivity and specificity of each particular assay. These data are widely used both by manufacturers to market and sell their assays and in papers where assay performance is compared. However, it is important that you understand how these figures are calculated and their limitations.

Table 5.1 Sensitivity, specificity and predictive values

		True TTI status +	True TTI status –	
Assay results	+	**a** **True-positives**	b False-positives	a+b
	–	c False-negatives	**d** **True-negatives**	c+d
		a+c	b+d	

sensitivity: The probability that a test result will be reactive in an infected individual.

specificity: The probability that a test result will be non-reactive in an individual who is not infected.

Sensitivity is the probability that the test result will be reactive in an infected individual: the sensitivity of an assay is therefore its ability to detect the weakest possible positive sample.

Specificity is the probability that a test result will be non-reactive in an individual who is not infected: the specificity of an assay is its ability not to detect false or nonspecific positives.

To calculate the sensitivity and specificity of an assay, two simple formulae are used, as outlined in Table 5.1 above:

$$\text{Sensitivity} = \frac{a}{(a+c)} \times 100\%$$

$$\text{Specificity} = \frac{d}{(b+d)} \times 100\%$$

ACTIVITY 18

Using the formula given above, calculate the specificity of an assay you use frequently. You will need to find out the number of true negatives and false positives for at least the last 100 tests you have performed. Compare your result with the specificity given in the manufacturer's instructions.

If you have external quality control samples, try to work out the sensitivity for the same assay, using the formula given above. You will need to find out the number of true positives and false negatives for at least the last 100 tests that you have performed. Compare your result with the sensitivity given in the manufacturer's instructions.

If you do not have external quality control samples, leave out the second part of this activity.

If your results differ from the specificity or sensitivity given in the manufacturer's instructions, talk to your supervisor about your findings. Note down your recommendations on your Action List.

An important point to remember when considering the sensitivity and specificity of assays is that they are generally inversely related; that is, as the sensitivity is increased, the specificity decreases and as the specificity is increased, the sensitivity decreases.

The importance of sensitivity in an assay should be clear and needs no further discussion. Specificity is a more complicated issue, however, but it is essential that all staff are aware of the importance of using specific assays. One of the main problems is to determine what causes the lack of specificity. At the very simplest level, the specificity of an assay is related to the particular assay format and the source of bound antigen or antibody. For example, the use of purer antigen preparations (such as synthetic peptides) reduces nonspecific reactions because there are very few, if any, non-peptide molecules present.

A final point is that, no matter how good the reported sensitivity and specificity of an assay, its final performance depends largely on the skilful handling of the assay by the operator. Figures quoted by manufacturers are usually very accurate, but often reflect the performance of an assay in an experienced dedicated laboratory under very closely controlled conditions. It is important for you to know how the assay will perform in your laboratory using this equipment and staff available.

5.2 PREDICTIVE VALUES

Another way of looking at the performance of an assay is to look at its predictive values. The predictive values, positive predictive value (PPV) and negative predictive value (NPV) are measures of the actual performance of an assay in a laboratory and therefore can be helpful in giving some idea of the sort of performance that you can expect from the assay in routine use.

The positive predictive value is the probability of a positive result being a true positive. The negative predictive value is the probability of a negative result being a true negative.

As with sensitivity and specificity two simple formulae are used to calculate the basic positive and negative predictive values, as outlined in Table 5.1 on page 64.

$$\text{Positive predictive value} = \frac{a}{(a+b)} \times 100\%$$

$$\text{Negative predictive value} = \frac{d}{(c+d)} \times 100\%$$

5.3 SELECTING AN ASSAY

The first step in selecting an assay is to find out which commercial assays are available or may be obtained in your locality or country.

ACTIVITY 19

Find out and write down the name of each assay available in your country, the manufacturer and, if possible, the cost per test (including any local taxes that apply). If this information is not readily available, your Ministry of Health or reference laboratory should be able to supply the information you require.

Then find out the details of one of each of the three types of assay and use the information to complete the table below, so that you can

	EIA	*Particle agglutination*	*Simple rapid assays*
Assay name			
Manufacturer			
Sample type			
Sample predilution			
Wash requirements			
Initial incubation time and temperature			
Composition of conjugate			
Conjugate incubation time and temperature			
Substrate			
Substrate incubation time and temperature			
Reading of results			
Specialized equipment required			
Overall assay time			
Cost			

make a direct comparison of these assays. You can return to this table later on to help you decide which would be the most suitable assay to use for the routine screening of donated blood.

There is a great deal of misleading information available about the importance of using the most sensitive assays. It is more important to use the most appropriate assay for your circumstances – those with which you will be able to obtain the most accurate and reliable results. This is not always the assay that is theoretically the most "sensitive" available. We shall therefore provide some simple guidelines to help you determine which assay is suitable for your own situation. In most circumstances, it would be inappropriate, for instance, for a small laboratory that tests 10–20 units of blood each week to use EIAs. The expense of obtaining the necessary equipment and the time needed to set it up and maintain it, as well as the amount of staff training required, are not justified.

A *suggested* and *approximate* guide is as follows:

- laboratories testing 1–10 donations a day or 1–60 per week should consider the use of simple rapid assays or particle agglutination assays
- laboratories testing 20 or more donations a day or 100 or more a week should consider the use of EIAs.

Manufacturers and suppliers often use the claim of greater sensitivity to encourage laboratories to use EIAs. In many cases, however, the sensitivity of some EIAs is not greater than some of the simple rapid assays or particle agglutination assays. Even if it is greater, the figures quoted to support these claims are often those obtained in large laboratories with highly skilled staff and with optimal testing conditions. As mentioned previously, the most important thing is to use an assay that gives reliable and accurate results in your own laboratory.

ACTIVITY 20

Look back at the table that you completed in Activity 19. Then note down any additional factors that specifically relate to your laboratory and that you feel are relevant to the choice of a TTI assay. You should consider:

- *the number of donations each week*
- *the facilities and equipment available in your laboratory*
- *the support service available: for example, water supply, electricity and so on.*

Assess the different types of assay on their appropriateness to your situation and consider which type of assay you would select. If you feel that your laboratory is not currently using the most appropriate type of assay, note down your recommendations on your Action List and discuss your findings with your supervisor.

Be wary of making the wrong decision simply because you cannot see the immediate solution to a problem. Instead, consider all the possibilities first. For example, you may decide that EIAs would be the most suitable type of assay to use in your laboratory, but feel you have to choose another option because you have no suitable equipment. However, you could perhaps ask the supplier of the assay to provide the equipment. Buying or hiring the equipment is often too expensive and you may end up with equipment that you have difficulty in getting serviced and maintained. A more common approach today is to lease the equipment as a package with the purchase of the kits. A major advantage of this is that the equipment remains the property of the supplier and it is in its interest to make sure that it is kept in good condition and is regularly maintained and serviced. This is both because of the financial value of the equipment and also because the equipment is needed to perform the screening tests. You will not continue to buy tests if the equipment is not working.

It is important that you select the type of assay that you feel is best suited to your particular circumstances. The information provided so far should have helped you to understand what choices have to be made.

5.4 FACTORS INFLUENCING SCREENING PROGRAMMES

Whatever type of assay is chosen as being the most appropriate for your circumstances, there are a number of other factors to be considered in setting up and maintaining an effective TTI screening programme.

ACTIVITY 21

What other important factors do you think need to be considered in order to maintain a successful TTI screening programme?

Consider the factors listed below, and any others you can think of, and note down how they relate to your particular circumstances.

- *staff training*
- *the equipment and reagents required*
- *the supply of assay kits*
- *the storage of assay kits*
- *the suitability and condition of the samples to be tested*
- *the supply of external quality control (QC) samples*
- *time constraints on screening*
- *the cost of screening.*

Staff training

All staff need training to improve their effectiveness, even if they are trained to do only one specific job. To be able to perform TTI testing successfully, staff need to be trained not only in the performance of the assay, but also in the other actions necessary as a result of TTI testing,

such as recording the results accurately. A successful TTI screening programme does not simply mean being able to perform the assay without mistakes or failures. What training programmes are there in your laboratory?

ACTIVITY 22

Make some notes on the training provided in your laboratory on screening for TTIs, including:

- *who provides the training*
- *whether there is a member of staff who is in charge of training*
- *the kind of training that is provided*
- *the duration of training*
- *the way in which trainees' skills are assessed*
- *whether regular training updates are held.*

How would you develop a training programme for new staff working in the area of screening for TTIs or for staff who are already performing TTI testing and testing for other infectious agents? Note down your ideas for a training scheme on your Action List. It need only be very simple, covering the most important areas. Then discuss your ideas with your supervisor.

It is important to keep a record of all training provided so that the trainer knows what the trainees should be capable of and the trainees understand what they are expected to know. Good training records not only enable monitoring of the actual training sessions, but also provide a log of when the training was provided so that regular updates can be held. This should help new staff to feel confident because it demonstrates that they will not be expected to undertake tasks for which they have not been trained. In addition, if the laboratory is likely to be inspected at some time, training records not only show that staff have been trained, but can also give the inspector confidence that the laboratory is run properly and that the quality of the results is important to the staff in the laboratory.

Equipment and reagents

All assays, except the simple rapid assays, require the use of certain pieces of equipment or of reagents that are not supplied with the assay kit. The reagents are usually only simple items, but they increase the cost of the assay and there may be problems if their supply cannot be guaranteed. The necessary equipment and reagents may not be readily available in all laboratories, especially in small laboratories in rural hospitals or those that are not part of a hospital.

ACTIVITY 23

From what you have already learned about EIAs and particle agglutination assays, list the items of equipment that are necessary to perform the assays correctly. Although the assays are very different, some items of equipment are common to both of them, while some are specific to each type of assay. Check your answers with those in the Activity Checklists and Answers on page 146.

When you have checked your answers, identify the items of equipment needed to perform the assays correctly that are already available in your laboratory. Then identify any equipment to which you have unrestricted access within the hospital or the institution to which your laboratory belongs.

If it is possible to use equipment in another part of your hospital or institution, it is important to check that access to it is not restricted. For example, equipment in another laboratory may be available during the week. However, if that laboratory is locked at weekends, the equipment would not be suitable for use since blood donations may need to be tested at any time if emergencies arise.

If you do not already have unrestricted access to the equipment needed to perform certain assays, the supplier of the assay may be willing to provide the required equipment. If your laboratory is setting up a large screening programme, this may be the best method of obtaining the appropriate equipment and support needed to maintain the programme. Remember, however, that the choice of assay should depend on whether it is appropriate to your circumstances and should not be influenced by the promise of the supply of equipment.

In addition to the equipment needed to perform the assay correctly, it is also necessary to ensure that there is an adequate supply of the other reagents and disposables that are required but that are not supplied with the assay. Simple rapid assays are usually supplied with all the required reagents and disposables, and the same may also apply to the reagents required for particle agglutination assays, although disposables are not usually provided. However, your laboratory will need to supply its own additional reagents and disposables for EIAs.

The disposables needed will vary, depending on the assays used, but they will include such items as:

- disposable pipette tips for multi-channel pipettes
- reagent troughs
- plastic bottles for the preparation of small volumes of reagents supplied in concentrated or two-part solutions
- microplates for particle agglutination assays.

The reagents required will also vary, depending on the assays used, but they will include such items as:

- deionized or distilled water to prepare the wash buffer and, in some cases, to wash the plates directly: a possible source would be injection or irrigation water from the pharmacy
- suitable acid solution to stop the final reaction and fix the developed colour.

Where the supply of these disposables and reagents cannot be guaranteed within the laboratory, it is again worth approaching the supplier of your assay kits for the necessary disposables and reagents to perform the assays. You will then have a complete supply system and all that will be needed are the staff to perform the assay and the samples to test. A system of this kind will obviously cost money and may take some time to set up and operate efficiently. You may find that buying the required items through the assay supplier is cheaper than using other sources and it will certainly save valuable staff time in trying to set up separate delivery schedules to coincide with the delivery of the kits.

Supply of assay kits

The supply of assay kits needs to be well organized in order to ensure that the screening strategy is maintained efficiently. Section 7 in the Introductory Module explains how to maintain a simple stock control system, but there are four important issues that also need to be considered.

1 *Calculating the actual usage of kits*
When calculating the number of kits that are required, you must take the following factors into account:

- the number of donations that you would expect to test in a specified period of time
- the repeat testing of initially reactive samples (you may feel that it is necessary to repeat the test in duplicate on these donations)
- any internal quality control (QC) samples (prepared within the laboratory) or external QC samples (prepared by a separate laboratory or institution) that you use
- the number of assay failures.

2 *Determining an appropriate stock level*
The actual stock level that it is possible to hold is determined by the following factors:

- the physical space available under suitable storage conditions
- the financial constraints: how much money is available and whether the supplier requires payment before delivery
- the expiry dates of the assays: the usual length of remaining shelf-life on the assays when they are delivered.

3 *Delivery times*
The length of time between ordering the assays and their delivery will depend on a number of factors, many of which may be outside the control of the supplier. For example, transportation may become impossible for a period during rainy seasons. An allowance should be made for this when ordering kits; for instance, it may be advisable to organize a delivery schedule that normally ensures delivery of a new supply of assay kits when you have used about 80% of the previous stock.

4 *Control of the batches of kits supplied*
If your laboratory uses a large number of test kits, it is worth assessing a particular batch before delivery and reserving sufficient kits from that batch for subsequent use.

ACTIVITY 24

Imagine that you are working in a laboratory that tests about 100 donations per month using a simple rapid assay. The incidence of HIV is 10% in the population from which the blood donors are drawn. The anti-HIV kits contain 20 tests each and arrive with a two-month shelf-life. Approximately 2% of assays fail. The kits usually take two weeks to arrive from the date of ordering, except in the rainy season when they take three weeks. There is one rainy season a year that lasts for two months. You have room to store only 8 kits for each marker.

When would you order more kits and how many would you order on each occasion? Design a suitable ordering schedule, covering a whole year, that ensures that you do not have less than 20 tests in stock at any time. Assume that you have seven kits in store at the beginning of the year. Check your answer with the suggestions given in the Activity Checklists and Answers on pages 146–147.

Then review the ordering schedule for assay kits in your laboratory. If you can suggest any ways in which it could be improved, note down your recommendations on your Action List and discuss them with your supervisor.

Storage of assay kits

It is vital that all assay kits are stored in appropriate conditions to ensure that the assay performs correctly and that the results are reliable. Whenever any kits are delivered, it is the responsibility of the user to store them exactly according to the manufacturer's instructions.

Most assays require storage at a temperature of +2°C to +6°C. Ideally, a walk-in, well-insulated cold store should be used, preferably not one that is also used to store clinical products, such as blood. The power supply should be reliable and the temperature should be monitored regularly. The kits should be stored on shelves above floor level and access should be restricted to authorized personnel.

In most countries, however, storage facilities of this kind are rarely available and a large laboratory (or even domestic) refrigerator is often used instead. It is important to ensure that any refrigerator used to store assay kits is reliable, is large enough to store the required number of kits and that the temperature is monitored regularly, at least twice a day. Temperature monitoring and the maintenance of refrigerators are covered in detail in Section 5 of the Introductory Module.

An unreliable power supply is a major problem for many laboratories, even if suitable storage facilities are available. It is important to try to maintain the temperature if the power fails by increasing the insulation of the refrigerator and not opening the door unnecessarily. However, the power may be cut frequently and for long periods and, in this case, these measures will not be sufficient to maintain the required storage conditions and long-term storage of assay kits may then not be advisable. A possible solution is simply to store sufficient kits for a short period of time so that they are not subjected to too many temperature fluctuations. However, this approach is only possible if the kit supplier can be relied on to supply kits regularly.

Temperature monitoring is an important part of a laboratory quality system. If continuous monitoring is not possible, regular checks using a suitable thermometer are sufficient. It is important to monitor the temperature so that assay failures that are the result of changes in storage temperature outside the manufacturer's recommended range can be identified. In some cases, manufacturers or other users of certain assays will say that the storage temperature is not particularly important and that the kits can be safely stored at room temperature. There are two points to remember, however:

1 Room temperature is a defined temperature range of 18°C–25°C, but the actual room temperature in laboratories in many countries is often higher than this.

2 It is essential to follow the manufacturer's instructions rather than to follow conflicting advice from anyone else.

ACTIVITY 25

Examine the assays used in your laboratory and note down the conditions in which they are stored. Are these storage conditions the same as those recommended by the manufacturers? If they are not, note down on your Action List your recommendations on how the storage conditions might be improved.

Sample type and quality

All commercial assays clearly state whether plasma or serum is required for the assay. It is very important to use only the type specified by the manufacturer. All commercial TTI assays are validated for use with serum and most with plasma. In effect, this means that, wherever possible, a clotted sample should be used for testing. A number of assays are also

validated for use with plasma samples. In addition, some of the simple rapid assays are validated for use with whole blood samples.

It is clear, however, that there is considerable variation in the collection procedures in different countries and sometimes even between different blood collection centres within the same country. In some situations, extra samples are not taken after the blood is collected from the donor, and any laboratory testing has to be performed using the bleed line attached to the blood pack. If this is the case in your own laboratory, it is advisable to change the system and to collect separate samples. The collection of a single clotted sample does not cost much money or adversely affect the donor, and it provides the laboratory with its own sample for testing without the need to sample the blood pack. Even if only one sample is available, TTI screening can be performed first and serology or any other testing can then be performed on the remaining sample.

ACTIVITY 26

What types of samples are collected from your blood donors and what are they each used for?

If testing in your laboratory is performed using the bleed line attached to the blood pack, talk to your supervisor about changing the system and collecting separate samples from donors. Note down your recommendations on your Action List.

The standardization of sample collection is very important in order to ensure that all samples are in optimal condition for use and are correctly identified. If either serum or plasma is suitable for an assay, it is critical to ensure that, if serum is used, the serum is fully clotted first. Assays are validated for use on fully clotted blood, not on clotting blood. The use of samples that are not fully clotted may lead to false positive results.

In general, samples should be stored at 2°C–6°C for 12–16 hours before testing. If it is not possible to wait this long before testing and serum is required for the assay, incubation at 37°C for 2 hours is usually sufficient to ensure that the sample has fully clotted. If a centrifuge is available, it is best to spin the samples first to remove any microclots from the serum.

Haemolysed or lipaemic samples are not suitable for use in most assays because they may give false-positive or false-negative results due to inhibition or, in some cases, obscure the results.

In all cases, the general rule is that good quality samples should be used. Assay results are better when using fresh, fully clotted, correctly stored samples.

Quality control samples

Every laboratory using a TTI screening assay should also use whatever external or internal QC samples are available for each marker. QC samples are important because they offer a way of monitoring the performance of an assay, both in terms of overall sensitivity and specificity and in highlighting gradual trends in the results that would not normally be noticed on a day-to-day basis.

External QC samples are stabilized samples provided by an independent laboratory or institution. Internal QC samples are prepared within the home laboratory/institution. They are sera of which the status is known and characterized against a number of assays, and which give reactions that can be reproduced and the results from different assays compared. They are generally diluted strongly reactive positive samples or less commonly weak true reactive samples. It is important that the samples are stabilized so that the reactions can be reproduced, even after extended storage.

External samples are not available in all countries. They may, however, be available through the supplier of the assay kits. If QC samples are obtained from the kit supplier, it is important to make sure that they are not simply commercial samples provided to validate the manufacturer's assay. Such samples may not represent true samples as they may have been selected just to give a range of different results with the one assay. They may therefore not react with other assays which may actually be more sensitive. Care must be taken to ensure that supplied samples do not contain preservatives which may interfere with the performance of the assay. Sodium azide, for example, is often used to preserve serum samples, especially if long-term storage at 4°C is intended, but will inactivate the enzyme horseradish peroxidase which is commonly used as the enzyme label in commercial EIAs.

If external QC samples are not readily available, internal QC samples can – and should – be prepared. These can be prepared by diluting confirmed positive sera, although it must be remembered that some assays do not work well with diluted samples. Standard negative QC sera should also be prepared.

ACTIVITY 27

If external QC samples are available to your laboratory, note down:

- *the markers that they are available for*
- *where they are obtained from*
- *how many samples are available*
- *whether you have to pay for them*
- *the assays with which they are meant to be used.*

Are internal QC samples used in your laboratory? If they are, how are they prepared and standardized?

Can you suggest any ways of improving the system for obtaining external QC samples or preparing and standardizing internal QC samples? If you can, note them down on your Action List and discuss them with your supervisor.

Time constraints

To be effective, the TTI screening of donations must take place before the blood is transfused. This may seem to be a very obvious statement. However, in some areas where a formal blood transfusion service does not exist, stocks of blood are not always maintained. Blood is often only donated when required in emergency, usually by the family or friends of the patient, and is transfused into the patient immediately. It is in such situations that the greatest risk of transfusion-transmitted infection exists. The use of untested blood must therefore be stopped if there is to be any hope of reducing the transmission of TTIs by blood transfusion.

In situations of this kind, it is generally most appropriate to use simple rapid assays. Even if time is short, there should be time to perform an assay, and blood or plasma could be sampled from the bleed line for testing. In a well-organized blood programme, a target period of 24 hours storage would provide sufficient time for routine TTI screening prior to transfusion.

The only way to develop an effective screening programme to reduce the risk of post-transfusion infection is, however, to use voluntary donors who provide blood for stock rather than for immediate use. All the evidence clearly indicates that, in all countries, the use of regular, voluntary, non-remunerated donors results in a safer blood supply. The collection and storage of their blood enables an efficient screening programme to be maintained and a safe blood supply to be provided.

To guarantee that this system works at all times, however, a planned blood collection programme is necessary. It is never easy to plan blood collection to avoid having either inadequate or excessive supplies of blood, and it is even harder to predict when blood will be required, particularly if a lot of blood is used for obstetric patients who often do not present more than a few days before they are due to deliver. Module 1 provides detailed guidance on building up a panel of voluntary non-remunerated donors who are willing to give blood regularly. Ways of estimating blood requirements are covered in Section 3 of Module 1 and Section 6 of Module 3.

ACTIVITY 28

In your blood bank, what is the current average storage time for a unit of donated blood before it is issued for transfusion?

Is untested blood ever transfused in your hospital? If it is, why is it necessary to use this blood so quickly that testing cannot be performed?

How do you think a storage period of at least 24 hours could be maintained to provide sufficient time for routine screening for TTIs before transfusion and prevent the use of untested blood? Note down your recommendations on your Action List and discuss them with your supervisor.

The cost of screening

It is difficult to make an accurate assessment of the cost of screening because there are so many factors that affect it and the prices of individual assays change frequently.

The actual cost of the assay is only part of the total cost of a screening programme, although the proportion of the overall cost varies in different countries. In many countries, little or no money is available for testing. Probably the best way to estimate the cost of screening is to consider all the factors that contribute to the overall cost; these factors can then be applied to any assay in order to determine the relative cost.

Number of donations to be tested

The number of assays required will be determined by the number of donations to be tested in a specified period. The expected number of donations to be tested therefore forms the basis for estimating the cost of a screening programme. The more assays that are performed on a given number of donations, the greater the overall cost of the screening programme.

Type of assay

The type of assay selected is important because each type has different needs in terms of equipment and reagents. Simple rapid assays are more expensive than EIAs and particle agglutination assays, but all the required reagents and disposables are supplied with the kit.

Additional equipment and reagents

The purchase of additional equipment and reagents can clearly increase the cost of testing, depending on the type of assay used. The cost of additional equipment increases with the complexity of the assay. Simple rapid assays usually come with all the equipment and reagents required to perform the assay. None of the other assays is self-contained in this way. These non-simple assays, however, are generally much cheaper than the simple rapid assays. This is particularly true where suppliers provide equipment “free of charge” with the assay rather than selling or leasing it, as no additional costs are involved. This may sometimes also apply to the supply of additional reagents. If this is not possible, and if the required reagents are not readily available within the laboratory, they must be purchased, again increasing the cost of the assay.

Reliability of the assay

The reliability of the assay is important because it is necessary to repeat failed assays. This increases the actual number of assays used to test the same group of donors and therefore also increases the cost.

Specificity of the assay

To some extent, the specificity of the assay determines the number of initially reactive samples that need to be repeated in order to confirm the result. Assays with lower specificity will result in a larger number of initially reactive samples that need to be repeated, even though they may be negative on repeat. Too low a specificity will therefore result in increased costs due to both unnecessary repeat testing and the loss of repeatedly reactive but confirmed negative donations.

Prevalence of the infectious agent in the donor population

The prevalence of each infectious agent screened for in the donor population plays an important part in the cost of screening because all initially reactive samples need to be repeated, ideally in duplicate, to confirm the results. Where there is a high prevalence of one or more TTIs, more repeat testing will be required and consequently more assays will be used. A larger number of units of infected or potentially infected blood will also have to be discarded following testing.

Collection of donated blood

The cost of the collection of donated blood depends on a number of other factors, such as the number of mobile blood collection sessions and the number of staff involved. Where there is a high prevalence of TTI in the donor population, the cost of blood collection will be proportionately higher because a larger number of units of blood will need to be discarded.

Staffing

Staff costs must always be considered, particularly if a new screening programme is to be set up and additional staff have to be employed.

ACTIVITY 29

Make brief notes on how you think each of the factors listed below might affect the overall cost of your screening programme:

- *the number of donations to be tested*
- *the types of assay used*
- *the additional equipment and reagents needed*
- *the reliability of the assays*
- *the specificity of the assays*
- *the prevalence of TTIs in the donor population*
- *the cost of collecting the donated blood*
- *staffing.*

The expected number of donations to be tested is the most obvious factor to consider when estimating the cost of a screening programme.

All the other factors are also important, however, because they involve expenditure, either to purchase the items required or to cover other costs, such as staff salaries. Remember that a further factor to take into account when calculating the overall cost of screening is the cost of the blood that has to be discarded as a direct result of screening.

SUMMARY

1 The sensitivity of an assay is the probability that the test result will be reactive in an infected individual. The specificity of an assay is the probability that the test result will be non-reactive in an individual who is not infected.

2 It is important to use the most appropriate assay for your particular circumstances.

3 All staff need appropriate training to be able to perform assays accurately.

4 Assays must always be stored in appropriate conditions to ensure that they perform correctly and that the results are reliable.

5 Fresh, fully clotted, or properly anticoagulated, and correctly stored samples should always be used for assays.

6 If external quality control samples are not available, internal quality control samples should be prepared.

7 Whatever the time constraints, TTI screening should always be performed before transfusion. When there is little time available, simple rapid assays may be the most appropriate assays to use.

8 The cost of screening is dependent on a combination of factors, not simply the cost of the assay.

SELF-ASSESSMENT

16 What is the relationship between sensitivity and specificity?

17 What factors contribute to the design and maintenance of an effective anti-TTI screening programme?

18 What are the important conditions in the storage of assay kits?

19 What is the difference between external and internal QC samples?

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 Define the terms "sensitivity" and "specificity" and explain their relevance to TTI screening assays.

2 Identify the most suitable type of primary screening assay for use in your own laboratory.

3 Explain the factors that have to be considered when choosing a screening assay.

4 Assess the factors that contribute to the overall cost of screening in your laboratory.

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 or other senior colleagues, if there is anything you are still not sure about.

6

Using Screening Assays for Transfusion-Transmissible Infections

The purpose of this section is to help you to develop an effective TTI screening programme by considering:

- the performance of screening assays and the results obtained
- the confirmation of assay results
- the permanent recording of assay results
- the importance of good record-keeping as an integral part of any screening programme
- potential safety hazards associated with commercial TTI screening assays.

This section considers the actual use of whatever assay is used as the primary screening assay. It does not include details on the assay methods themselves, but focuses on general aspects of screening that are important in ensuring that an assay is performed correctly.

LEARNING OBJECTIVES

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

1. Use assays correctly to develop an effective screening programme for transfusion-transmissible infections.
2. Determine the correct screening results for a set of donor samples.
3. Maintain accurate and complete records of the screening results.
4. Review the health and safety procedures in your laboratory for handling and disposing of TTI-positive donations, assay components and waste.

6.1 USING SCREENING ASSAYS

Assuming that the right choice of assay has been made and that the necessary equipment is available, performing the assay is simply a matter of following the manufacturer's instructions exactly.

Reliable results can be obtained only if the instructions are followed precisely. If it is later found that the test has failed to detect a positive sample, it is essential to identify the cause of the failure so that action can be taken to prevent it from happening again. There are a number of possible reasons for such a failure and many are due to error by the person performing the assay. However, assay failure may also result from a defect in one or more components of the assay kit.

It is important to keep an error log to record assay failures and the actions taken to prevent them from happening in the future. The log should record the following information:

- the assay
- the operator
- the reason for the failure
- the consequences of the failure
- the changes made to laboratory practice to prevent a similar failure in the future.

ACTIVITY 30

Is an error log kept in your laboratory to record assay failures? If you can suggest any ways that it can be improved, note your recommendations on your Action List.

If an error log is not kept in your laboratory, talk to your supervisor and your colleagues about introducing one and identify the precise information that will need to be recorded. Note your recommendations on your Action List.

Handling screening results

After performing the assay and obtaining a set of initial screening results, the next stage should be to repeat the assay on all initially reactive samples. The reason for this is to confirm the initial result. Whether you repeat the assay singly or in duplicate depends on the type, specificity and cost of the assay. Initially reactive samples detected by simple rapid assays are usually repeated singly, while those from particle agglutination assays and EIAs are repeated in duplicate. From the results of the repeat assays, the final test results can be determined. Repeatedly reactive samples should be considered to be TTI-positive and the donation should be discarded. It is also important to test the unit of blood itself to make sure that the correct pack has been identified. This can normally be done by taking a sample from the bleed line. Remember that this sample will be plasma and not serum.

It is important to understand that the screening results can be considered from two standpoints:

- the result that determines the status of the *donation*
- the result that determines the status of the *donor*.

Although the results may appear to be the same, this is not always the case.

A repeatedly reactive sample is considered to be TTI-positive and the donation is discarded. If confirmatory testing is available, however, it may subsequently show that the screening result was a false positive result and that the donor is actually TTI-negative. The donation would be correctly discarded as TTI-reactive using the screening test, but the donor would subsequently be cleared as being TTI-negative. This situation is common in countries with a low prevalence of TTIs where the **predictive value** of a positive result is relatively low because of the relatively high incidence of false positive reactions.

predictive value: The likelihood of a result being a true result. Both positive and negative predictive values are used.

6.2 CONFIRMATORY TESTING

Confirmatory testing should be performed by a separate reference laboratory and using different assays from that used for the original screening. The level of reference service varies greatly from country to country, but laboratories offering a confirmatory service are expected to work to a high standard and to have experienced scientists on their staff. They should also be willing and able to give advice on the performance of, and problems associated with, a range of diagnostic assays, including aspects of quality control and local evaluation.

If no confirmatory service is available in your country, the results must be accepted on the basis of the screening tests performed, although it is important to be aware of their potential limitations, such as the possibility of repeatedly false positive reactions using the primary assay. If your laboratory does not have access to a confirmatory service, a possible solution is to keep a small stock of an alternative assay that is at least as sensitive as the primary assay, for use in confirming the status of samples that are found to be repeatedly reactive by the primary assay. This is not a particularly cheap solution, but it does allow a basic confirmation of screening results, using an alternative assay format.

An excessive number of false positive reactions may be caused by a number of factors, including:

- poor quality test kits
- poor quality samples
- poor washing
- dirty reagent containers
- dispensing of incorrect volumes of samples or reagents
- incorrect reader set-up (for EIAs).

ACTIVITY 31

Do you have access to a reference laboratory? If you do, what assays are used?

Look at your records for the last 25 or 50 assays. How many of them were confirmed as positive by your reference service (if one is available) or by an alternative assay? How many assays were false positives? Can you suggest any ways of reducing the number of false positives? Note down your recommendations on your Action List and discuss them with your supervisor.

Confirmatory assays

In theory, a confirmatory assay can be any reliable TTI assay *as long as it is different from the assay that was used as the original screening assay*. There is often discussion about a standard assay, and the term "gold standard" has been widely used to describe what is considered to be the definitive assay for each TTI, both in terms of sensitivity and specificity.

The assay that was at one time considered to be the "gold standard" for antibody assays is the Western blot. The technique is one that combines the basic principles of the antibody EIA with the separation and immobilization of specific proteins from the infectious agent on an inert support membrane. Western blotting is, however, a relatively specialized and expensive technique which is not appropriate when screening blood donations. Consequently, it is not recommended as a routine confirmatory test, except in central reference laboratories where there is a high level of technical expertise and a wide range of techniques are available.

WHO has a set of recommendations for specific anti-HIV testing strategies which you may find helpful if you do not already have a strategy for blood screening. They are outlined in *Revised Recommendations for the Selection and Use of HIV Antibody Tests* (WHO, 1997), which is reproduced in Appendix 1. *Read this now*.

Dispatch of samples for confirmatory testing

The regulations for the dispatch of samples for confirmatory testing vary from country to country, but the basic approach is the same in all cases. As you saw in Section 3 of the Introductory Module, all blood and serum samples and other pathological material should be packed in such a way that if the sample container is damaged or breaks, the sample itself will be completely contained with no risk of leakage out of the package. The following five precautions should be observed.

1 Use a sample container that is strong and watertight and has a leak-proof screw lid. Clearly label the container.

2 Wrap the container in sufficient absorbent material to soak up the sample in case of spillage.

3 Pack the wrapped container in a second watertight container or seal it in a leak-proof plastic bag. Heat-

sealing is the best method, although the necessary equipment may not be available. Snap-tight bags or well-taped bags are alternatives. Seal the accompanying documentation in a protective pouch and attach it to the outside of the container or plastic bag.

4 Put this in an outer package that is capable of protecting the contents from physical damage while they are in transit.

5 Label the outer packaging to indicate that it contains pathological material. On the outside, write the name and address of your own laboratory as well as the name and address of the reference laboratory.

In addition to the precautions taken to safeguard against leakage, it may be necessary to take special measures to ensure that samples arrive in a suitable condition for testing. Delays in delivery are a common problem which may be difficult to overcome because they can be due to many, often unpredictable, reasons. The use of special delivery services would solve the problem, but they tend to be too expensive for most laboratories to use.

A second common problem is the exposure of samples to too high or too low a temperature during transportation. This can be overcome to a certain extent by using well-insulated containers that can prevent fluctuations in temperature for short periods. If serum samples are being sent, low temperatures are generally not a problem. The addition of solid carbon dioxide to the container can prevent a rise in temperature, but only for a limited period, and this will depend on the actual temperatures to which the samples are exposed. If whole blood samples are being sent, it is important to maintain a very specific temperature range (normally 2°C–6°C). Again, this can be managed for short periods, but not for long periods or in extreme temperatures.

ACTIVITY 32

If there is no reference service in your locality, how do you ensure that samples arrive at the reference laboratory that you use in a suitable state to give reliable confirmatory results?

What are the local or national regulations about sending pathological material by post? If there are no regulations, how do you think you should send samples so that there is no risk of leakage during transit?

From your records of the last 25 or 50 samples sent to your reference service, note down the following:

- *the time it takes for samples to arrive at the reference service*
- *the proportion of samples that arrive damaged*
- *the time it takes for you to receive the results.*

Note down on your Action List any improvements that could be made in the safe dispatch of samples.

6.3 RECORDING TEST RESULTS

The recording of the assay results is important to ensure that the right results are linked to each sample. This may appear to be a very obvious statement, but it is important to remember that the majority of errors in clinical laboratories are clerical.

The first step in recording test results is obviously to have a suitable record sheet. We shall consider the overall requirements of laboratory worksheets in Section 7 when we consider quality assurance in TTI testing. At this stage, we shall simply focus on the recording of the individual test results.

In the case of simple rapid tests and particle agglutination tests, the results sheet is the only record of the results. EIAs may give a printout of the OD values obtained, depending on the plate reader used.

In order to record testing data adequately, a results sheet should record the following information:

- the donation numbers
- the date the donations were tested
- the test used including lot/batch number
- the initial results
- the final results on samples that needed repeat testing
- the fate of the donations: whether they were issued for transfusion or discarded
- the person who performed the testing.

Whatever the design of the record, the important rule is to make it as simple and as clear as possible. Figure 45 on page 87 is an example of a record sheet which gives a clear indication of the results and, importantly, whether the donation is to be accepted or rejected. It also includes two columns for signatures, one to be completed by the person who tests the samples and enters the details, and the other by a second person who checks all the details. This helps to minimize clerical errors.

ACTIVITY 33

Look at the record sheet used in your laboratory for test results. Does it enable you to record all essential information? Compare it with Figure 45. Note down on your Action List any improvements that you think could be made to your record sheets.

Donation no.	*Assay name and lot/ batch no.*	*Initial result*	*Repeat needed*	*Repeat result*	*Accept/ reject donation*	*Tested by*	*Checked by*

Figure 45: TTI assay record sheet

Accurate record-keeping is clearly crucial. Checks are needed to ensure that the correct assay results are recorded against each sample, that the records are a true picture of the testing performed and the results obtained, and that the correct decision is made about the final fate of the donation. The importance of accuracy cannot be overemphasized. If an error is made, the worst could happen – a patient could be transfused with a TTI-positive unit of blood. This situation is inexcusable when a good screening programme is being used.

The record sheet shown in Figure 45 is sufficient to record current testing data. However, it is also necessary to store these data in a form that allows them to be retrieved in the future. It should therefore be added to the donor records that contain all the necessary information about individual donors, but in a suitable format that maintains confidentiality. Section 8 in Module 1 gives more detailed information on donor records.

Previous records can be referred to if there is any doubt about the screening results obtained from a specific sample from a donor. This could be important in the situation described earlier, where the screening result is positive but confirmatory testing shows that the result is a false positive and the donor is TTI-negative. Whether the donor should still be bled in future and what, if anything, should be said to him or her about the results is a decision that remains with each transfusion service.

It is crucial, however, to ensure that donors who are confirmed as TTI-positive are clearly identified and are permanently excluded from donating blood again. Previous records containing information about donors who are permanently unacceptable must therefore always be kept up to date and checked by donor clinic staff before donors are bled. It is essential to use the screening data to update the donor records but, in order to maintain confidentiality, the specific reason for permanent exclusion may not be included in a donor's individual record. This applies equally to the results of screening for any of the infectious agents. Confidentiality is covered in more detail in Sections 2 and 4 of the Introductory Module and Section 7 of Module 1.

ACTIVITY 34

What system is used in your laboratory for retrieving data from previous tests on donors? Can you suggest any ways of improving the retrieval system? Note any recommendations down on your Action List.

Very often, the TTI screening results have to be entered onto a separate sheet, such as an overall worksheet that contains all the current testing information. This may include:

- blood grouping
- antibody screening
- relevant medical information
- the results of screening the blood for infectious agents.

If your screening programme includes screening for several infectious agents, a number of different systems may be used to report the screening results which then all need to be coordinated. Two basic approaches can be used to record the screening results. A worksheet can be used for each different assay and the results gathered together at the completion of screening. Alternatively, a single worksheet can be used that enables the results of all the different assays to be entered against each donation. The number of different assays performed and the total number of samples screened will determine which of these approaches would be most suitable in your laboratory.

The results from the screening laboratory can be reported either as individual assay results or as a single positive or negative result. Using a single result is a very effective way of reporting because the screening laboratory is then solely responsible for gathering and assessing all the screening results and thereby determining the overall suitability of the donation for issue.

6.4 STORING SCREENING RESULTS

The screening results provide a permanent record of the testing of each donation and need to be stored confidentially for future retrieval, if necessary. What does storage mean? It does not mean putting records in a room or cupboard, shutting the door and forgetting them. If there is no intention of ever referring to the records, there is no point in keeping any records at all. The purpose of record-keeping is to ensure that accurate records are stored in a logical way so that a particular set of results can be retrieved quickly and easily and there is full traceability.

There are a number of factors that need to be considered before any records can be stored correctly:

- the records must be complete
- the length of storage time must be decided
- a suitable storage area must be selected

- the storage area must be kept secure in order to maintain confidentiality
- the storage system must enable the data to be retrieved easily
- the conditions must be suitable for the long-term storage of records
- a suitable disposal system must be available.

In some countries, the decision on how long to store records is made at national level and guidelines are issued which must be followed in all centres. In other countries, there is no policy on the length of time for which records must be stored, or the results are only kept until the blood has been transfused.

In countries which produce plasma for the preparation of other therapeutic products, records need to be stored for a longer period of time than in those which simply collect and transfuse essentially unprocessed blood. Red cells are usually used within one month, but some of the other products prepared from harvested plasma may not be used for a number of years; immunoglobulin preparations, for example, may have an expiry date up to five years after the date of donation. Clearly, some laboratories are unable to store records for such long periods, but it is still important to provide facilities for long-term storage, even if only for about one year.

ACTIVITY 35

How are records of screening results stored in your centre so that they are kept confidential, but are easy to retrieve? Think carefully about the factors listed above. If you can suggest any improvements that could be made to the storage system, note them on your Action List.

6.5 HANDLING TTI-POSITIVE DONATIONS

Once a TTI-positive donation has been identified, it must be removed and destroyed as soon as possible. When handling positive material, remember that there is always a risk of accidental infection. All positive donations must be clearly marked to avoid them re-entering the issuable blood stocks and the appropriate safe handling procedures should be observed at all times. Each laboratory must design and enforce suitable guidelines to ensure that the risks of infection of laboratory staff from the material that they handle, both pathological samples and units of blood, are reduced to a minimum.

As you saw in Section 3 of the Introductory Module, the method used to dispose of positive donations safely has to be determined by each laboratory and depends on the facilities available. However, in order to ensure that a safe system is in operation, you should take the following steps.

1 Confirm the identity of the positive donation. Make a second check of the assay worksheets to ensure that the correct donation has been identified.

2 Physically retrieve and remove for disposal the donation and any products that have been prepared from it. If unsuitable or unsafe donations are stored for a short time before disposal, the storage area must be separate from the main blood bank. Mark all unsuitable donations in some way to make it clear that they should not be transfused.

3 Safely dispose of the donation. Ideally, it should be destroyed by autoclaving, followed by incineration. If autoclaving is not possible, incineration is the next best method of disposal. Avoid cutting the bag open and washing the blood away or burying the blood unless no suitable facilities for disposal are available; these methods can be dangerous and do not destroy the potential infectivity of the blood. If there is no alternative to burying, make sure that the infected blood bag is securely packaged and buried deeply so that it cannot be dug up by animals. If blood bags are opened, the infected blood must be poured into a suitable deep pit with strong disinfectant, such as sodium hypochlorite in concentrated form. The bags should then be burned immediately.

   You will find WHO guidelines for the safe disposal of HIV-infected blood in *Biosafety Guidelines for Diagnostic and Research Laboratories Working with HIV* (WHO AIDS Series, No. 9, 1991).

4 Maintain adequate completed records to demonstrate that the donation was actually removed from stock and destroyed.

5 Make all staff aware of the importance of the correct identification and safe disposal of all unsuitable blood. The procedure should be reviewed regularly.

ACTIVITY 36

What procedure is used in your centre to ensure that TTI-positive donations are correctly identified, removed from stock and disposed of safely? If you can suggest any improvements to the procedure, note them down on your Action List.

If there is no standard procedure in your laboratory, it is important to develop a system which is followed by all staff. Talk to your supervisor and colleagues about designing and implementing a suitable system for your laboratory. Note down your recommendations on your Action List.

6.6 HEALTH AND SAFETY ASPECTS OF COMMERCIAL ASSAYS

Health and safety are covered in detail in Section 3 of the Introductory Module. We shall focus here on the health and safety aspects of the commercial TTI screening assays.

Assay components

ACTIVITY 37

From what you have already learnt about TTI assays in general, list the components of the assays that you think might present a health and safety risk. In what way might they present a risk?

It is often difficult to identify the reagents that present a genuine risk as virtually all chemicals can be considered dangerous. However, we need to consider only certain components of the commercial assays here. Clearly, these components are not found in every kind of assay, and the assays are presented in a number of different formats.

The components that could present health and safety risks are:

- wash buffer
- substrate
- acid stopping solution
- control sera.

Particle agglutination assays do not produce wash fluid waste or use acid.

Wash buffer

Wash buffer is supplied as a concentrated solution and therefore is potentially far more hazardous than it is when at working dilution. Since there is a high concentration of chemicals in the stock solution, it may be caustic or cause other skin irritation or it may be toxic. Azide-containing solutions have potentially toxic levels of azide and should not be disposed of through copper waste pipes because explosive compounds may result.

Substrate

Some of the synthetic substrates (chromogens) used in modern assays have been investigated for possible carcinogenic or teratogenic effects. Although the volumes in each assay kit are small, repeated exposure to these chemicals could be harmful.

Acid stopping solution

Stopping solution is usually dilute acid solution, but at a concentration that can still cause irritation or skin damage. All acid solutions should be handled with care and neutralized with sodium bicarbonate before disposal.

Control sera

Control sera are screened for the presence of other infectious agents and are treated to inactivate any potentially infectious material present. However, they are human in origin and should therefore always be handled as if they are capable of transmitting infection.

It is important to realize that these components present what are only *potential* risks and that they need not be hazardous if basic laboratory safety procedures are observed. Unless specifically stated in the manufacturer's instruction leaflet, no specialized facilities are required to handle the reagents in the assays. Remember that the safe disposal of the used assay components is important to protect not only laboratory staff but also any other individual who may come into contact with the used assay components or other waste material.

ACTIVITY 38

List the assay materials that may present a hazard in their disposal and suggest ways of disposing of them safely.

Disposal of potentially hazardous materials

There are a number of components that require safe handling and disposal after screening has been performed. The subject of safe handling has been covered extensively in the WHO publication *Biosafety Guidelines for Diagnostic and Research Laboratories Working with HIV* (WHO AIDS Series, No. 9, 1991). There are however, some additional points to consider.

Acid

Laboratories that use EIAs invariably end up with a number of finished assay plates containing acid. These should be put into bicarbonate solution to neutralize the acid. If disinfectant is to be used, it can be added later when the acid has been completely neutralized. Care must be taken when adding hypochlorite solutions to acids as chlorine gas can be released. Excess bicarbonate must be used to ensure that all the acid is neutralized.

Fluid waste

Laboratories using EIAs also accumulate a large volume of infectious waste wash fluid. Remember that the wash fluid contains the original serum from the first stage of the assay procedure. Disinfectant may be added to this to the correct final concentration before disposal.

Solid waste

All assays generate an amount of contaminated solid waste. Do not attempt to reuse any disposable items, but dispose of them correctly and safely.

ACTIVITY 39

If there is a health and safety policy in your laboratory, check that it covers all the necessary areas and working practices in your screening programme.

If it does not, talk to the person who is responsible for health and safety and ask how regularly the policy is rewritten and updated. If you can suggest any ways of improving the handling and disposal of potentially hazardous waste, note them down on your Action List.

Remember that laboratories contain many potential hazards because of the nature of the substances used and the tasks performed. All members of staff must be appropriately trained so that they are aware of all these potential hazards and follow the safety procedures at all times.

SUMMARY

1 The instructions provided by the manufacturer of a screening assay must always be followed exactly to ensure that the assay has been performed correctly and the results are reliable.

2 Initially reactive samples should be retested to confirm the result, using a different assay from the initial test.

3 Safety precautions must always be observed when dispatching samples for confirmatory testing.

4 Accurate and complete records must be kept of screening results. They should be stored safely, using a system that enables easy retrieval for future reference to ensure that TTI-positive donors can be identified and excluded from donating blood in the future, while maintaining confidentiality.

5 TTI-positive donations must be correctly identified, removed from storage and disposed of safely. An accurate record should always be kept of the disposal of positive donations.

6 Health and safety procedures should be followed at all times when handling assay components and waste.

SELF-ASSESSMENT

20 How does confirmatory testing differ from repeat testing?

21 What are the five precautions that should be taken when despatching pathological specimens through the post?

22 Why is it important to keep accurate records of assay results?

23 *Why is it important to be able to retrieve previous testing data?*

24 *If autoclaving is not possible, what is the next best method of safely disposing of TTI-positive donations?*

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 Use assays correctly to develop an effective TTI screening programme.

2 Determine the correct screening results for a set of donor samples.

3 Maintain accurate and complete records of the screening results.

4 Review the health and safety procedures in your laboratory for handling and disposing of TTI-positive donations, assay components and waste.

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

Quality Systems in Screening for Transfusion-Transmissible Infections

The purpose of this section is to consider the importance of quality systems in the maintenance of an effective TTI screening programme, regardless of how many tests are performed or how many staff are employed.

LEARNING OBJECTIVES

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

1 Review the overall quality system in your laboratory.

2 Review the system for documenting screening procedures in your laboratory.

3 Contribute to the preparation of standard operating procedures (SOPs) and follow them correctly.

4 Identify any weak areas in your own laboratory quality system.

7.1 THE NEED FOR QUALITY IN TTI SCREENING

Section 4 of the Introductory Module deals in detail with quality and implementing a quality system. Reread that section before continuing with this module, particularly if you are still unclear about the terminology used, and are not familiar with standard operating procedures.

As you saw from the Introductory Module, there are many aspects of the collection and processing of donated blood which must be constantly monitored to ensure quality throughout the blood transfusion service. In this section, we shall focus on quality in the specific context of the screening of blood to ensure that the blood and blood products are safe for transfusion or other specified purposes.

No matter what assay is used or how many tests are performed, a quality-orientated approach is essential in the successful operation of any screening programme. Quality systems must be developed and applied in *all* blood transfusion centres, laboratories and hospital blood banks. However it is important to develop a quality system that is appropriate to the individual BTS/laboratory and its specific activities.

Let us consider some of the constraints that a screening laboratory may face. These will vary from laboratory to laboratory, but in the area of screening for infectious agents they are most likely to include technical, financial and time constraints.

1 Technical constraints include the ability of a laboratory to perform a certain type of assay and to ensure the sensitivity of the assay that is used.

2 Financial constraints include the amount of money available to use for screening in relation to the actual costs of screening for the laboratory.

3 Time constraints include the amount of time available to test the blood before it is required for transfusion to a patient.

It may be difficult to overcome some of these constraints, but it should still be possible to develop and maintain a quality system that is appropriate and feasible in your particular circumstances.

7.2 QUALITY SYSTEMS

The introduction of a quality based approach, or even the improvement of an existing quality system, starts with a recognition of the importance of a consistent quality focused approach to procedures and working practices in order to provide a safe and effective product at all times. As you saw in the Introductory Module, introducing a quality system essentially involves five stages.

1 Seeking and obtaining high-level support and resources.

2 Assessing what is required in order to achieve quality.

3 Planning the action that needs to be taken and identifying the best way to do it.

4 Implementing the changes required, including establishing systems for monitoring and control.

5 Monitoring the system to assess how well it is operating and to identify any further changes needed in order to ensure that quality is maintained.

The basis for any quality system is that procedures are defined and documented and can then be monitored or recorded in some way as, for example, in the case of an assay failure. Improvements can then be made to the procedure because there is a full record of its operation. Many aspects of laboratory work can be documented and form the basis for a quality system, including:

1 The sample collection date.

2 The sample test date.

3 The identity of the test run in which each sample was included.

4 The identity of all samples in each testing run and a record of the position of these samples in any testing system.

5 The manufacturer, product number, lot or batch number and expiry date of the assay kits used.

6 The name of the operator and the supervisor.

7 The assay procedure.

8 The results obtained (eye-read and manually-transcribed results, if necessary).

9 The preparation of any reagents or buffer solutions used in the assay which were prepared in the laboratory.

10 The preparation and maintenance of any equipment used to perform the assay. If incubators are used, temperature calibration checks should be included.

11 Temperature monitoring records for the laboratory itself (this is important if room-temperature incubations are required), incubators, water-baths and refrigerators used for the storage of assay kits or reagents.

12 Maintenance and calibration records for the equipment used, including any mechanical pipettes.

13 Records of the disposal of any positive donations, with details of the retrieval of the packs and any products prepared from them, and the actual destruction of the packs.

These are some of the main areas where written records are important. You may be able to think of some more.

ACTIVITY 40

Look at the list above of areas where written records are important. Are they all documented in your laboratory?

If any of these aspects of laboratory work are not documented, talk to your supervisor about the importance of recording them in the future and about any suggestions you may have about additional procedures that should be documented. Note down your recommendations on your Action List.

7.3 STANDARD OPERATING PROCEDURES

Standard operating procedures (SOPs) are an important part of the written quality system. An SOP is a set of written instructions on how to perform a specific task. Thus, any laboratory should have a number of SOPs covering all the important tasks within the laboratory.

A laboratory that tests 60 donations per day using an EIA, for example, should at least have SOPs for the following:

- the reception and handling of samples
- performing the assay
- use and maintenance of the plate washer
- use and maintenance of the plate reader
- use and maintenance of the pipettes
- issuing the results
- retrieving and disposing of positive donations
- cleaning and decontaminating the laboratory.

It is important to understand that the instructions given in an SOP are not provided simply as a guide to help someone perform the particular task, but that they set out the *only* way that the task is to be performed in that laboratory. They are therefore a set of instructions to be followed at all times by all staff.

It seems sensible to be strict about the way tasks are performed when you consider all the tasks that are necessary to ensure that the screening programme runs smoothly. However, all commercial assays are provided with a set of instructions and all equipment comes with the necessary instructions. Why then do we need SOPs for these aspects of screening?

The reason is that the manufacturer's instructions are instructions for general use and they may not be sufficient or appropriate in every situation because they do not relate to each laboratory's specific requirements. For example, a laboratory's SOP covering the use of a particular assay may include such additional information as:

- the types of samples tested
- the preparation of any reagents
- the checking and recording procedures needed
- the handling of samples before testing
- the use of the kit controls and any external controls
- the use of the equipment required to perform the assay (this would simply be mentioned briefly in the assay SOP,

with references to separate SOPs for the use of each piece of equipment)

- the use of the assay itself
- the recording of any testing data on laboratory worksheets
- the method of reading the assay results
- the calculation of the final results
- the use of these results (this would simply be mentioned briefly in the assay SOP with reference to a separate SOP for overall result handling)
- clearing and cleaning up at the completion of testing.

As you can see, an SOP provides detailed instructions on the way in which the assay should be performed in your particular laboratory and within your screening requirements. Furthermore, the same applies to any equipment used; the SOP covers the way in which the equipment should be used as part of your assay procedure. In all cases where manufacturers' instructions are provided, they should be incorporated into the SOP and can be attached as an appendix to it.

You will find an example of an SOP covering the performance of a simple EIA anti-HIV screening assay in Appendix 2. Further examples of SOPs are included in the Introductory Module and Module 1.

Preparing an SOP

Ideally, the preparation of an SOP should be a team effort. The first draft should be written by the people performing that particular job. The final draft should then be prepared by the head of the laboratory, but this should be rechecked by a member of staff who performs that job, before the SOP is issued. In order to ensure an SOP is written in the correct way, a set of general guidelines should be provided so that all staff understand exactly what should be included in the draft, and in what order.

The contents of an SOP obviously depend on the particular procedure that it covers but, as the Introductory Module showed, SOPs can usually be broken down into six main sections including:

- **purpose**: the purpose for which the SOP has been written
- **responsibilities**: the responsibilities of the different grades of staff who may use the SOP or be in charge of the work area in which the SOP is used
- **restrictions**: who may and who may not have use of the SOP, where the SOP is to be used, what the SOP is to be used for
- **definitions**: the definitions of words, phrases or abbreviations used in the SOP
- **items required**: items required to be able to follow the SOP, including other documentation required (such as SOPs, forms, health and safety guidelines), equipment
- **procedure**: precise details of the procedure, clearly described in numbered steps that logically follow the

working sequence, including any quality control procedures involved, the procedure for interpreting and reporting the results and the action to be taken if problems occur.

In addition to the basic framework and contents, the SOP should be uniquely identified with:

- a unique SOP identity number including version number
- the date when the SOP was written or revised
- the name of the person who prepared or revised the SOP.

Appendices may be included which provide any additional relevant documentation or information, such as copies of any standard forms or labels to be completed or used during the procedure, operating instructions and methods of use recommended by manufacturers of equipment and diagnostic reagents. However, they should only be included if there is a clear benefit to the user of the SOP.

Instructions in the SOP should be written in the imperative.

Each SOP should be reviewed regularly, ideally at least once each year, in case any modifications are required as a result of direct changes in the procedure, such as amendments in the manufacturer's instructions for performing an assay, or indirect changes in the procedure, such as alterations in an associated procedure that affect certain parts of the SOP.

ACTIVITY 41

Are SOPs used in your laboratory? If they are, assess how effective they are in contributing to a quality system, by answering the following questions:

- *Are there SOPs for all the major activities in the screening programme, such as those listed on page 98?*
- *Do the SOPs cover all aspects of the procedures?*
- *Are they followed at all times by all members of staff involved in performing the procedures?*
- *How frequently are they reviewed and, where necessary, updated?*

If you can suggest any ways in which the preparation and use of SOPs could be improved in your laboratory, note them on your Action List.

If SOPs are not yet used in your laboratory, identify an important task that you perform regularly as part of your job and try to develop a simple SOP. Discuss it with any colleagues who also perform the same task and then ask your supervisor to review it and amend it, where necessary. Once it has been finalized, ensure that all members of staff follow it at all times.

Note down on your Action List any other areas where you think that SOPs are required and discuss them with your supervisor.

7.4 LABORATORY WORKSHEETS (FORMS)

It is clear that there are many areas within the laboratory where a written record is either essential or would be beneficial. Some laboratories, however, face the problem of having insufficient staff to maintain complete records since ideally at least two people are required: one to perform the action and the other to check the action. In a laboratory with only one main or regular staff member, how can a quality system be maintained in the screening programme?

It can be argued that in a very small laboratory, even though only small numbers of donations may be screened, quality is even more important since all the responsibility rests with just one person. It could be easier for mistakes to go unnoticed. Some sort of quality system is therefore essential.

In this kind of situation, laboratory checklists that can also serve as laboratory worksheets are perhaps the simplest and most appropriate means of maintaining a quality system. All the assays performed in the laboratory could be included on one checklist or a different checklist could be used for each assay. The checklist should detail all the required actions in the correct sequence, and should have space or boxes for recording whatever other information may be required, including signatures and dates.

The checklist can then be used to record the actions that have been taken when performing an assay, as well as to record any other details required. After the screening has been performed, the completed worksheet becomes the record of what has happened and thus completes the quality circle providing part of the traceability through the laboratory. It becomes the quality record of that batch of testing.

ACTIVITY 42

Design a simple checklist that would be suitable for use for any screening assay in your own laboratory.

7.5 QUALITY AUDITS

The use of SOPs and laboratory worksheets forms the basis of a quality system, but there are many areas within the laboratory that also require regular monitoring to ensure that a full quality system is in place. The purpose of a quality audit is to check the integrity of the quality system, that it is comprehensive, effective and, importantly, actually followed. A key part of this is traceability, the ability to trace what has happened to a unit of blood from donor to patient. If, for example, a particular pack of blood was selected that had been fully tested and cleared for transfusion, could the testing details of that donation be traced back throughout the laboratory screening programme and, ultimately, to the donor? As well as checking the screening results for the donation, the validity of the testing itself would be examined: for example, did the controls perform as expected and were the results obtained within the defined ranges? Was the test run that included that particular donation valid?

Clearly, it would not be possible to trace the testing details of a donation unless all the relevant information were recorded together with the screening results. What other information is needed to demonstrate that the assay was not only performed correctly, but that all the associated actions were performed and checked, and the complete testing run was valid? The answer to this question depends on the type of assay in use. The more complex assays require more complex associated actions. However, the list on page 97 covers the basic information that would be expected to be available.

If these records are maintained, they will be able to validate the test run and thus complete the microbiology part of the audit. As well as providing validation for quality audits, they are important in the continuous monitoring of the performance of the laboratory so that appropriate action can be taken if any problems occur.

ACTIVITY 43

From your transfusion records, select a donation that was tested three or four months ago. Follow the testing records back to the donor.

Were you able to do this? If you could not follow the records of the donation back to the donor, at what point did the record-keeping system fail?

Talk to your supervisor about your findings and try to suggest ways of improving the record-keeping system to ensure that an audit trail could always be followed. Note down your recommendations on your Action List.

SUMMARY

1 A quality based approach must be applied to every screening programme, even in the smallest laboratories.

2 Written records are the basis of a quality system because they enable procedures to be monitored.

3 SOPs should be prepared for all the main tasks undertaken in the laboratory.

4 A simple means of maintaining a quality system is to use laboratory checklists that also serve as worksheets for recording any actions taken and then become the final records for the work performed.

5 Quality audits enable the quality system to be assessed and its effectiveness determined.

SELF-ASSESSMENT

25 Why are SOPs required for the use of assays when instructions are provided by the manufacturer?

26 Why is a quality system particularly important in a small laboratory with only one member of staff?

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 Review the system for documenting screening procedures in your laboratory.

2 Contribute to the preparation of standard operating procedures (SOPs) and follow them correctly.

3 Identify any weak areas in your own laboratory quality system.

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, if there is anything you are still not sure about.

8

Screening for Other Transfusion-Transmissible Infections

The purpose of this section is to consider the other most significant infectious agents that can be transmitted by blood and blood products:

- hepatitis B virus (HBV)
- hepatitis C virus (HCV)
- human T cell leukaemia viruses I + II (HTLV I + II)
- *Treponema pallidum* (syphilis)
- *Plasmodium* species (malaria)
- *Trypanosoma cruzi* (Chagas disease).

The detailed aspects of the main screening tests used have already been considered in earlier sections. In this section, therefore, we shall simply outline the natural history of these infectious agents, including the routes of transmission and the risks of transmission by blood transfusion. Although some of these agents may not be a problem in your country, they represent those commonly considered to be both transmissible and transmitted by transfusion. You may wish to study only those agents that are known to be prevalent in your country although, with changes in the global distribution of infectious diseases, it is important that blood transfusion services should be aware of potential threats to the blood supply and have monitoring programmes in place to identify any emerging transmissible diseases in their country or region.

Please note that there are no activities in this section although you may wish to repeat some of the relevant activities in Section 3: *The Human Immunodeficiency Viruses*, but applying them to these other infectious agents.

The principles discussed in this module apply to screening for any infectious agent that may be transmitted by blood transfusion. However, the range of commercially available screening tests varies according to the agent.

In countries where the prevalence and incidence of certain infectious agents are very low, it may not be necessary to screen routinely for them. In other countries, where infection by an agent is endemic, screening may not be considered to be sensible or practical. Such considerations are important because, although blood safety is important, the resources available to ensure the safety of the blood supply are often limited and must be used carefully and wisely to ensure maximum effectiveness.

The information given in this section about these infectious agents is not intended to be comprehensive, but should enable you to understand the agents, the mechanisms of infection, the laboratory screening needed and the significance for transfusion practice. The figures in this section are included to provide additional information, but you do not need to memorize them. If you need further information, consult an appropriate textbook.

LEARNING OBJECTIVES

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

1 Describe the basic features of infection with HBV, HCV, HTLV, *T. pallidum, Plasmodium* species and *T. cruzi*.

2 Explain the significance of these agents for blood transfusion practice.

3 Describe the laboratory screening needed to identify donations from infected donors.

8.1 HEPATITIS B VIRUS

The infectious agent

Hepatitis B virus (HBV) is a DNA virus of the hepadnavirus family. The virus is transmitted parenterally, including through intimate contact, and infection may follow one of two courses:

- acute infection with the subsequent clearance of the virus and development of immunity
- chronic infection with the persistence of virus replication for extended periods, even the lifetime of the individual.

HBV infection can lead to severe disease: cirrhosis, hepatocellular carcinoma (HCC) and ultimately liver failure. Asymptomatic infections are very common, however, with most individuals resolving infection and developing immunity without any symptoms or with only mild symptoms. The prevalence of HBV varies across the world from 0.1–0.2% in low endemic countries, up to 3% in some Mediterranean countries and up to 15% in Africa and the Western Pacific region. The rates are even higher in some isolated communities. It is estimated that there are at least 250 million chronic carriers worldwide and that approximately half of the global population has been infected by HBV.

Structure

Two main types of circulating virus particles are identified following HBV infection: the "infectious" virion, the Dane particle, and a vast excess of smaller particles that consist only of viral surface protein with no DNA and that are therefore not considered to be "infectious".

The virus particle (virion) is 42 nm in diameter and is known as the "Dane particle" after the scientist who first identified it in the serum of infected individuals. This is the "infectious particle" in HBV infection. In the centre of the virion is the capsid core, which is 27 nm in diameter. It contains both the viral nucleic acid and the enzyme DNA polymerase which is essential for successful infection by the virus.

A number of important proteins are found in the particle. The major protein of the virion is known as hepatitis B "surface" antigen (HBsAg) which completely encloses the capsid. HBsAg is produced in excess by cells infected with the virus and is released into the circulation as the small "noninfectious" particles of pure HBsAg. The capsid contains two major proteins: hepatitis B "core" antigen (HBcAg), and hepatitis B "e" antigen (HBeAg). HBcAg itself cannot be detected in the circulation, but is detectable in infected liver cells. Circulating HBeAg appears fairly early on in infection and, in general, indicates viral replication and the release of infectious virions.

The smaller particles (either spherical particles of 22 nm diameter or small tubular particles) containing only the major viral protein, HBsAg are released into the serum of infected individuals. They are produced in a large excess to that needed to form new virions and this excess is continually released into the circulation during infection. These smaller particles are therefore considered uninfectious, although they act as a marker of infection – both acute and chronic.

Transmission of HBV

The transmission of HBV is essentially by the parenteral route which involves direct contact with body fluids. Thus, the most common routes for infection are:

- contact with infected blood; either by exposure of wounds to infected blood or to contaminated needles, syringes or knives used, for example, in surgical interventions, injecting drug use, tattooing, body-piercing, acupuncture or ritual scarification
- sexual contact
- neonatal or perinatal transmission, usually at birth and not during pregnancy, from cervical secretions or from close contact afterwards
- transfusion of infected blood or blood products.

Clinical course of infection

Following infection, there is an incubation period which can last from 30 to 180 days. During this time, no symptoms are seen but virus may be detected in the bloodstream. Symptoms such as fever, rash and jaundice may appear during the acute phase of infection, but the severity and duration of infection vary greatly. In mild cases, jaundice often does not develop. More severe cases can produce serious disease. Acute infection is self-limiting and usually lasts no longer than 4 months. As the infection resolves, immunity develops. This generally provides lifelong protection. Figure 46 shows the consequences of HBV infection.

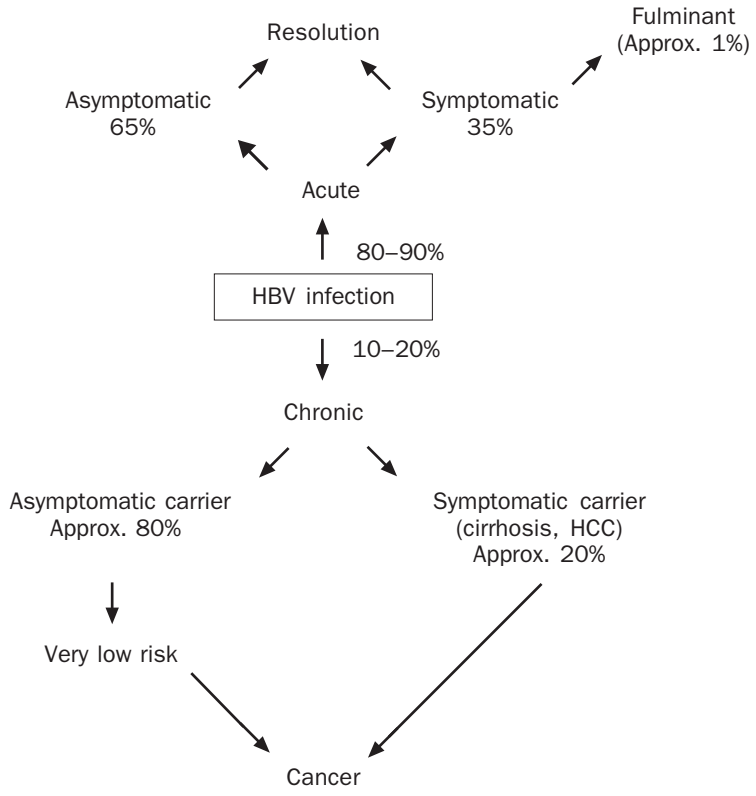


Figure 46: Consequences of HBV infection

However, approximately 10–20% of individuals who have been clinically diagnosed as having HBV infection do not resolve their acute infection and a period of chronic infection begins. In developing countries with a high incidence of HBV infection, as many as 90% of perinatally-infected infants and 20%–50% of childhood infections develop into chronic infection. Chronic infection may last from months to years and then:

- spontaneously resolve with the development of immunity
- reactivate with a new acute episode and the possibility of fulminant hepatitis.

In some individuals, chronicity may simply persist for life. It is in these persistently chronically-infected individuals that serious consequences of infection may develop, often slowly over a period of years. In some individuals, no ill-effects are seen. In many cases, however, chronic liver disease eventually develops which may lead to cirrhosis, primary liver cancer and then death. The risk of death from cirrhosis or liver cancer is approximately 40% in male HBV carriers and at least 250 000 cases of hepatocellular carcinoma are reported annually, making it one of the most common cancers in the world.

Laboratory testing for HBV infection in blood donations

Following infection with HBV, a range of circulating markers are detectable, which reflect the replication of the virus and the body's immune response to it. These markers are not static but change over time as the infection progresses. Figure 47 on page 109 shows the usual profiles of these markers in acute and chronic infections. However, these normal patterns may not be seen in all infected individuals, atypical patterns are seen. The stage and the progression of infection can usually be determined by identifying the markers present in a serum/plasma sample or in sequential samples from the infected individual. Specific test kits are available for all circulating markers of HBV infection and these are used in the laboratory to determine the stage of infection at any point in time.

The first circulating marker to appear is viral DNA, which appears as replication begins and virus is released from infected cells. This is followed soon afterwards, sometimes simultaneously, by the appearance of HBsAg which very quickly reaches high titres. HBeAg appears next and the presence of HBsAg and HBeAg are considered to indicate active viral replication. Within 1–2 weeks, IgM anti-HBc usually appears and this is used as an indicator of a recent infection. IgM levels drop within about 4 weeks and IgG anti-HBc persists, usually lifelong, irrespective of subsequent acute or chronic infection. The end of the acute stage is marked by the gradual fall in titre of HBsAg, with the subsequent appearance, 4–6 weeks later, of anti-HBs. Persistence of HBsAg for at least 6 months is considered to indicate chronic infection. The appearance of anti-HBs indicates resolution of infection and immunity. HBeAg levels fall as anti-HBe appears, but may persist if the infection becomes chronic; this is known as infectious carriage. Chronic infections that are anti-HBe positive are termed noninfectious carriage.

The detection of HBV infection in donated blood is achieved primarily by screening for HBsAg as it is the first serological marker to appear in the

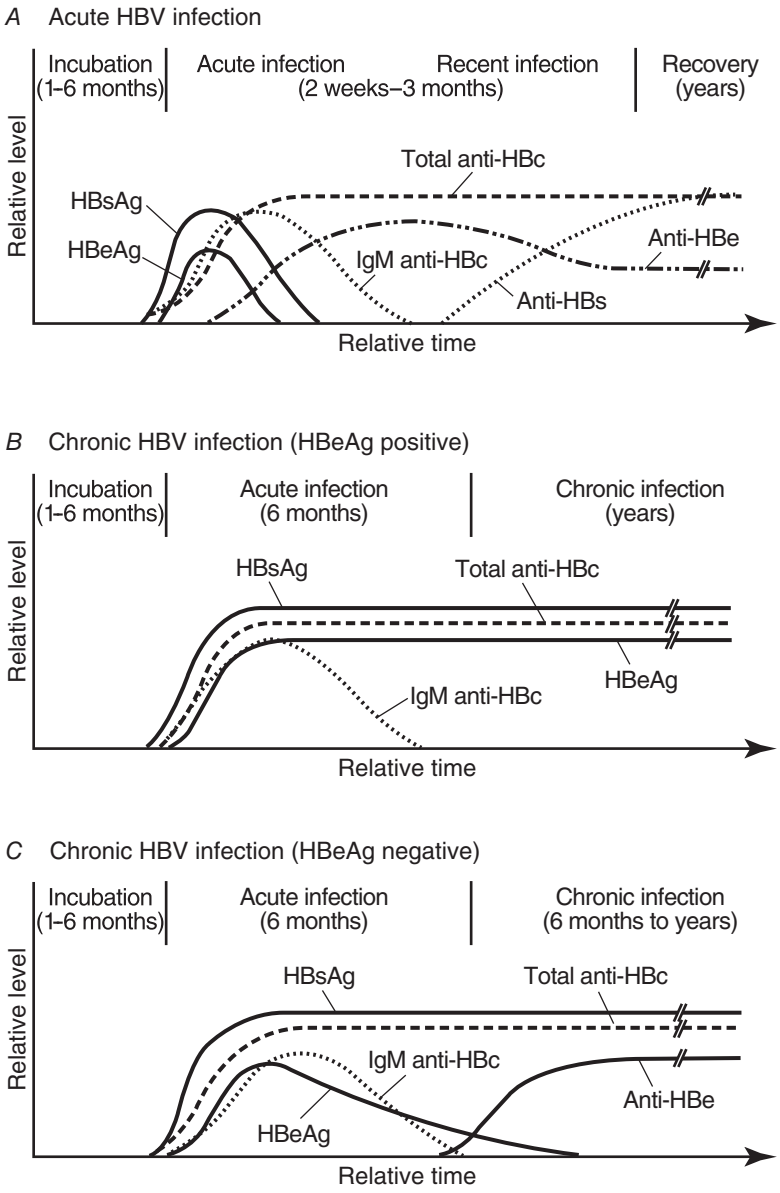


Figure 47: Serological profiles of acute (A) and chronic (B,C) hepatitis B virus infections

bloodstream. It then rises rapidly to a high titre, and persists in chronic infections, making it an ideal screening marker. In general, the other serological markers of HBV infection are of use in confirming infection and determining the type and stage of infection but, apart from anti-HBc, have no value in routine blood screening.

The use of anti-HBc, in addition to HBsAg, in the screening of donations has been the subject of discussion for many years. Before HCV was identified and anti-HCV screening was introduced, anti-HBc screening was considered by some to be a surrogate marker for post-transfusion non-A, non-B hepatitis (PTNANBH) which was at that time a significant problem in transfusion medicine. It was subsequently shown to have little screening value in most populations. However, anti-HBc screening may have a value in identifying the small number of donors who are either resolving an acute infection or clearing a chronic infection: these are apparently HBsAg-negative on screening, but may still have a low level viraemia and be infectious – often referred to as "tail end carriers". In

such individuals, anti-HBc may be the only detectable circulating marker of infection and thus may be identifiable only by anti-HBc screening. This could explain the occasional reported cases of post-transfusion HBV resulting from transfusion of donations screened as HBsAg-negative; in these situations, unfortunately, it is often difficult to demonstrate that the patient had no other risks of infection.

Significance for transfusion practice

In the past, transmission via the transfusion of blood and blood products was a significant route of HBV infection. A clear example was the incident in which a large number of American army personnel were vaccinated against yellow fever using a vaccine prepared from plasma from individuals who had recovered from the disease but at least one of whom also had acute HBV infection. This was subsequently transmitted to most of the recipients of the vaccine. The screening of donations for the presence of HBsAg is now routine in many countries and this has helped to reduce considerably the occurrence of post-transfusion hepatitis. It has been shown that the severity of infection is related to a number of factors, one of which is the size of the infecting dose. Blood transfusion can therefore be an efficient route of transmission of HBV because a large amount of infected material is passed directly into the bloodstream of the recipient.

HBsAg mutants

Variants of HBV have been described which result from mutations in the viral genome. Very broadly, these can be divided into two groups: those affecting the core region of the genome and those affecting the surface antigen region. The mutations in the core region of the genome have normal HBsAg production and are currently not a major concern in blood transfusion. However, the mutations in the HBsAg region are of concern to blood screening since HBsAg expression may be altered such that some assays may fail to detect some HBsAg mutant forms. The extent of the problem is hard to assess critically and, although mutants are being identified and there are reports of variable reactivity with assays, their frequency is very low; a proportion of those that have been identified were as a result of specific searches for such mutants and not as a result of transmissions from donations screened as HBsAg-negative.

Hepatitis delta virus (HDV)

Hepatitis delta virus (HDV) is a small RNA virus, currently not firmly classified, that requires co-infection with HBV, replicating only in the liver cells of individuals already infected with HBV. Although transmissible by transfusion, screening for HBsAg will also prevent transmission of HDV because the virus needs the presence of HBV to replicate.

Prevention of spread of infection

Vaccination is the approach that is now being used globally to reduce the rate of transmission, especially through childhood vaccination programmes. This is a long-term approach, however, and the programmes take some time to reach full effectiveness. The screening of all blood and blood products, tissues and organs is therefore an important means of

reducing transmission by these routes. General public health education programmes help to minimize transmission by the sexual and injecting drug use routes. Specific HBV immunoglobulin is still also used in some situations where immediate protection is required, such as the prevention of infection in infants born to infected mothers or resulting from needlestick injuries where there is a significant risk of HBV infection and the injured person has not been vaccinated for HBV.

8.2 HEPATITIS C VIRUS

The infectious agent

Hepatitis C virus (HCV) is an RNA virus of the flavivirus family. Although infections with HCV have been recognized for many years, the virus was finally identified and characterized only in 1989. The virus is transmitted parenterally, mainly through injecting drug use and via transfusion, although there are still a significant number of cases where parenteral routes cannot be identified. Currently, like HBV, infection is known to follow one of two courses: acute infection which is both fairly rapid and relatively mild in its course or chronic infection which may persist for many years with a significant number of cases progressing to cirrhosis and hepatocellular carcinoma.

The main difference between HBV and HCV is that only about 20–40% of HCV cases are acute; the majority progress to chronic infection. The carrier rate varies across the world from 0.05–0.5% in low endemic countries such as western Europe, north America and Australasia; 1–5% in parts of southern Europe, south America, Asia and Africa; and as high as 20% in Egypt and specific regions of some Asian and African countries. Current estimates are that at least 200 million people are infected worldwide and, in many areas, the long-term significance of subsequent disease due to cirrhosis and HCC in these individuals is greater than that of HBV.

Structure

Although HCV was finally identified in 1989, the intact virion has still not been specifically isolated and characterized. The virus is a single-stranded, enveloped, RNA virus 50–80 nm in diameter with a genome of approximately 9 400 nucleotides. Work with the virus has all arisen from the sequencing of the viral RNA and the expression of viral proteins from that RNA. Proteins expressed from the nucleic acid sequence of the virus are used to develop assays to detect the presence of antibody to the virus and, more recently, viral antigen.

Transmission of HCV

The transmission of HCV is essentially by the parenteral route, which involves direct contact with body fluids. Thus, the most common routes for infection are:

- contact with infected blood; by exposure of wounds to infected blood or to contaminated needles, syringes or knives used, for example, in surgical interventions, injecting drug use, tattooing, body-piercing, acupuncture or ritual scarification

- transfusion of infected blood or blood products (a significant route of infection where screening is not performed)
- transplantation of organs and tissues
- neonatal or perinatal transmission; probably at birth, and not during pregnancy, but breastfeeding has not been identified as a route of infection; the risk of infection appears to correlate to the level of circulating RNA during the pregnancy
- sexual and close domestic contact.

There are sporadic cases in which no defined route of infection can be identified. In some countries, up to 30% of cases fall into this category although, in a proportion of them, it is quite likely that one of the above defined routes may be the cause, although the precise event(s) is not obvious and cannot be specifically identified. Many healthy blood donors, who have not been transfused and have no other stated lifestyle risks, have been found to be HCV-positive.

Clinical course of infection

Clinically, acute HCV infection resembles HBV infection, although it is generally much milder. Infected individuals often primarily present with fatigue and an overall feeling of being unwell. Some symptoms are related to circulating antigen–antibody immune complexes giving rise to the cryoglobulinaemia strongly associated with chronic HCV infection. Fluctuating liver enzyme (transaminase) levels, which can be used to give an indication of the current disease process, are a significant feature of HCV infection.

It is estimated that up to 80% of HCV-infected individuals may develop chronic infection, of whom up to 60% may develop chronic liver disease leading to death from cirrhosis or primary liver cancer. Figure 48 shows the consequences of HCV infection.

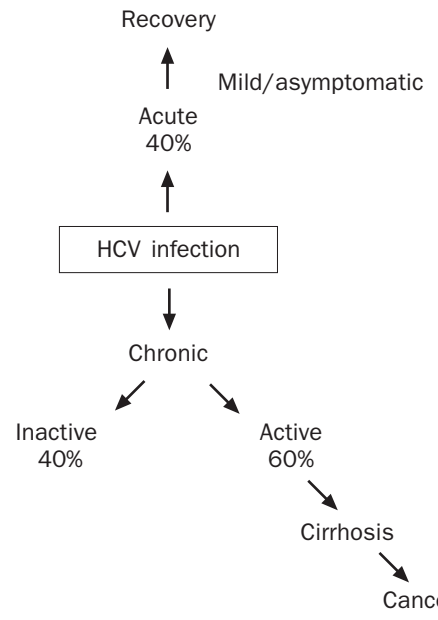


Figure 48: Consequences of HCV infection

Laboratory testing

It is important to understand how the tests for HCV have been developed because this process has been quite unique to HCV and has had an impact on diagnostic testing. As mentioned above, to date, the virus itself has not been isolated intact or cultured in vitro. The nucleic acid sequence has been used to construct recombinant and peptide antigens which have then been used in the laboratory tests available. This approach differs from the usual development of viral serology tests which generally originate with the isolation of the virus itself, from which crude antigen preparations are then made. Following this, recombinant and peptide antigens are subsequently produced, but with knowledge of the behaviour of the crude "native" antigens being used in the design of the recombinant and peptide antigens. This has not been the case with HCV; "native" antigens have not been isolated and the tests used jumped straight to the use of recombinant and peptide antigens. The first screening tests for anti-HCV were relatively basic and used only one HCV recombinant antigen, which was not very specific. These tests have developed significantly since the first commercially available screening test was developed in 1990 and now use a combination of antigens from different regions of the genome.

Currently, following infection with HCV, three specific circulating markers of infection can be used to identify infected individuals. The first marker is viral RNA, which appears as replication begins and virus is released from infected cells. This is followed soon afterwards, sometimes simultaneously, by viral antigen (HCVAg) which reaches reasonable titres, but not as high as with HBV infection. Within 1–4 weeks, specific antibody appears. IgM can be demonstrated very early in the immune response, but only at low titres and these fall rapidly, and appears to persist for life in most individuals although this may subsequently not prove to be the case. Cases of seroreversion have been reported in individuals who have resolved their infection and chronicity has not developed. As antibody titres rise, antigen titres fall as the free antigen is rapidly complexed with the antibody. Viral antigen can still be detected in individuals in whom viral replication is continuing if an antigen test utilizing an immune complex dissociation step (ICD) is used.

The detection of HCV infection in donated blood was initially achieved by screening for anti-HCV and this remains the main test performed today. The detection of antibody indicates both current and past infection. As technology has developed, however, some countries now augment the serology with the detection of HCV RNA. Because there can be a relatively long window period for HCV, it is possible to encounter a donor who has circulating viral RNA, but no detectable antibody. While tests for viral RNA will detect such donors, this type of testing requires a much higher level of staff skills and expertise, infrastructure and resources than serology, and is significantly more expensive. In many countries, any benefits of such testing may actually be outweighed by the costs. A test for HCV antigen has now been developed and made commercially available for blood screening; the test is a standard EIA format. Although not quite as sensitive as HCV RNA, testing for HCV Ag can reduce the window period significantly and provides a far simpler and less expensive alternative to HCV RNA testing in situations in which nucleic acid testing is too expensive or not feasible.

Significance for blood transfusion

In the past, transmission via the transfusion of blood and blood products was a significant route of HCV infection. This is clearly demonstrated by the large numbers of individuals in multi-transfused groups with evidence of active or past HCV infection – haemophiliacs and thalassaemics being two of the groups most severely affected. The screening of donations for the presence of antibody to HCV (anti-HCV) is now routine in many countries with developed healthcare systems, and this has reduced significantly the occurrence of PTHNANB. In many countries, cases of PTHNANB are now virtually unknown, but blood transfusion remains a significant route of infection in countries that do not screen blood donations for HCV.

Prevention of spread

Apart from the screening of all blood and blood products, tissues and organs, the only other intervention currently available to prevent HCV transmission is the education of infected and at-risk individuals, mainly injecting drug users in whom transmission via shared needles and other devices is still common in many countries. The risk of transmission of HCV from mother to infant is not clear; nonetheless, advice about breastfeeding may be appropriate. Specific HCV immunoglobulin is not available as the antibodies do not appear to confer immunity, although neutralizing the viral antigen so far detected.

8.3 HUMAN T CELL LEUKAEMIA VIRUSES I + II

The infectious agent

oncogenic virus: A virus that causes the formation of tumours.

Human T cell leukaemia virus (HTLV-I) was the first identified human retrovirus. It is an **oncogenic virus** causing adult T cell leukaemia and lymphoma (ATLL or ATL) and tropical spastic paraparesis (TSP), also known as HTLV-I associated myelopathy (HAM). A second virus, HTLV-II, has also been identified in specific groups of individuals, such as injecting drug users, although no significant disease process has yet been associated with this virus. HTLV-I and -II are distinct, but closely related, viruses with a number of proteins in common as well as unique individual proteins. The distribution of both viruses is very localized, both geographically and in specific populations within defined areas across the world – mainly the tropics, Japan, Caribbean, parts of south America, Africa and Australia.

Structure

Both HTLV-I and HTLV-II are enveloped RNA viruses with essentially identical structures surrounding a central core. The virus is approximately 100 nm in diameter and has a spikey appearance formed by projections of viral glycoprotein from the surface of the viral envelope. The genome consists of two single-stranded linked RNA molecules that are contained within the core together with the enzyme reverse transcriptase, which is essential for viral replication and integration.

Transmission

It is thought that virus is virtually always cell-associated, infecting the CD^{4+} lymphocytes, and is transmitted in these cells parenterally via

blood or semen, or from mother to infant via breast milk. Once in the host, the virus integrates into the cell DNA and remains there for life; the appearance of antibody marks the resolution of acute infection, but not immunity. The transmission by breast milk is a major route of infection in some areas where HTLV-I is endemic. Studies on the transmission of HTLV-I indicate that the virus is not normally transmitted in utero, but is transmitted in early life through breast milk equally to both male and female children. Later in life, however, sexual transmission is almost exclusively from male to female.

Blood transfusion is another potentially significant route of infection. Early studies demonstrated the efficiency of transfusion transmission, but indicated that cell-free products, such as plasma, did not transmit infection. Fresher components from infected individuals are those most likely to transmit the virus. Additionally, recent findings appear to indicate that transfusion transmission may have a much shorter incubation period for the development of ATLL – as short as 1 year in some cases.

Clinical course

Most infections with HTLV-I in otherwise healthy individuals are asymptomatic and remain so. However, there is small risk that disease may develop at any time up to 40 years after infection. ATLL can present as an acute leukaemia of CD^{4+} lymphocytes and death usually occurs within a year of the onset of symptoms. TSP is a progressive disease involving the degeneration of neurones in the spinal cord, leading to gradual paralysis of the lower limbs.

Similarly, virtually all HTLV-II infections in healthy individuals are asymptomatic. More recently HTLV-I and II infection have been associated with certain inflammatory diseases.

Laboratory testing

Following infection with HTLV, there is an incubation period from 30 to 90 days before seroconversion. Prior to seroconversion, viral RNA can be detected in lymphocytes. At seroconversion, antibody to HTLV appears; this is the major target for the diagnosis of HTLV infection. After seroconversion, the antibody generally persists at high titres for life, even if clinical disease subsequently develops only much later in life, although it is not protective. The serological responses to HTLV-I and HTLV-II are very similar but, like HIV-1 and 2, there are sufficient differences to require specific tests for the detection of anti-HTLV-I and anti-HTLV-II.

Significance for transfusion

The potential significance of blood transfusion as a route of transmission has meant that, in a number of endemic countries, the screening of donations for anti-HTLV-I and II has been carried out for some time. However, because the virus is cell-associated, only cellular products are implicated in transmissions; virus has not been found in either acellular products, such as fresh plasma, or in any fractionated products that have been prepared in accordance with modern fractionation procedures and following current internationally agreed guidelines.

Screening has also been introduced in some nonendemic developed countries with mixed populations; in some instances, it is restricted to previously untested donors. In many other countries, debate continues on the need and value of screening donations.

Prevention of spread

The main focus in the prevention of spread of HTLV in endemic areas is the education of infected and at-risk individuals. Breastfeeding is known to transmit HTLV, a significant route in endemic areas, and advice must be given to infected mothers not to breastfeed. General public health awareness programmes can also help to limit the risk of sexual transmission and transmission through injecting drug use. Blood and organ screening is important in endemic areas and in nonendemic areas where the donor population may contain infected individuals. Specific immunoglobulin is not available, but anti-retroviral therapy can be effective if given early enough in infection.

8.4 SYPHILIS (*TREPONEMA PALLIDUM* INFECTION)

The infectious agent

Syphilis is the disease caused by infection with the bacterium *Treponema pallidum* which is a member of the class of bacteria known as spirochaetes. There are four major human treponemal pathogens which are so closely related that they are described as subspecies of *T. pallidum*:

- *T. pallidum* subspecies *pallidum* (syphilis)
- *T. pallidum* subspecies *pertenue* (yaws)
- *T. pallidum* subspecies *carateum* (pinta)
- *T. pallidum* subspecies *endemicum* (bejel).

Here we are primarily concerned with infection due to *T. pallidum pallidum* (which we shall simply refer to as *T. pallidum*) as it is the most important pathogen in the group. The other subspecies, which we shall also consider briefly, are notably different in their routes of transmission and their clinical significance and sequelae. There are also many nonpathogenic species that commonly infect all mammals.

Structure

Spirochaetes are long, thin, highly motile bacteria that have flexible, **Gram-negative** type, cell walls, composed of an outer membrane, a peptidoglycan layer and an inner cytoplasmic membrane. The outer membrane contains the antigenic proteins of the bacterium. Spirochaetes are generally relatively fragile organisms that are very sensitive to temperature and die rapidly outside their host. They cannot be cultivated on artificial media, although they can be cultivated in cell culture or in animals. They are very characteristic organisms and can easily be seen under dark-field microscopy.

Gram-negative: The Gram stain is a method of staining bacteria which is used to classify them. Bacteria are either Gram-negative or Gram-positive.

Transmission

Syphilis is endemic in many parts of the world. In some areas, the incidence of disease is increasing as a result of the breakdown of public

health systems and treatment programmes as a result of natural disasters, war and other civil unrest.

Syphilis is essentially a sexually-transmitted disease, although it can also be spread by other forms of close contact with mucous-membrane lesions. Congenital infection is a significant route, especially in endemic countries with poor healthcare systems and minimal or absent treatment programmes. The organism can pass across the placenta and infect the fetus. If this does not kill the fetus directly, the infant will be born with congenital syphilis. Since part of the life cycle of treponemal infection involves release into the bloodstream, parenteral transmission may occur and, in the past, blood transfusion was a potential route of infection, especially if fresh blood was transfused. However, after donated blood is stored for 24–48 hours at 4°C, any infection risk is essentially eliminated as the organism is very sensitive to temperature and is killed quickly at low temperatures, including freezing.

Clinical course of infection

The normal course of syphilis can be divided into early and late infection, the division being approximately two years from initial infection. Infection then follows the stages of primary, secondary and tertiary infections.

1 *Primary syphilis*: following initial contact, the spirochaetes pass through the mucous membranes and enter the lymphatic system, leaving a lesion full of treponemes (chancre) at the original site of entry. The normal incubation period ranges from 9 to 90 days. Without treatment, this heals in 4 to 8 weeks but the disease progresses to the secondary stage.

2 *Secondary syphilis*: 6 to 8 weeks after the chancre first appears, new secondary lesions begin to appear on the skin and mucous membranes. These are highly infectious as they are full of treponemes. There may also be a rash and/or generalized lymphadenopathy. Non-venereal spread of the infection can occur at this stage. Without treatment, latent syphilis follows; this is classed as early latent until the 2-year mark has passed when it then becomes late latent; late latency may last for many years.

3 *Tertiary syphilis*: this may occur any time from 5 to 40 years after the initial infection. This is the most destructive stage of the disease. Lesions may occur in the central nervous system, cardiovascular system, bones, skin, viscera and eyes where they can cause severe damage. Cases of tertiary syphilis, even in endemic areas, are not commonly seen.

In cases of congenital infection, the disease can lead to a number of severe disabilities, including blindness, deafness and bone disease if the fetus does not die during pregnancy and if the disease is untreated.

Laboratory testing

Although direct observation of spirochaetes in fluid from the lesions is possible using dark-field microscopy, this can be performed only at

certain stages of infection. Serology is therefore the major diagnostic method. After infection, specific IgM antibodies appear after about two weeks, with IgG levels rising from three to four weeks. IgG antibodies tend to persist for life, whether or not treatment has been given. Detectable antibody levels drop only in late tertiary syphilis, possibly because of the spread of the disease throughout the body with the significant tissue destruction that then occurs.

Two groups of tests are available: nonspecific tests and specific tests. The particular screening strategy developed needs to reflect whether the testing is for blood donors alone as well as the incidence of infection in the population from which the donors are drawn.

Nonspecific tests, such as the Venereal Disease Reference Laboratory test (VDRL) or Rapid Plasma Reagin test (RPR) use a mixture of highly purified cardiolipin/lecithin/cholesterol to detect a substance called reagin which circulates in the bloodstream of recently/actively infected individuals. The tests are based on flocculation or aggregation of the cardiolipin reagent, either free in suspension or absorbed to carbon microparticles, by the reagin. Reagin is a mixture of specific IgM and IgG antibodies and is a fairly sensitive marker of recent/active syphilis infection, although it is not particularly specific. Approximately 1–2% of normal adults produce nonspecific antibodies which may lead to false positive reactions.

Specific tests, such as the *Treponema pallidum* Haemagglutination Assay (TPHA) or conventional EIAs, use native *T. pallidum* or *T. pallidum* proteins (native or recombinant) as the antigens in a number of different types of test, all of which are aimed at the detection of specific antibody to the organism.

Many particle agglutination tests, using red cells, latex or gelatin particles, have been developed which are inexpensive, specific and sensitive. Since most of these tests can be used in a microwell format, they are suitable for the testing of a large number of samples. Many high quality EIAs have also been developed, but the cost of these assays is generally higher and most transfusion services in developed countries currently use particle agglutination assays to screen for syphilis.

Significance for transfusion

Syphilis was the first infectious agent shown to be transmitted by blood transfusion (1910) and, in the past, there was a reasonably significant number of transmissions. Occasional cases still occur even today in some countries with a high incidence of syphilis. However, it is very unlikely that transfusion has ever been a major factor in the spread of the disease.

In low-incidence countries, the vast majority of cases of syphilis identified in blood donors are due to “old” infections that have been treated successfully and present no risk of transfusion-transmission, although cases of recent primary acute syphilis are occasionally identified. With the exclusion of at-risk donors, screening for *T. pallidum* and the storage of most blood components at or below +4°C before transfusion, the risk of post-transfusion syphilis is generally very low and is almost negligible

in many countries. The only potential risk occurs with products that are not stored at or below +4°C prior to transfusion. The only product not routinely stored at low temperatures is platelet concentrates, but whole blood is sometimes transfused fresh and without testing. However, syphilis is a disease that can be treated effectively and completely so that, if transmission does occur, effective clinical intervention is available.

The infection of donors with syphilis is commonly used as a marker of donor suitability. Although syphilis is not a specific marker of HIV infection, it indicates donors who are at risk of sexually transmitted disease because of their particular sexual behaviour. Since this may increase their risk of exposure to HIV, donors with such patterns of risk behaviour should be deferred.

Prevention of spread

Prevention of the spread of syphilis is primarily by education and by the development of effective screening and treatment programmes. Sexually-transmitted diseases in general are a major cause of significant disease in many populations throughout the world and many countries are developing such programmes through STD (Sexually-Transmitted Disease) or GUM (Genito-Urinary Medicine) clinics. While the screening of all blood, tissues and organs is important, transfusion is only a very minor route of infection.

Other pathogenic treponemal infections

Yaws – infection due to T. pallidum pertenue

T. pallidum pertenue is the causative agent of yaws and is morphologically indistinguishable from *T. pallidum pallidum*. Yaws is restricted to tropical areas where it is spread by direct contact with open ulcers or by vectors such as flies. Bone involvement with considerable disfigurement of the face is commonly found in the tertiary stage. In areas where yaws is endemic, diagnosis is based on the clinical findings. The tests used for syphilis will give the same results with yaws.

Pinta – infection due to T. pallidum carateum

T. pallidum carateum is the causative agent of pinta, which occurs mainly in central and south America. It is also indistinguishable from *T. pallidum pallidum*, although the skin lesions are flat red or blue areas that do not ulcerate and eventually lose their pigment. The lesions are confined to the skin. Transmission is by direct person-to-person contact, notably through open sores, and probably via insect vectors. In areas where pinta is endemic, diagnosis is based on the clinical findings. The tests used for syphilis will give the same results with pinta.

Bejel – infection due to *T. pallidum endemicum*

T. pallidum endemicum is the causative agent of bejel, a non-venereal disease occurring in Africa and the eastern Mediterranean region. The disease occurs mainly in children living under poor standards of hygiene and is spread from person to person through the use of common eating and drinking utensils. The primary and secondary lesions are usually in

the mouth. Tertiary lesions are often more widespread and can be found on the skin and bones. In areas where bejel is endemic, diagnosis is based on the clinical findings.

Yaws and pinta may potentially be transmitted by transfusion, but few data exist. Transmission will generally occur only in endemic areas where patients may already be infected. Bejel is unlikely to be transmitted and infected individuals usually have obvious symptoms that would lead to donor deferral.

8.5 MALARIA

The infectious agent

Malaria is a systemic disease caused by infection with one of a number of members of the class of protozoa called sporozoa. Approximately 150 to 200 million individuals each year are infected with malaria and about two million die from the disease.

The four species of sporozoa recognized as causative agents of malaria in humans are:

- *Plasmodium vivax*
- *Plasmodium ovale*
- *Plasmodium malariae*
- *Plasmodium falciparum*.

Although there are some basic similarities in the lifecycles of the organisms and symptoms arising, there are also significant differences.

Structure and life cycle

Sporozoa are transmitted in various ways, but they are all parasitic for one or more animal species. An unusual finding is that they require two different animal hosts to complete their reproductive cycle: humans and mosquitoes. The natural history of infection with *Plasmodium* can be divided into two parts: within the human host and within the mosquito carrier. Figures 49 and 50 on page 121 show the life cycle in each host. We shall consider the life cycle within the human host first.

Life cycle in the human host

Infection starts when the individual is bitten by a carrier female *Anopheles* mosquito and the sporozoite form of the parasite is injected into the bloodstream. The sporozoites invade the liver where, depending on the species, they undergo a number of cycles of asexual reproduction before being released back into the bloodstream. After entering the liver cell, the sporozoite changes shape to form a round trophozoite. This enlarges and the nucleus divides to form thousands of individual nuclear bodies that form a schizont. The individual nuclear bodies are surrounded by cytoplasm and a membrane and are released as merozoites. These can then infect either red blood cells or other liver cells. This cycle of asexual reproduction in the liver cells is known as the pre-erythrocyte cycle.

The erythrocyte cycle starts when a merozoite infects a red blood cell. The cycle is very similar to that in the liver cell. The merozoite changes form

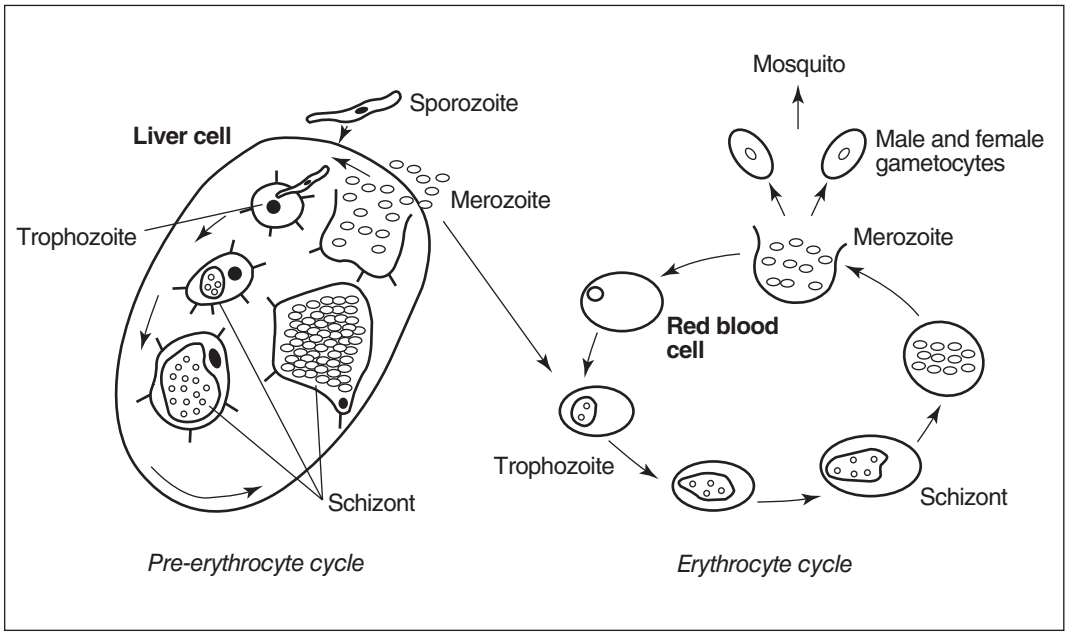


Figure 49: Life cycle of malarial parasite in a human host

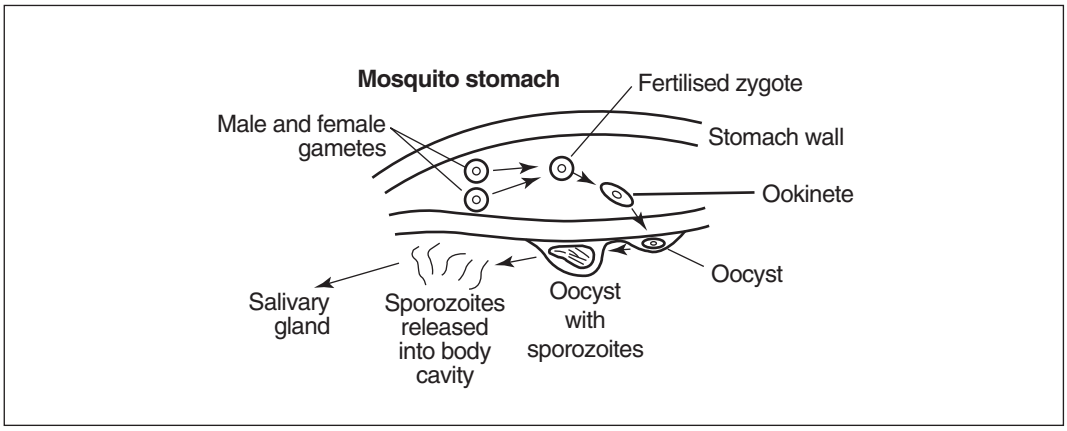


Figure 50: Life cycle of malarial parasite in an insect host

into an immature trophozoite which appears as the characteristic ring form in the red cell. This then develops into a large, more compact form and the nucleus divides into a number of nuclear bodies to form a schizont, the exact number depending upon the species. Schizonts are then enveloped and released from the red cells as merozoites.

After a number of cycles of asexual reproduction in the red blood cells, some of the merozoites form male and female gametocytes. These survive in the bloodstream for only 6–12 hours, after which they degenerate. If they are not ingested by a mosquito in that time, the life cycle of the *Plasmodium* ceases. Transmission to the mosquito cannot occur without these male and female gametocytes.

Life cycle in the mosquito host

When the gametocytes are ingested by the mosquito, they change to male and female gametes and fuse in the stomach of the mosquito, forming a zygote. This then changes form to become an ookinete which leaves the stomach and embeds on the outside of the stomach wall to form an oocyst. Inside the oocyst, sporozoites are formed which are

eventually released and spread throughout the body of the mosquito. Those that reach the salivary gland can then be transmitted to the next human host. The whole cycle within the mosquito generally takes 10–12 days.

Transmission

The parasite is spread to humans primarily through the bite of an infected female *Anopheles* mosquito. There are over 200 species of *Anopheles*, of which approximately 60 are known to be vectors of the malaria parasite. Humans themselves are the reservoirs of infection; a mosquito that has bitten an infected individual can pass malaria to noninfected individuals when feeding on them. The transmissibility of malaria through blood transfusion has long been recognized because of the phase of the life cycle of the parasite that involves the red cells. Donations from infected individuals may transmit malaria if the parasite is present in red cells in the donation.

Clinical course of infection

The clinical picture of infection varies with each species, but is usually cyclic in nature. The common symptoms are chills and fever at regular intervals and profuse sweating. Recurrent episodes can lead to severe anaemia. The incubation period ranges from 12 days for *P. falciparum* to 15 days for *P. vivax* and *P. ovale* and to as long as 30 days for *P. malariae*. Infection caused by *P. falciparum* is generally considered to have the most serious clinical consequences in humans and may be fatal in a significant number of cases when cerebral involvement occurs. Only *P. malariae* persists for extended periods in humans (up to 30 years); in general, *plasmodia* of the other three species are finally destroyed by the immune system after 1–3 years and die. The individual, if not reinfected, is then free from malaria.

Laboratory testing

Laboratory testing can be performed to detect either the malaria parasites in the bloodstream or specific antibody produced in response to infection. Parasites can be detected in the bloodstream as quickly as 48 hours after infection in heavily infected individuals. Antibody levels start to rise 3–4 weeks after infection and all infected individuals generally have specific antibody within 5–6 months. The development of specific antibodies may provide some degree of protection and amelioration of the disease process in multiply-infected individuals. The antibody response usually differs between individuals who have lived in an endemic area for a significant amount of time and who have been multiply-infected, and those from nonendemic countries who have been infected through a single exposure episode. Infected individuals from endemic areas are referred to as semi-immune and generally have low-titre antibody. This reflects the repeat exposure episodes in such individuals with frequent, if not persistent, low-level parasitaemia. Immunity is quickly lost on moving to a nonendemic area. Infected individuals from nonendemic areas, however, produce much higher titre antibody which then declines over the next one to two years, more quickly if treated, and in many cases disappears totally.

The direct detection of parasites in blood is possible by looking at blood films, using a specific diagnostic test for parasitaemia or even nucleic acid detection. In most cases, however, these are not sensitive enough to identify infected donors, except in endemic countries where infected donors may have high numbers of circulating parasites (parasitaemia). These approaches are also impractical for the mass screening of blood donations in most countries. However, donors with a high parasitaemia are usually symptomatic and should be identified through the donor selection procedures.

Screening for specific antibody can therefore be used to identify currently or recently infected donors – those likely to donate blood that may transmit malaria. Any donor who last returned from a nonendemic area at least six months previously can be tested for specific antibody. Donors from endemic areas can be treated similarly although the six-month deferral may need to be increased, depending on the country involved.

Significance for transfusion

Blood transfusion has been clearly documented as the cause of many cases of malaria, most of which have been *P. falciparum*. The extent to which transmission by transfusion is a problem depends on the endemicity of malaria in that area and the number of infected individuals who are likely to donate. In countries where malaria is endemic, it is highly likely that most individuals are infected during childhood and that most donors and most patients would already be infected. The significance of transmission of malaria by blood transfusion is therefore very different from that in nonendemic countries where most patients are not infected and in whom infection would may be serious and even fatal.

Most nonendemic countries protect against malaria by donor selection, identifying those donors who have been to malarious areas and deferring them for a suitable period before accepting them again. Whilst this is generally effective, the number of donors being deferred is rising and may be too high to sustain in some countries. There is an increase in travel to malaria risk areas as well as the spread of malaria itself into new areas as climate changes occur or back into old areas as eradication programmes break down. The use of antibody testing in addition to donor deferral is now becoming more acceptable in nonendemic countries in an effort to prevent transmission of malaria without significantly affecting the sufficiency of the blood supply.

Prevention of spread

Malaria is a significant problem worldwide and, for the last 30 years or more, prevention of spread in endemic areas has focused on the removal of the mosquito vector by attacking its breeding sites. Prophylaxis is used to prevent infection in travellers from nonendemic countries. This is generally effective, although not always totally successful, usually due to individuals' failure to follow the regimen or to the presence of resistant *Plasmodium* strains. Treatment of malaria in individuals from nonendemic areas is usually successful if diagnosed early enough.

Transmission via blood and organs is minimized through a combination of donor exclusion and specific testing.

8.6 CHAGAS DISEASE

The infectious agent

Chagas disease, also known as American trypanosomiasis, is a systemic disease caused by infection with the protozoan *Trypanosoma cruzi* (*T. cruzi*) which can be severe and even fatal. It is transmitted to the vertebrate host by blood-sucking insects known as triatomine or reduviid bugs which transmit it via their faeces which they deposit as they bite and feed. The disease is endemic in the Americas where it is thought that 16–18 million people are infected, most of these living in the poorer areas of south and central America. Infection of nonhuman vertebrates is also common from the southern areas of Chile and Argentina to as far north as California. In the USA, however, the better living conditions mean that human infection by natural transmission is uncommon, although it does occur. It is estimated that in most central and south American countries between 4% and 8% of the population are infected, although the figure can be as low as 1.2% and as high as 20% in certain countries.

Structure and life cycle

T. cruzi is one of a group of protozoa that are called haemoflagellates. These are flagellated protozoa that are transmitted to humans through the bites of infected insects. They are further grouped into two categories, *Trypanosoma* and *Leishmania*, although the differences between the two groups are not well defined. Here, we shall only consider *Trypanosoma*, although cases of transfusion-transmission of leishmaniasis have been suspected in some groups of military personnel serving abroad in endemic areas.

The life cycle of *T. cruzi* and the different forms of the haemoflagellates during the cycle are shown in Figures 51 and 52 on page 125. The essential difference between the life cycles of *T. cruzi* and the African forms of trypanosomiasis is that *T. cruzi* cannot multiply in the bloodstream of the human host. Instead it replicates inside the cells of almost every organ of the human body.

Infection is usually acquired by contamination, through contact with the faeces of the triatomine bug. The trypanosomes grow in the gut of the bug and are excreted in the faeces. As the bugs defecate while feeding, the trypanosomes in the faeces are able to infect the human host through the site of the bite. After entering the bloodstream, the organisms change their shape from the trypomastigote form and produce the rounded amastigote form. The organisms first infect the lymph nodes close to the site of infection where they initially multiply as the amastigote form. From there, they subsequently spread to other organs via the bloodstream. The liver, splenic macrophages and, importantly, the heart are most commonly infected. The infected cells eventually rupture and release the amastigotes into the bloodstream, where they change to trypomastigotes. Although infectious, the protozoa do not multiply during this stage. If the infected individual is then bitten at this stage, the triatomine ingests the trypomastigotes. The trypomastigotes multiply in the mid-gut of the insect as epimastigotes and then pass through the insect to be finally excreted in the faeces as the trypomastigote form. The cycle then starts again.

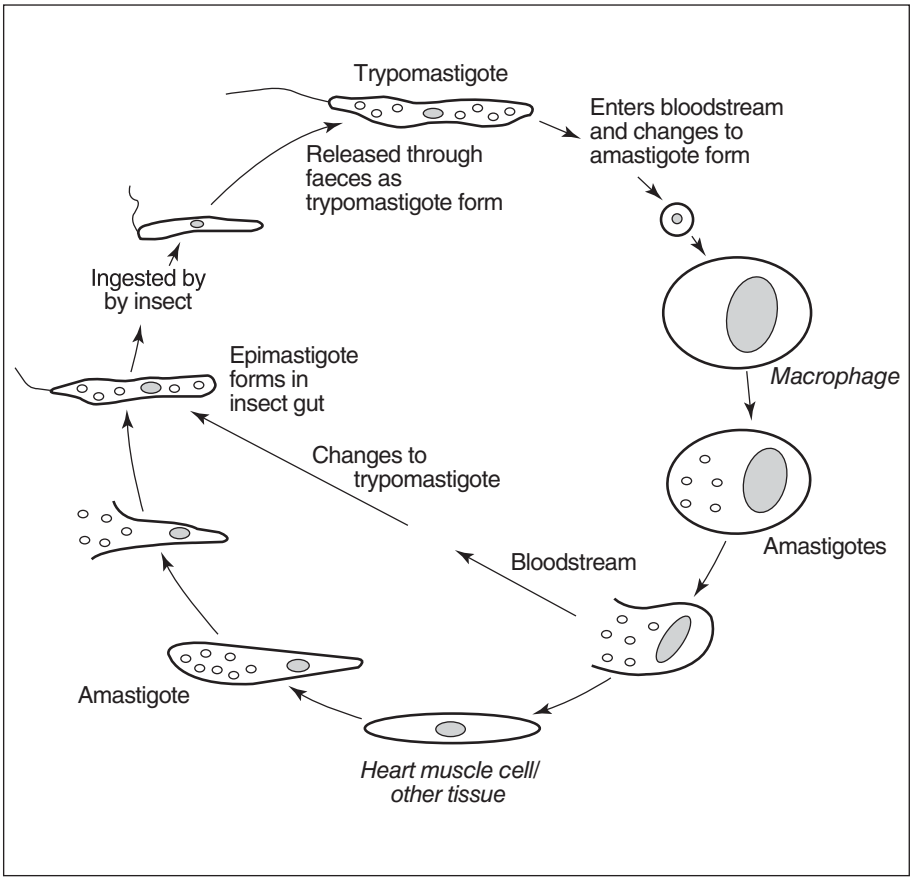


Figure 51: Life cycle of T. cruzi

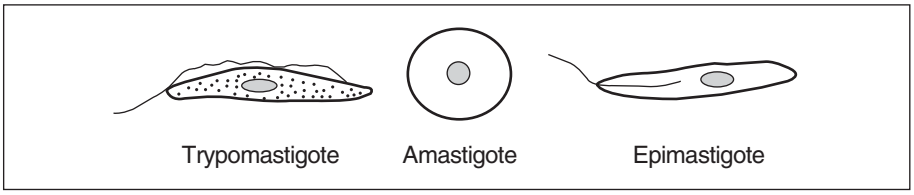


Figure 52: Different forms of the haemoflagellates

Transmission

Although insect transmission is the major route of infection, transmission via blood transfusion was a significant route in the past and some cases may still occur today. Similarly, transmission via organ transplants may also occur. Congenital infection and breastfeeding are effective routes of transmission. Parenteral transmission may occur through needles and surgical instruments.

There are many varied reservoirs for *T. cruzi*, including animals such as rodents, armadillos and opossums, which are common in endemic areas, but the triatomine bugs are totally restricted to the Americas and this restricts natural spread of the disease across the rest of the world. The domestic transmission cycle is responsible for maintaining infection in humans and occurs mostly in houses in rural or suburban areas where the triatomines live and multiply in cracks in the walls, holes in the roof and similar places.

In infected individuals, the bloodstream acts as the transport system disseminating the organism around the body and provides a new source of organism for any feeding reduviids.

Clinical course of infection

Initial infection is often asymptomatic and, in the few instances when symptoms appear and are recognized as *T. cruzi* infection, the site of entry shows only a small reddish painful nodule (primary chagoma) with swelling of the adjacent lymph nodes. Fever and enlargement of the spleen may follow. The systemic nature of the disease is due to the multiplication of the organism in the cells of tissues and organs virtually anywhere in the body. Mortality is low, except for children with cardiac or central nervous system involvement. Most people recover from acute infection without treatment but then develop chronic symptoms after a latent period that often lasts many years. Although the liver and spleen are usually infected, the most characteristically infected organ is the heart; congestive heart failure plays a significant part in the morbidity and mortality of the disease with 20–40% of infected individuals developing characteristic cardiac or gastrointestinal symptoms. If primary infection is untreated, the individual will remain infected for life and parasitaemia may be detected in as many as 50% of untreated infected individuals, and years after the initial infection.

Laboratory testing

The disease can be diagnosed early in infection by looking for the protozoa in blood films (amastigote form). Later, in acute infection, the organism can be cultured from blood samples (trypomastigote form). Specific antibodies appear approximately three to eight weeks after infection and serve as a marker of an infected individual rather than as a protective response. Even if an individual has been treated, the parasite may persist somewhere in the body and continue to replicate at a low level because infection is usually so widespread.

The identification of an infected donor is a major problems as far as blood transfusion is concerned. The techniques available to detect parasites are impractical for the screening of blood donors. In addition, it is highly unlikely that a donor would have been recently infected, which is the only situation in which the direct detection of parasites would be of any real use. The number of parasites present in the blood of an individual with parasitaemia following infection earlier in life would be far fewer than in a recently infected individual, but would still be equally capable of transmitting infection.

Screening for specific antibody is therefore a relatively effective way of identifying individuals who have at some time been exposed to the parasite. Whether individuals have been treated or not, it is generally considered that any donor with evidence of infection at any time should be deferred permanently because of the possibility of a very low-level asymptomatic infection with release of parasites into the bloodstream.

Significance for transfusion

The trypanosomes can also be acquired by blood transfusion when blood is collected from an asymptomatic infected donor with parasitaemia. This route of infection is now considered to be the second most common route of infection, after natural infection.

The importance of transmission by this route is related to the prevalence of the organism in the population and to the movement of people from

endemic areas to nonendemic areas. Migration by people from central and south America into north America and Europe has resulted in the presence of infectious individuals in previously nonendemic areas. While it is very unlikely that these individuals will provide a reservoir for domestic cycles of transmission involving the triatomines, they do present a source of transfusion-transmitted infection. Transmission by this route into nonendemic areas is now well documented.

The actual transmission rate of Chagas disease by blood transfusion is difficult to assess as there are few reliable data available, although it is estimated that, until recently, there were up to 10 000 cases per year in Brazil. The exclusion of infected donors can help to reduce the risks of infection but, since many cases are largely asymptomatic, it may not be clear which donors are infected or are potential infection risks. Furthermore, in many countries the rate of natural infection is high and cannot be ruled out as the source of infection.

Prevention of spread

Control of infection in endemic areas generally focuses on improving housing and the elimination of the triatomine bugs from homes, and the prevention of spread via blood or organs. In nonendemic areas, spread by blood and organs is limited, depending on the risks within the donor population, such as donors from endemic countries or who have travelled extensively in endemic areas. Public health education programmes play a part in the control of the disease, especially in the early identification and reduction of risk of infection via breastfeeding.

In endemic countries, the screening of blood donors is a good control measure that can help to reduce the spread of infection. In nonendemic countries, screening may not be very useful unless there is a large number of donors from, or who have travelled in, endemic countries and recipients of the blood are likely to be immunosuppressed. A more suitable approach may be to exclude potentially infected donors by predonation questioning although, with the increase in world travel, this may result in the deferral of too many donors and selective screening may have to be introduced.

An important method of preventing transmission by transfusion is practised in parts of Latin America where the disease is still endemic and the exclusion of infected donors would significantly decrease the blood supply. The addition of a drug that kills trypanosomes to the donated blood has proved very effective. Crystal violet (also known as gentian violet) at a concentration of 200 μg/ml in blood stored at +4°C for 24 hours before use prevents transfusion-transmitted infection. Thousands of transfusions have been performed using blood treated in this way without any serious side-effects being reported.

Since the transmission of Chagas disease by blood transfusion has been clearly demonstrated, there is no reason to suppose that other members of this protozoan group would not be transmitted under the correct conditions. The other organisms that infect humans are:

- *T. gambiense* which causes west African sleeping sickness (Gambian trypanosomiasis)

- *T. rhodesiense* which causes east African sleeping sickness (Rhodesian trypanosomiasis).

However, no cases of transmission by transfusion of either *T. gambiense* or *T. rhodesiense* have yet been reported.

The spread of infection and the disease process caused by *T. cruzi* are very different from the African types of trypanosomiasis.

SUMMARY

1 The basic principles that apply to the screening of blood for anti-HIV also apply to screening for other transfusion-transmitted infections.

2 The range of screening tests that are available for anti-HIV is not always available for other infectious agents.

SELF-ASSESSMENT

27 What is the significance for transfusion practice of HBV infection?

28 What is currently the most effective method of screening for malaria?

29 What is the significance for transfusion practice of Chagas disease?

PROGRESS CHECK

Before moving on to Section 9, spend a few minutes thinking about whether you have achieved the learning objectives for Section 8. These were to:

1 Describe the basic features of infection with HBV, HCV, HTLV, syphilis, malaria and Chagas disease.

2 Explain the significance of these agents for blood transfusion practice.

If you feel confident that you have understood everything in this section, turn to Section 9.

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.

9

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 programme for screening for anti-HIV and other infectious agents 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 2.
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 screening service provided by your laboratory.

9.1 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 44

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

Module objective	*Rating (1–4)*	*Comments*
Section 2 Explain the role of microorganisms as infectious agents in human disease and their significance for blood transfusion		
Section 3 Describe HIV infection and the significance of infection for blood transfusion		
Section 4 Outline the principles of the diagnostic assays most commonly used to detect transfusion-transmissible infections and the differences between them		
Section 5 Select the most suitable type of screening assay for transfusion-transmissible infections for use in your own laboratory		
Section 6 Develop an effective screening programme for transfusion-transmissible infections and maintain accurate records of the screening results		
Section 7 Help to develop an appropriate quality system for your laboratory to maintain an effective screening programme		
Section 8 Recognize the basic features of other infectious agents and their significance for blood transfusion practice		

discuss any remaining problems with your supporter or trainer before continuing with your Action Plan.

9.2 MAKING YOUR ACTION PLAN

The Action Plan provides you with an opportunity to make practical improvements in your own workplace, within any financial, resource or staffing constraints that exist. As you worked through this module, you have been noting down your ideas on the Action List on page 132. 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 45

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 133. 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 133. 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 at this stage.

When you have reached agreement about the actions you are going to take, set a date by which 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.

ACTION LIST

Activity number	Ideas for improvement

ACTION PLAN

Ideas for improvement	*Planned action*	*Expected results*	*Planned completion date*	*Actual completion date*	*Actual results*

9.3 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 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 46

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 any further changes or take any follow-up action needed to ensure that they continue to lead to improved quality in your screening programme.

As you work through the remainder of the learning programme, you will be asked to complete an Action List and Action Plan for each module. This approach can be applied to almost any situation and you may decide to use it in other areas of your work to improve the quality of the service that you provide.

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 2.

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 screening service provided by your laboratory.

Activity Checklists and Answers

SECTION 1

Activity 1

Purpose

To identify a personal "supporter" for your work on Module 2.

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 2 and checked that he or she is 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 2 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 2.

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 7
- 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 2.

Checklist

You should have:

- Quickly looked at other sections to get an idea of the content, level and approach of the module 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 when you plan to complete each section and the dates of meetings with your trainer and supporter.

SECTION 2

Activity 4

Purpose

To identify the infectious agents in your locality which you think can be transmitted by blood transfusion.

Checklist

You should have:

- Listed the infectious agents that are prevalent in your locality, including at least one example of each of the four types of infectious agent
- Identified the normal routes of transmission of each of these agents

- Indicated the agents that you think are likely to be transmitted by blood transfusion.

Activity 5

Purpose

To identify the infectious agents which can be transmitted by blood transfusion.

Checklist

You should have:

- Made any amendments you now think necessary to your list from Activity 4 of infectious agents that can be transmitted by blood transfusion
- Checked your answers with your supervisor or another senior member of staff.

Activity 6

Purpose

To check your understanding of the markers of infection at different stages following infection.

Answers

- 2 weeks: antigen
- 4 weeks: antigen and possibly low-titre antibody
- 6 weeks: antibody, with possibly some remaining antigen
- 8 weeks: antibody
- 18 months: low-titre antibody or no remaining markers.

If an infectious agent is likely to be transmitted only during the period of acute infection, it would be better to screen donors for the agent itself because the presence of antibody only indicates immunity – not an infectious state.

SECTION 3

Activity 7

Purpose

To check your understanding of the structural features of HIV.

Answers

1 = nucleic acid

2 = capsid

3 = p24

4 = p7/p9

5 = reverse transcriptase (p66)

6 = p17 matrix protein

7 = gp41

8 = gp120

9 = lipid bilayer

10 = complete virion

Activity 8

Purpose

To check your understanding of the entry of HIV into a susceptible cell.

Answers

1 = HIV

2 = CD4 receptor

3 = uncoated virus (capsid)

4 = viral RNA

5 = DNA copy of viral RNA

6 = viral DNA integrated into genome

7 = viral proteins

8 = viral RNA

9 = virus capsid

10 = budding virus particle

11 = new HIV virion

Activity 9

Purpose

To investigate the clinical presentation of HIV and AIDS in your country.

Checklist

You should have tried to find out:

- The prevalence of HIV infection and the number of AIDS cases in your country
- The most common clinical course of HIV infection and its progression to AIDS
- The average time taken for AIDS to manifest following the initial infection

- The main opportunistic infections found in AIDS patients and whether they are found in all areas of your country or there are local variations
- How the incidence of Kaposi's sarcoma 25 years ago compares with the incidence today and how it relates to the current prevalence of AIDS in your population.

Activity 10

Purpose

To consider possible reasons for any under-reporting of HIV infection and AIDS in your country.

Checklist

You should have:

- Identified any possible reasons for under-reporting of HIV infection and AIDS in your country, using the following categories:
  - economic
  - political
  - practical
  - technical
  - cultural.

Activity 11

Purpose

To identify the principal route of transmission of HIV infection in your country.

Checklist

You should have:

- Identified the main route of transmission of HIV in your country
- Identified the reason why this is the main mode of transmission.

Activity 12

Purpose

To illustrate the importance of screening for transfusion-transmitted HIV infection.

Answers

1 Short-/long-term: the patient is infected with HIV and is likely to die within three to five years.

2 Short-/long-term: his wife (and any other sexual partner) is likely to be infected following normal sexual contact.

3 Long-term: his wife may infect any subsequent children.

4 Long-term: a recipient of any blood donated by the husband or wife is likely to be infected if the blood is not tested before transfusion.

Activity 13

Purpose

To investigate the approaches taken to reduce the spread of HIV in your country.

Checklist

You should have:

- Listed all the approaches taken in your country to reduce:
  - the spread of HIV infection
  - its transmission through blood transfusion
- Found out what advice is given about avoiding the sexual transmission of HIV
- Noted whether condoms are widely available
- Noted how effective condom distribution has been in preventing the transmission of HIV infection or whether people are reluctant to use condoms.

SECTION 4

Activity 14

Purpose

To identify the types of TTI screening assay used in your laboratory.

Checklist

You should have:

- Listed the names of all the assay kits used in your laboratory for screening donated blood for TTIs
- Correctly identified each assay type.

Activity 15

Purpose

To standardize the terminology used in your laboratory.

Checklist

You should have:

- Listed the words used in your laboratory to classify screening results
- Noted any words that are different from those used by the manufacturers of the assays used in your laboratory
- Discussed the terminology to be used in your laboratory with your supervisor and colleagues and noted down your recommendations on your Action List.

Activity 16

Purpose

To calculate the results of an antiglobulin/sandwich-type EIA.

Checklist

You should have:

- Used the cut-off value of 0.36 to complete Column B of Figure 36 with the final assay results
- Looked at Figure 37 to see the results from Figure 36 plotted on a simple graph.

Activity 17

Purpose

To calculate the signal/cut-off ratios of an antiglobulin/sandwich-type EIA.

You should have:

- Used the cut-off value of 0.36 to complete Column C of Figure 36 with the signal/cut-off ratios
- Looked at Figure 37 to see the results from Figure 36 plotted on a simple graph.

SECTION 5

Activity 18

Purpose

To calculate the specificity and, if possible, the sensitivity of an assay used in your laboratory.

Checklist

You should have:

- Found out the number of true-negatives and false-positives for the last 100 assays performed in your laboratory

- Used these figures correctly in the formula given to calculate the specificity of the assay
- Compared the answer with the specificity given in the manufacturer's instructions
- Found out the number of true-positives and false-negatives for the last 100 assays performed, if external quality control samples are used in your laboratory
- Used these figures correctly in the formula given to calculate the sensitivity of the assay
- Compared the answer with the sensitivity given in the manufacturer's instructions
- Talked to your supervisor about your results if they differed from the specificity or sensitivity given in the manufacturer's instructions and noted down your recommendations on your Action List.

Activity 19

Purpose

To collect data on the assays available in your country.

Checklist

You should have:

- Listed all the assays available in your country, together with the names of the manufacturers and, if possible, the cost per test
- Selected one assay from each of the three types: EIA, particle agglutination assay and simple rapid assay
- Completed the table on page 66 with the relevant information about each of these three assays.

Activity 20

Purpose

To identify the most appropriate type of assay for use in your laboratory.

Checklist

You should have:

- Noted any additional factors relevant to the choice of a TTI assay that specifically relate to your laboratory, including:
  - the number of donations per week
  - the facilities and equipment available in your laboratory
  - the support services available in your laboratory, such as water supply and electricity

- Assessed the different types of assay on their appropriateness to your situation
- Identified the most suitable type of assay for use in your laboratory
- Noted down your recommendations on your Action List, if you feel that your laboratory is currently not using the most appropriate type of assay, and discussed them with your supervisor.

Activity 21

Purpose

To examine other factors that need to be considered in setting up and maintaining an effective TTI screening programme.

Checklist

You should have:

- Noted how each of the factors listed in the activity relate to the particular circumstances in your laboratory
- Added any other factors that you think might be relevant to setting up and maintaining an effective TTI screening programme.

Activity 22

Purpose

To review the training provided in your laboratory on screening for TTIs.

Checklist

You should have:

- Made notes on the training provided in your laboratory including:
  - — the name of the person who provides the training
  - — the name of the person who is in charge of training
  - — the kind of training that is provided
  - — the duration of training
  - — how trainees' skills are assessed
  - — whether regular training updates are held
- Outlined how you would develop a simple training programme for new staff or for staff who are already performing testing for TTIs
- Noted down your ideas for a training scheme on your Action List and discussed them with your supervisor.

Activity 23

Purpose

To identify the items of equipment that are needed to perform TTI screening assays correctly.

Checklist

You should have:

- Listed the following items of equipment needed to perform EIAs and particle agglutination assays:
  - suitable mechanical pipettes (ideally multi-channel)
  - EIA plate reader
  - EIA plate washer (manual/automatic)
  - reagent troughs
- Noted the items of equipment that are already available in your laboratory
- Identified any equipment to which you have unrestricted access elsewhere in your hospital or institution.

Activity 24

Purpose

To plan an ordering schedule for the supply of assay kits.

Answer

Five or six kits would be required each month. Assuming that seven kits are in stock at the beginning of the year, a suitable ordering schedule might be as shown on page 147.

Checklist

You should have:

- Reviewed the ordering schedule for assay kits used in your laboratory
- Identified any ways in which it could be improved
- Noted down your recommendations on your Action List and discussed them with your supervisor.

Activity 25

Purpose

To review the storage conditions for assays in your laboratory.

Checklist

You should have:

Ordering schedule for assay kits

	January	7 kits in stock on 5/1
		↓
	February	Order 6 kits when only 3 are left
		↓
	March	Order 6 kits when only 3 are left
		↓
	April	Order 6 kits when only 3 are left
		↓
	May	Order 6 kits when only 3 are left
		↓
	June	Order 6 kits when only 4 are left
Rainy season		↓
	July	Order 6 kits when only 4 are left
		↓
	August	Order 6 kits when only 3 are left
		↓
	September	Order 6 kits when only 3 are left
		↓
	October	Order 6 kits when only 3 are left
		↓
	November	Order 6 kits when only 3 are left
		↓
	December	Order 6 kits when only 3 are left
		↓
	January	Order 6 kits when only 3 are left

- Made a note of the conditions in which assays are currently stored, including the storage temperature, the reliability of the power supply, temperature monitoring, the amount of space, restrictions on access and facilities for storage above floor level if a walk-in cold store is used
- Compared these storage conditions with those recommended by the manufacturer of each assay and identified any differences
- Identified any ways in which the storage conditions for assays could be improved in your laboratory and noted your recommendations on your Action List.

Activity 26

Purpose

To review the use of samples collected from your donors.

Checklist

You should have:

- Listed the types of sample collected from your donors
- Identified what each type of sample is used for

- Talked to your supervisor about collecting separate samples from donors, if testing is currently performed using the bleed line attached to the blood pack, and noted your recommendations on your Action List.

Activity 27

Purpose

To review the use of external and internal QC samples in your screening programme.

Checklist

You should have:

- Noted the external QC samples available, including:
  - where they are obtained from
  - how many samples are available
  - whether you have to pay for them
  - the assays with which they are meant to be used
- Explained how any internal QC samples used in your laboratory are prepared and standardized
- Suggested possible improvements in the system for obtaining external QC samples or preparing and standardizing internal QC samples
- Noted your recommendations on your Action List and discussed them with your supervisor.

Activity 28

Purpose

To identify ways of preventing the use of untested blood.

Checklist

You should have:

- Noted the average time that a unit of blood is currently stored in your blood bank before it is issued for transfusion
- Noted whether untested blood is ever transfused in your hospital and, if it is, the reasons why this is necessary
- Suggested ways of maintaining a storage period of at least 24 hours to provide sufficient time for routine screening for TTIs before transfusion
- Noted your recommendations on your Action List and discussed them with your supervisor.

Activity 29

Purpose

To assess how various factors might affect the overall cost of your screening programme.

Checklist

You should have considered the following factors:

- the number of donations to be tested and therefore the number of assays that will need to be performed
- the cost of the different types of assay
- the cost of additional equipment and reagents required
- the reliability of the assays
- the specificity of the assays
- the prevalence of TTIs in your donor population
- the costs of collecting the donated blood
- staff costs.

SECTION 6

Activity 30

Purpose

To review the system used in your laboratory to record assay failures.

Checklist

You should have:

- Suggested any ways in which an error log kept in your laboratory could be improved and noted your recommendations on your Action List
- Discussed introducing an error log with your supervisor and colleagues, if one is not kept at present
- Identified the following information that should be recorded in an error log:
  - — the name of the assay
  - — the name of the operator
  - — the reason for the failure
  - — the consequence of the failure
  - — the changes made to laboratory practice to prevent a similar failure in the future
- Noted your recommendations on your Action List.

Activity 31

Purpose

To identify ways of reducing the number of false-positives in your screening programme.

Checklist

You should have:

- Noted the assays used by your reference service
- Consulted the records for the last 25 or 50 assays that were subjected to confirmatory testing, either by a reference laboratory or by use of an alternative assay
- Noted the number of these assays that were confirmed positive
- Noted the number of false-positives
- Suggested ways of reducing the number of false-positives by ensuring that:
  - — washing is performed correctly
  - — clean reagent containers are used
  - — the correct volumes of samples and reagents are dispensed
  - — the reader set-up (for EIAs) is correct
  - — good quality samples are used
- Noted your recommendations on your Action List and discussed them with your supervisor.

Activity 32

Purpose

To review the procedures used for the dispatch of samples to a reference laboratory.

Checklist

You should have:

- Noted the procedures used in your laboratory to ensure that samples arrive in a suitable state to give reliable confirmatory results
- Noted any local or national regulations for sending pathological material by post
- Suggested ways of sending samples so that there is no risk of leakage during transit
- Reviewed the records of the last 25 or 50 samples sent to your reference service and noted the time taken for

delivery, the proportion that arrive damaged and the time taken to receive the results

- Identified any ways of improving the safe dispatch of samples and noted your recommendations on your Action List.

Activity 33

Purpose

To review the TTI assay record sheet used in your laboratory.

Checklist

You should have:

- Examined the record sheet used in your laboratory for test results
- Compared it with the example given in Figure 45
- Identified any ways of improving your TTI assay record sheets and noted your recommendations on your Action List.

Activity 34

Purpose

To review the system used in your laboratory for retrieving previous test data.

Checklist

You should have:

- Described the system used in your laboratory for retrieving data from previous tests on donors
- Identified any ways of improving the system for retrieving previous test data and noted your recommendations on your Action List.

Activity 35

Purpose

To review the system used in your centre for storing screening results.

Checklist

You should have:

- Assessed your centre's system for the storage of records in relation to the factors listed on pages 88–89
- Identified any ways of improving the system for storing screening results and noted your recommendations on your Action List.

Activity 36

Purpose

To review the system used in your centre for identifying, removing from stock and disposing of TTI-positive donations.

Checklist

You should have:

- Compared the procedure used in your centre with the guidelines given on page 90
- Identified any ways of improving the current procedure and noted your recommendations on your Action List
- Talked to your supervisor and colleagues about designing and implementing an appropriate system, if none currently exists, and noted your recommendations on your Action List.

Activity 37

Purpose

To recognize the importance of the safe handling and disposal of assay components and waste.

Checklist

You should have:

- Listed the components of TTI screening assays that might present a health and safety risk
- Suggested why these components might present a health and safety risk.

Activity 38

Purpose

To identify methods for the safe disposal of potentially hazardous assay materials.

Checklist

You should have:

- Listed the materials used in TTI screening assays that may be hazardous in their disposal
- Suggested ways of disposing of these materials safely.

Activity 39

Purpose

To review the handling and disposal of potentially hazardous waste from TTI screening assays in your laboratory.

Checklist

You should have:

- Read the health and safety policy for your workplace and checked whether it covers all the necessary areas and working practices in your screening programme
- Contacted the person who is responsible for health and safety and found out how regularly the health and safety policy is rewritten and updated
- Identified any improvements you would like to see in the handling and disposal of potentially hazardous waste and noted your recommendations on your Action List.

SECTION 7

Activity 40

Purpose

To review the documentation of procedures in your laboratory.

Checklist

You should have:

- Identified any of the areas listed on page 97 for which written records are not kept in your laboratory
- Discussed with your supervisor the importance of recording any of these aspects of laboratory work that are not currently documented
- Identified any further procedures that you think should be documented and noted your recommendations on your Action List.

Activity 41

Purpose

To review the use of SOPs in your laboratory.

Checklist

If SOPs are used in your laboratory, you should have:

- Noted whether there are SOPs for all the major activities in the screening programme, such as those listed on page 98
- Assessed whether each SOP covers all aspects of the procedure
- Noted whether SOPs are followed by all members of staff involved in performing those procedures

- Noted how frequently SOPs are reviewed and updated, where necessary
- Suggested any ways in which the preparation and use of SOPs could be improved in your laboratory and noted your recommendations on your Action List.

If SOPs are not used in your laboratory, you should have:

- Identified an important task that you perform regularly
- Developed a simple SOP
- Showed it to your colleagues and your supervisor for amendment, where necessary
- Once it was finalized, ensured that all members of staff follow it at all times
- Identified any additional areas where SOPs are required, noted your recommendations on your Action List and discussed them with your supervisor.

Activity 42

Purpose

To design a checklist for performing an assay.

Checklist

You should have:

- Designed a checklist for a screening assay with which you are familiar
- Listed all the required actions in the correct order
- Included space or boxes for recording the actions taken and adding any other information that may be required, including signatures and dates.

Activity 43

Purpose

To review the efficiency of the record-keeping system used in your laboratory.

Checklist

You should have:

- Used your transfusion records to select a donation that was tested three or four months earlier
- Attempted to follow the testing records back to the donor
- Noted any point at which it was impossible to follow the records back to the donor
- Discussed your findings with your supervisor

- Suggested ways of improving the record-keeping system to ensure that an audit trail could be followed in the future and noted your recommendations on your Action List.

SECTION 9

Activity 44

Purpose

To assess the progress you have made as a result of your work on Module 2.

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 130
- 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 before continuing with your Action Plan.

Activity 45

Purpose

To plan how to implement the improvements that you have identified as being necessary to ensure quality in your screening programme.

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 each planned action will be completed.

Activity 46

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 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 Latency is the property of infectious agents, generally viruses, to remain hidden in a previously-infected individual and in an inactive state for an extended period of time – often the lifetime of the individual. Although the individual may be immune to the agent, specific antibody can be detected and reactivation of the agent may occur at any time.

2 The three conditions that will determine whether an infectious agent is likely to be transmitted by transfusion are:

- the agent must be capable of using the bloodstream as a means of entry into its host, the patient
- the infected donor will be essentially free of any signs and symptoms of disease
- the agent must exist naturally for a period of time, either free in the plasma or present in a cellular component in the bloodstream of an infected donor.

3 Blood transfusion can never be the primary route of infection because most people do not have a blood transfusion during their lifetime. Any agent that depends solely on transfusion for transmission will therefore not persist in the population.

4 Antigens are foreign substances that enter the body and induce an immune response against them. Antibodies are molecules that are made up of proteins and carbohydrates. They are produced by the immune response of an individual as a reaction to stimulation by a foreign protein – antigen.

5 The five types of immunoglobulin are: IgG, IgM, IgA, IgD and IgE.

6 The main reason for screening blood is to ensure that the available blood supply is as free as possible from any infectious agents.

7 The risk of transmission of infection by transfusion can be minimized by:

- the careful selection of donors
- the direct screening of the donated blood for infectious agents
- the removal of specific components of blood thought to harbour infectious agents
- the physical inactivation of any contaminating agents that may be present.

8 The presence of antibody does not necessarily protect against infection and the infectious agent may still be present in the unit of blood.

SECTION 3

9 HIV enters susceptible cells by binding to a receptor (CD4) on the cell surface.

10 The two HIV-specific antibodies that have been found to be the best confirmation of HIV infection are anti-p24 and anti-gp41.

11 Antibodies may be present as early as 14 days following infection with HIV, but may not appear until 28 days or more after infection.

12 The three main routes of transmission for HIV infection are:

- unprotected penetrative sexual contact with an infected person, either between men or between men and women
- the inoculation of infected blood by transfusion or the use of contaminated needles, syringes or knives
- from an infected mother to her child, either in the uterus, during birth or by breastfeeding.

13 Three routes of potential transmission that are not implicated in the transmission of HIV are:

- non-sexual social or domestic contact
- insect vectors
- handling pathological material, provided that the correct safety procedures are applied at all times and good laboratory practice is followed.

SECTION 4

14 The three main types of screening assay are:

- enzyme linked immunosorbent assays (ELISA) or enzyme immunoassay (EIA)
- particle agglutination assays
- simple rapid assays.

15 The term "positive" describes the status of the donation or donor after the initial result has been confirmed by further assays. The term "reactive" is used until the results are confirmed.

SECTION 5

16 Sensitivity and specificity are inversely related. As the sensitivity increases, so the specificity decreases. Similarly, as the specificity increases, the sensitivity decreases.

17 The factors that contribute to the design and maintenance of an effective TTI screening programme are:

- staff training
- provision of necessary equipment and reagents
- regular supply of assay kits
- suitable facilities for the storage of assay kits
- availability of fresh, fully clotted, correctly stored samples
- supply of external or internal quality control samples
- adequate time for TTI testing
- adequate finance.

18 Assay kits should be stored according to the manufacturer's instructions in a walk-in cold store or a refrigerator. The following conditions are required:

- a temperature of +2°C to +6°C
- a reliable power supply
- a facility for temperature monitoring
- sufficient capacity
- restricted access
- facilities for storing kits above floor level in a walk-in cold store.

19 External QC samples are stabilized samples provided by an independent laboratory or institution. Internal QC samples are prepared within the home laboratory.

SECTION 6

20 Repeat testing means that when a sample is initially reactive, the assay is repeated in order to confirm the initial result. A repeatedly reactive sample is considered to be TTI-positive and the donation should be discarded.

Confirmatory testing is performed by a separate laboratory, using a different assay from that used in the original screening, in order to confirm the test result from the primary assay. Confirmatory testing may show, however, that the screening result was a false-positive result and that the donor is actually TTI-negative.

21 When pathological specimens are dispatched through the post:

- the sample container should be strong and watertight and have a leak-proof screw lid; the container should be clearly labelled
- the container should be wrapped in sufficient absorbent material to soak up the sample in case of spillage
- the wrapped container should be packed in a second watertight container or sealed in a leak-proof plastic bag, with the accompanying documentation attached to the outside of the container or bag
- the package should be packed in a strong container that will protect the contents from physical damage while they are in transit
- the outer packaging should be labelled to indicate that it contains pathological material. The name and address of the laboratory sending the sample should be written on the outside, as well as the name and address of the reference laboratory.

22 Accurate records of assay results ensure that the correct assay results are recorded against the right samples, that the records are a true picture of the testing performed and the results obtained, and that the correct decision is made about the final fate of the donation. This is essential to prevent patients from being transfused with TTI-positive blood.

23 It is important to be able to retrieve previous testing data in order to check previous screening results obtained from a specific sample from a donor and to ensure that donors who have previously been confirmed as TTI-positive are clearly identified and permanently excluded from donating blood again.

24 If autoclaving is not possible, incineration is the next best method of safely disposing of TTI-positive donations.

SECTION 7

25 SOPs are required for the use of assays because manufacturers' instructions are intended for general use. These may not be sufficient or appropriate in every situation because they do not relate to each laboratory's specific requirements.

26 Quality assurance is particularly important in a small laboratory with only one member of staff because all the responsibility falls on one person and mistakes could easily go unnoticed.

SECTION 8

27 Blood transfusion is an efficient route for the transmission of HBV because a large amount of infected material is passed directly into the bloodstream of the recipient.

28 Currently the most effective method of screening blood for malaria is the examination of blood films for parasites within the red cells. However, this is not suitable for screening large numbers of blood donations.

29 Many cases of Chagas disease are largely asymptomatic and it may therefore not be clear which donors are infected or are potential infection risks.

Glossary

Antibody
A protective protein produced by the immune response of an individual to stimulation by a foreign substance. It plays a role in the defence against pathogens, often by neutralization or by identifying the pathogen as foreign and to be eliminated by the immune system.

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

Basophil
A type of white blood cell that contains many cytoplasmic granules which contain bioactive substances.

Bioactive
Biologically active.

Capsid
The inner protein core of a virus particle which contains the nucleic acid. It is made up of identical protein sub-units.

Chromogen
A synthetic soluble compound that changes colour following oxidation, reduction or other chemical modification by an enzyme.

Cross-reactivity
When an antibody recognizes not only its corresponding specific antigen, but also other antigens that may have certain similarities.

Cytoplasmic
Referring to the cytoplasm, the material surrounding the nucleus of a cell.

DNA (deoxyribonucleic acid)
The genetic material of most living organisms which determines hereditary characteristics by the control of protein synthesis.

Dormant
An inactive period in the life-cycle of organisms in which growth slows or ceases.

Envelope (viral)
An external protein coat that surrounds the viral capsid. Not all viruses are enveloped.

Epidemiology
The study of the occurrence, distribution and spread of infection and disease in the population.

Equivocal
A result that cannot be classified as clearly positive or negative.

Eukaryote
An organism in which the genetic material of the cell is contained within a distinct nucleus.

Gammaglobulin
The class of serum proteins that includes antibody molecules.

Genome
The complete genetic structure of an organism.

Glycoprotein
A protein molecule with a sugar molecule attached. Glycoproteins are common constituents of cell membranes.

Gram-negative
The Gram stain is a method of staining bacteria which is used to classify them. Bacteria are either Gram-negative or Gram-positive.

Histamine
A substance found in many cell types, especially mast cells and basophils, that is released when vessels are injured.

Hypersensitivity
Overreaction to an allergen that results in pathological changes in tissues.

Immunity
The state of being resistant to infection by an infectious agent due to previous exposure to the agent, with resultant production of a protecting immune response.

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

Incidence
The number of new infections in a population.

Integration
The joining of foreign nucleic acid into the genome of an organism.

Latency
The property of infectious agents, generally viruses, to remain hidden in a previously-infected individual and in an inactive state for an extended period of time – often the lifetime of the individual. Although the individual may be immune to the agent, specific antibody can be detected and reactivation of the agent may occur at any time.

Lymphocyte
A type of circulating mononuclear white blood cell. It plays a role in both the cellular and humoral immune responses.

Lymphoid cell
A cell of the lymphatic system.

Macrophage
A phagocytic cell type found in the bloodstream as well as tissues. It ingests bacteria and cell debris.

Markers of infection
The detectable signs of infection, including the body's own response to the infectious agent, appearing in the bloodstream during, or following, infection.

Mast cell
A cell found in the loose connective tissue running alongside blood vessels that produces a number of bioactive substances, e.g. histamine, heparin.

Morphology
The study of the shape or form of organisms.

Nucleic acid
A complex organic compound found in living cells that consists of chains of nucleotides. There are two types, DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), which make up the cells' genetic information.

Nucleotide
A compound formed from a nitrogen-containing base, either a purine or pyrimidine, phosphoric acid and a pentose sugar. DNA and RNA are formed from long chains of nucleotides.

Nucleus
Part of the cell that contains the cell’s DNA. The nucleus functions as the control centre of the cell.

Oncogenic virus
A virus that causes the formation of tumours.

Opportunistic infection
Uncontrolled infection by a normally present, but controllable, infectious agent.

Organelle
A permanent structure within a cell with characteristic morphology which is specialized to perform a specific function in the cell activities.

Pathogenic microorganism
Any disease-causing microorganism.

Phagocytosis
The process by which cells ingest solid matter, especially cell debris and pathogens.

Predictive value
The likelihood of a result being a true result. Both positive and negative predictive values are used.

Prevalence
The proportion of a specific population that is infected with the infectious agent at any particular time.

Prion
A small proteinaceous infectious particle that resists inactivation by procedures that modify nucleic acids.

Quality assurance
The overall range of activities and systems that provide confidence within the organization and authorities that all quality requirements are met.

Quality control
Checks put in place to ensure that processes, procedures and products meet the quality requirements.

Quality system
Organizational structure, processes, procedures and resources needed to implement quality requirements.

Retrovirus
A virus family that is characterized by RNA as the nucleic acid, a unique morphology, the presence of a unique enzyme (reverse transcriptase), and latency.

Reverse transcriptase
A naturally-occurring enzyme which translates RNA into DNA.

RNA (ribonucleic acid)
A complex chemical found in the cytoplasm and concerned with protein synthesis. In some viruses, it is the hereditary material.

Sensitivity
The probability that a test result will be reactive in an infected individual.

Seroconversion
A change in serostatus of an individual from seronegative to seropositive.

Serostatus
The serological findings in an individual following infection.

SOP
Standard operating procedure.

Specificity
The probability that a test result will be non-reactive in an individual who is not infected.

Spore
A minute reproductive cell of fungi and some plants. A protective state which some bacteria are able to assume in adverse conditions.

Surrogate testing
Testing for an indicator of infection which is thought to indicate the presence of an infectious agent, but which is not a specific marker of infection by that agent.

Transfusion-transmissible infection
An infection that is capable of being transmitted by blood transfusion.

Transfusion-transmitted infection
An infection that has been transmitted by blood transfusion.

Toxin
Any poisonous compound, usually produced by living organisms.

Unicellular
Consisting of a single cell.

Virion
A virus particle.

Window period
The period between infection and the first appearance of circulating detectable marker of that infection.

Appendices

World Health Organization

Joint United Nations Programme on HIV/AIDS
UNAIDS
UNICEF • WFP • UNDP • UNFPA • UNODC
ILO • UNESCO • WHO • WORLD BANK

REVISED RECOMMENDATIONS FOR THE SELECTION AND USE OF HIV ANTIBODY TESTS

Recommendations for the selection and use of HIV antibody tests were first issued by WHO in 1992. Since then the range of HIV antibody tests available has expanded. New types of assays have been developed and the overall quality has improved. HIV tests for other body fluids (saliva and urine) have been developed. However, the testing strategies described here should only be applied to tests using serum or plasma.

To serve the needs of blood transfusion services, which use the vast majority of all HIV tests worldwide, increasingly sensitive HIV antibody assays have been developed in order to shorten the window period (the interval between the point of infection and the development of detectable antibody).

As a result of this trend, less sensitive but highly specific HIV tests have been withdrawn from the market. This is unfortunate as these were ideal as second and third line tests; their withdrawal affects the practical implementation of the proposed WHO HIV testing strategies.

Choice of a testing strategy, the selection of the most appropriate test or combination of tests to use, depends on 3 criteria:

(1) the objective of the test;

(2) the sensitivity and specificity of the test(s) being used;

(3) the prevalence of HIV infection in the population being tested.

Objectives of HIV antibody testing

The 3 main objectives for which HIV antibody testing is performed are:

(1) *Transfusion/transplant safety.* Screening of blood and blood products, and of tissues, organs, sperm or ova from donors.

(2) *Surveillance.* Unlinked and anonymous testing of serum for the purpose of monitoring the prevalence of, and trends in, HIV infection over time in a given population.

(3) *Diagnosis of HIV infection.* Voluntary testing of serum from asymptomatic persons or from persons with clinical signs and symptoms suggestive of HIV infection or AIDS.

Sensitivity and specificity of antibody tests (Table 1)

Sensitivity and specificity are 2 major factors that determine a test's accuracy in distinguishing between infected and uninfected persons. A test with a high *sensitivity* will have few false-negative results. Therefore, only tests of the highest possible sensitivity should be used when there is a need to minimize the rate of false-negative results (e.g. in transfusion/transplant safety). A test with a high *specificity* will have few false-positive results and should be used when there is a need to minimize the rate of false-positive results (e.g. in diagnosis of HIV infection in an individual). Although the emphasis

Table 1. Sensitivity, specificity and predictive value of HIV serological tests

		True HIV status +	True HIV status –	
Test results	+	**a True-positives**	b False-positives	a+b
	–	c False-negatives	**d True-negatives**	c+d
		a–c	b+d	

Sensitivity = a/(a+c).
Specificity = d/(b+d).
Positive predictive value = a/(a+b).
Negative predictive value = d/(c+d).

might shift slightly towards sensitivity or specificity, depending on the objective of testing, both should meet minimum standards (>99%, >95%, respectively).

Prevalence of HIV infection

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the prevalence of HIV infection in the population, the greater the probability that a person testing positive is truly infected (i.e. the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of serum samples testing false-positive decreases; conversely, the likelihood that a person showing negative test results is truly uninfected (i.e. the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of samples testing false negative.

Quality assurance

All laboratories carrying out HIV tests should have a quality assurance programme. It is most important that quality control procedures be stringently complied with so as to maximize the accuracy of the laboratory results. Procedures for detecting both technical and clerical errors must be included in all protocols. For example, procedures that guarantee the correct identification of initially reactive units of donated blood, which must be discarded, are essential to the maintenance of a safe blood supply. It is recommended that laboratories submit to an external quality assessment at least once a year.

Strategies for HIV antibody testing

Several studies and field experiences have shown that minor modifications to the 3 testing strategies recommended in 1992 are needed. Both the selection of and the order in which the assays are used are of the utmost importance for the final outcome of the strategy. As HIV antibody assays have become more sensitive over the years, the probability of a false-positive reaction in 2 assays based on a different principle is not negligible. Therefore, if test combinations are not carefully selected, individuals may be wrongly diagnosed as HIV seropositive. Conversely, the more specific assays are presently slightly less sensitive as compared to the average HIV antibody test, which may result in a false-negative diagnosis. These observations cause concern with regard to strategies II and III of the 1992 recommendations. The choice of the most appropriate HIV tests also depends on the HIV variants present in a particular geographical region (e.g. HIV-1 group O). **Therefore, test combinations should always be evaluated in the context in which they will be used before wide-scale implementation.**

Studies have shown that combinations of ELISA and/ or simple/rapid assays such as dot immunoassays and agglutination tests can provide results as reliable as, and in some instances more reliable than, the ELISA/Western blot (WB) combination, and at a much lower cost. At present, a number of countries use a selection of different HIV screening assays in a particular order, to minimize the number of costly confirmatory assays.[1] Confirmatory assays should only be used to resolve indeterminate results for diagnostic purposes. UNAIDS and WHO therefore recommend that countries consider testing strategies for HIV antibody detection which use ELISA and/or simple/ rapid assays rather than ELISA/WB.

Recommendations

UNAIDS and WHO recommend 3 testing strategies to maximize accuracy while minimizing cost. Which strategy is most appropriate will depend on the objectives of the test and the prevalence of HIV in the sample population, as shown in *Table 2* and *Fig. 1*.

Strategy I

All serum/plasma is tested with one ELISA or simple/ rapid assay. Serum that is reactive is considered HIV antibody positive. Serum that is non-reactive is considered HIV antibody negative.

Transfusion/transplant safety

When applied for safeguarding the blood supply, the test selected for this strategy should preferably be a **combined HIV-1/HIV-2 assay** which is **highly sensitive**. Units of donated blood yielding **reactive** or **indeterminate** test results must be considered as probably infected with HIV and should be discarded according to universal safety instructions.[2] Strategy I is meant for testing the donations, but must not be used for notifying donors of a positive test result. **If a blood or tissue donor is to be notified of a test result, testing strategies II or III for diagnosis must be applied** (*Table 2, Fig. 1*). Whatever the final diagnosis, donations which were initially reactive should not be used for transfusion or transplants. Several studies have shown that careful selection of donors is more efficient than HIV antigen testing to minimize the risk of transfusion related infections.

Surveillance

Sensitivity is less crucial for surveillance purposes; however, for this and the above application the assay chosen should have a specificity of at least 95%. It is recommended that the same assay(s) be used over time to monitor fluctuations in HIV prevalence.

Diagnosis (see below)

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[1] Western blot or similar assays based on recombinant proteins and/ or synthetic peptides capable of detecting antibodies to specific HIV-1 and/or HIV-2 proteins.

[2] See WHO AIDS SERIES 9, *Biosafety guidelines for diagnostic and research laboratories working with HIV*.

Table 2. UNAIDS and WHO recommendations for HIV testing strategies according to test objective and prevalence of infection in the sample population

Objective of testing		Prevalences of infection	Testing strategy
Transfusion/transplant safety		All prevalences	I
Surveillance		>10%	I
		≤10%	II
Diagnosis	Clinical signs/symptoms of HIV infection[a]	>30%	I
		≤30%	II
	Asymptomatic	>10%	II
		≤10%	III

[a] World Health Organization. Interim proposal for a WHO staging system for HIV infection and disease (WER No. 29, 1990, pp. 221-228).

Strategy II

All serum/plasma is first tested with one ELISA or simple/rapid assay. Any serum found reactive on the first assay is retested with a second ELISA or simple/rapid assay based on a different antigen preparation and/or different test principle (e.g. indirect versus competitive). Serum that is reactive on both tests is considered HIV antibody positive. Serum that is non-reactive on the first test is considered HIV antibody negative. Any serum that is reactive on the first test but nonreactive on the second test, should be retested with the 2 assays. Concordant results after repeat testing will indicate a positive or negative result. If the results of the 2 assays remain discordant the serum is considered indeterminate.

Surveillance

When testing low HIV prevalence populations for surveillance purposes, even if one uses a test of high specificity the PPV will be very low. Therefore, an additional test is necessary in order not to overestimate the HIV prevalence in such regions. All samples remaining discordant after repeat testing with the 2 assays are considered indeterminate; unlike for diagnosis, no further testing is needed. The indeterminate results should be reported and analysed separately in the annual surveillance overviews.

Diagnosis (see below)

Strategy III

As in strategy II, all serum is first tested with one ELISA or simple/rapid assay, and any reactive samples are retested using a different assay. Serum that is nonreactive on the first test is considered HIV antibody negative. Serum that is reactive in the first test but nonreactive in the second assay should be repeated with both tests. Strategy III, however, requires a third test if serum is found reactive on the second assay or is reactive on the repeated first assay. The 3 tests in this strategy should be based on different antigen preparations and/or different test principles. Serum reactive on all 3 tests is considered HIV antibody positive. Serum that remains discordant in the second assay, or is reactive in the first and second tests but non-reactive in the third test, is considered to be indeterminate. Serum that is reactive on the first assay and non-reactive on the second and third assays is considered indeterminate for individuals who may have been exposed to HIV in the last 3 months and negative for those who have not been exposed to any risk for HIV infection.

Diagnosis (Strategies I, II and III)

Newly diagnosed HIV seropositives

An additional blood sample should be obtained and tested from all persons newly diagnosed as seropositive on the basis of their first sample. This will help eliminate any possible technical or clerical error.

Uncertain diagnosis: indeterminate result

Serum from people with clinical signs meeting the WHO criteria,[3] stages III or IV, may have an indeterminate result due to a decrease in antibodies. In this case serum does not normally need to be retested.

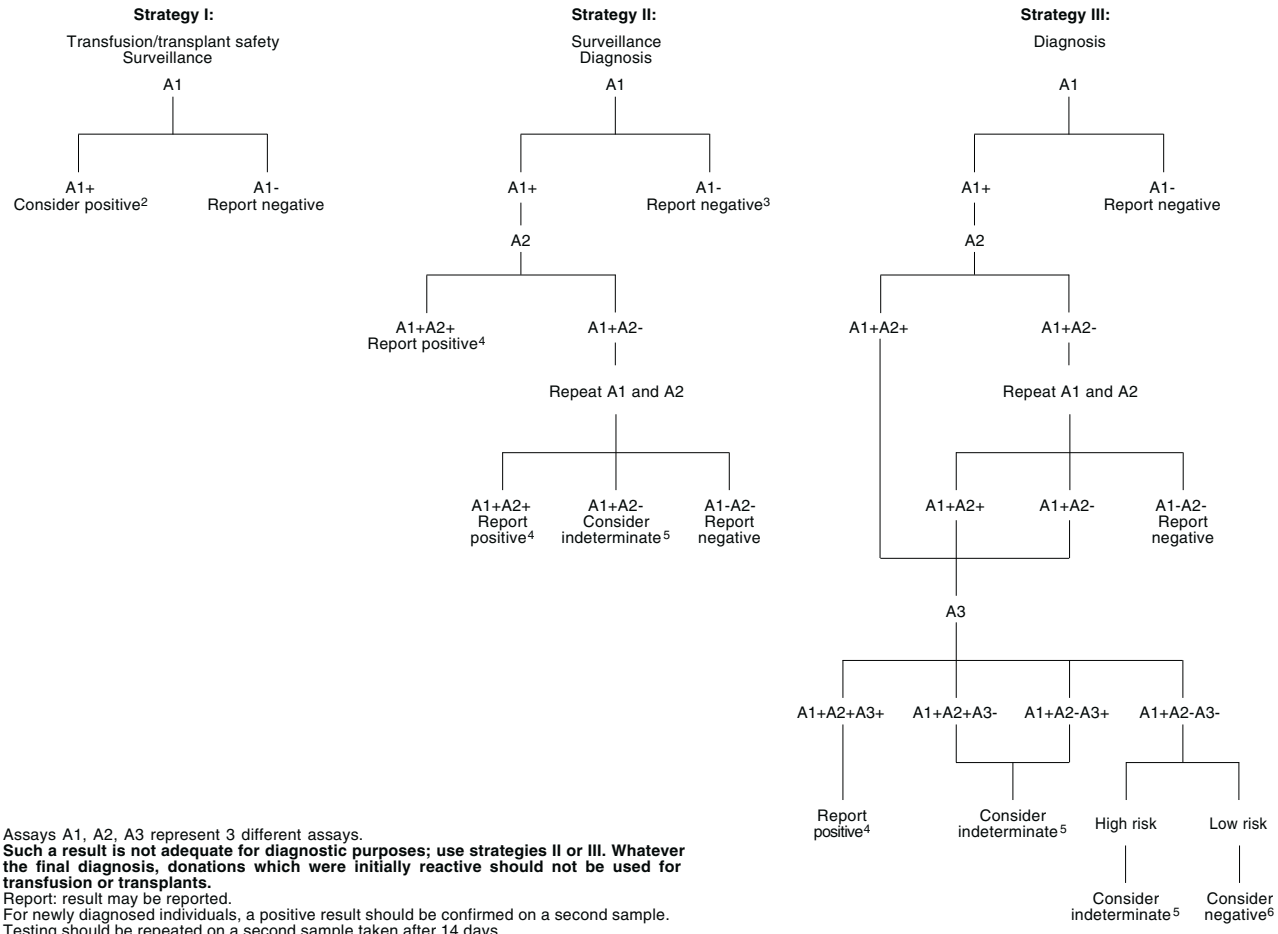
[3] World Health Organization. *Interim proposal for a WHO staging system for HIV infection and disease* (WER No. 29, 1990, pp. 221-228).

For diagnosis of HIV infection in asymptomatic individuals, with an indeterminate result, a second blood sample should be obtained after a minimum of 2 weeks following the first sample and should be tested using the appropriate strategy. If the second serum sample also produces an indeterminate result, it should be tested with a confirmatory assay. However, if this result is also indeterminate longer follow-up may be required (3, 6, 12 months). If the results remain indeterminate after 1 year, the person is considered to be HIV antibody negative.

General remarks about Strategies I-II-III

Strategy I can only be used to confirm the clinical diagnosis of individuals meeting the WHO criteria of stage III or IV of HIV infection and when the HIV prevalence in the sample population (e.g. patients from a tuberculosis ward) is above 30%. In lower prevalence populations, strategy II should be used to diagnose persons with the above-mentioned clinical symptoms. 1 World Health Organization. *Interim proposal for a WHO staging system for HIV infection and disease* (WER No. 29, 1990, pp. 221-228). In the selection of HIV antibody tests for use in strategies II and III, the first test should have the highest sensitivity, whereas the second and third tests should have a higher specificity than the first. The number of initial discordant, indeterminate results should not exceed 5%. If it does, quality assurance procedures should be checked and/or a new test combination should be adopted. An HIV test kit bulk-purchase programme has been established by WHO in collaboration with UNAIDS in order to provide national AIDS control programmes with tests giving the most accurate results at the lowest possible cost. This list of HIV test kits is updated annually. Tests other than those bulk-purchased by the programme, but meeting the minimum standards in terms of sensitivity and specificity, are also suitable for use with the testing strategies shown in *Table 2* and *Fig. 1*. Information concerning the performance of HIV antibody tests is available upon request from UNAIDS and WHO as are guidelines for counselling persons regarding HIV testing, infection and disease.

Fig. 1 Schematic representation of the UNAIDS and WHO HIV testing strategies[1]



1 Assays A1, A2, A3 represent 3 different assays.
2 **Such a result is not adequate for diagnostic purposes; use strategies II or III. Whatever the final diagnosis, donations which were initially reactive should not be used for transfusion or transplants.**
3 Report: result may be reported.
4 For newly diagnosed individuals, a positive result should be confirmed on a second sample.
5 Testing should be repeated on a second sample taken after 14 days.
6 In the absence of any risk for HIV infection.

Example of a Standard Operating Procedure

STANDARD OPERATING PROCEDURE SOP/BTS/LAB/008/01

Manual performance Detect-Wel anti-HIV EIA

This SOP replaces	Quality Manager	Date effective
New		Copy no

1 PURPOSE

To define the procedure for the manual performance of the Detect-Wel Enzyme Immunoassay (EIA) used in the BTS Blood Screening Laboratory.

2 RESPONSIBILITIES

All BTS Screening Laboratory Staff

All trained and authorized BTS Blood Screening Laboratory staff can use this SOP

Head of Blood Screening Laboratory

Head of the Blood Screening Laboratory must resolve any:

- Problems with the process
- Difficulties using the SOP
- Borderline interpretations.

3 RESTRICTIONS

This SOP must not be used by unauthorized Blood Screening Laboratory staff or by any non-Blood Screening Laboratory staff.

4 DEFINITIONS

Blood Screening Laboratory

The BTS laboratory whose responsibility it is to screen blood donations for infectious diseases and perform blood grouping and screening for irregular red cell antibodies.

Assay validation criteria

Manufacturer's criteria outlined in the assay instructions.

HIV standards/QC samples

Defined sample(s) used as an independent check on the performance of the assay.

5 ITEMS REQUIRED

Detect-Wel EIA kit
ABC microplate reader
DEF microplate washer
Appropriate HIV standards/QC samples

STANDARD OPERATING PROCEDURE SOP/BTS/LAB/008/01

Documentation:

- Sample Testing Worksheet (FRM/BTS/BSL/001)
- Screening Assay Monitoring Worksheet (FRM/BTS/BSL/002)
- Use and maintenance of DEF microplate washer (SOP/BTS/BSL/002/01)
- Use and maintenance of ABC microplate reader (SOP/BTS/BSL/003/01).

6 PROCEDURE

6.1 Remove the kit from the refrigerator and leave for 30 minutes to warm up.

6.2 Identify the samples to be tested, and list the samples on the Sample Testing Worksheet (FRM/BTS/BSL/001).

6.3 Perform the assay according to the manufacturer's instructions, using the DEF microplate washer (SOP/BTS/BLS/002/01) and ABC microplate reader (SOP/BTS/BSL/003/01). The ABC microplate reader is preloaded with assay interpretation software with profiles set up for each assay performed in the laboratory.

6.3a Prepare sufficient wash buffer for the number of samples to be tested.

6.3b Add 50 ul of sample diluent to each assay well including controls and standards.

6.3c Add 50 ul of each sample, controls and standards/QC samples to the appropriate wells as specified in the manufacturer's instructions and cover with the lid provided. Mix on a plate shaker for 10 seconds and incubate for 30 minutes at 37°C. Record the set-up time on the Screening Assay Monitoring Worksheet (FRM/BTS/BSL/002).

6.3d At the end of the incubation period, wash the plate using the DEF microplate washer (SOP/BTS/BSL/002/01), washing 5 times with a 30-second soak between washes. At the end of the wash, ensure that the wells are dry.

6.3e Add 100 ul of conjugate to each well and incubate for 30 minutes at 37°C. Record the set-up time on the Screening Assay Monitoring Worksheet (FRM/BTS/BSL/002).

6.3f At the end of the incubation period, wash the plate using the DEF microplate washer (SOP/BTS/BSL/002/01), washing 5 times with a 30-second soak between washes. At the end of the wash, ensure that the wells are dry.

6.3g Add 100 ul of substrate to each well and incubate for 30 minutes at 37°C. Record the set-up time on the Screening Assay Monitoring Worksheet (FRM/BTS/BSL/002).

6.3h At the end of the incubation period, add 50 ul of stop solution to each well and read the plate on the ABC microplate reader at 450/690 nm (SOP/BTS/BSL/003/01) within 15 minutes of adding the stop solution. Obtain a printout of the OD values and calculated results.

6.4 Check the results printout to ensure that the assay run is valid according to the manufacturer's validation criteria.

6.5 Pass the results and completed paperwork to a senior member of staff for checking and authorization.

6.6 As required, shut down the DEF microplate washer and ABC microplate reader as detailed in their respective SOPs (SOP/BTS/BSL/002/01, (SOP/BTS/BSL/003/01).