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ABBREVIATIONS

AIDS .... Acquired Immune Deficiency Syndrome
ALT .... Alanine Aminotransferase
Anti-HBc .... Antibody to Hepatitis B core Antigen
Anti-HCV .... Antibody to Hepatitis C Virus
API .... Annual Parasite Incidence
BTS .... Blood Transfusion Services
CE .... Continuing Education
CJD .... Creutzfeldt-Jakob Disease
CMIA .... Chemiluminescence Immunoassay
CMV .... Cytomegalovirus
DF .... Dengue Fever
DHF .... Dengue Haemorrhagic Fever
EEG .... Electro-Encephalogram
EIA .... Enzyme Immunoassay
ELISA .... Enzyme Linked Immuno Sorbent Assay
EQAS .... External Quality Assessment
FFP .... Fresh Frozen Plasma
GDBS .... Global Database on Blood Safety
GOP .... Government of Pakistan
HA .... Haemagglutination
HAM .... HTLV-1 Associated Myelopathy
HAV .... Hepatitis A Virus
HBCab .... Hepatitis B core Antibody
HbsAg .... Hepatitis B Surface Antigen
HBV .... Hepatitis B Virus
HCV .... Hepatitis C Virus
HGV .... Hepatitis G Virus
HIV .... Human Immunodeficiency Virus
HTLV-1 .... Human T-Cell Lymphotropic Virus Type-1
IAs .... Immunoassays
ICT .... Immuno Chromatography Test
ID .... Individual Donations
IFN .... Interferon
IgE .... Immunoglobulin E
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
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<tr>
<td>KS</td>
<td>Kaposi’s Sarcoma</td>
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<td>MP</td>
<td>Malarial Parasite</td>
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<td>NAT</td>
<td>Nucleic Acid Tests</td>
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<td>SBTP</td>
<td>Safe Blood Transfusion Programme</td>
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<td>NEQAS</td>
<td>National External Quality Assurance Scheme</td>
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<tr>
<td>NGO</td>
<td>Non-Governmental Organization</td>
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<tr>
<td>OD</td>
<td>Optical Density</td>
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<tr>
<td>PA</td>
<td>Particle Agglutination</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PTNANBH</td>
<td>Post-transfusion Non-A, Non-B Hepatitis</td>
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<tr>
<td>QA</td>
<td>Quality Assurance</td>
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<td>QC</td>
<td>Quality Control</td>
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<td>RNA</td>
<td>Ribo Nucleic Acid</td>
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<td>RPR</td>
<td>Rapid Plasma Reain</td>
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<tr>
<td>SARS</td>
<td>Severe Acute Respiratory Syndrome</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure (s)</td>
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<tr>
<td>TDR</td>
<td>Tropical Diseases Research</td>
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<tr>
<td>TPHA</td>
<td>Treponema Pallidum Haemagglutination Assays</td>
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<tr>
<td>TTI</td>
<td>Transfusion Transmitted Infections</td>
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<td>TTV</td>
<td>Transfusion Transmitted Virus</td>
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<tr>
<td>UNAIDS</td>
<td>Joint United Nations Programme on HIV/AIDS</td>
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<td>UNFPA</td>
<td>United Nations Funds for Population Activities</td>
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<tr>
<td>UNGASS</td>
<td>United Nation General Assembly Special Session</td>
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<tr>
<td>UPS</td>
<td>Uninterrupted Power Supply</td>
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<tr>
<td>VDRL</td>
<td>Venereal Diseases Research Laboratory</td>
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<tr>
<td>VL</td>
<td>Visceral Leishmaniasis</td>
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<tr>
<td>WHA</td>
<td>World Health Assembly</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>WNV</td>
<td>West Nile Virus</td>
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PREFACE

Screening for transfusion-transmissible infections (TTIs) is a critical part of the process of ensuring that transfusion is as safe as possible. Unsafe blood transfusion is very costly both from a human and an economic point of view. With every unit of blood transfused, there is a 1% risk of transfusion-associated problems, including transfusion-transmitted infections such as HBV, HCV, HIV and Syphilis. In Pakistan, with an only incipient culture of voluntary donations, a strong reliance on replacement and paid donors, and the lack of systematic screening strategy, the infection risks are at the upper end, as commercially remunerated blood donors and family replacement donors are more likely to transmit transfusion-transmissible infections than are voluntary donors. Morbidity and mortality rates resulting from the transfusion of infected blood have far-reaching consequences, not only for the recipients but also for their families.

The Safe Blood Transfusion Programme, Pakistan is currently a recipient of the WHO/OFID Joint Project (OPEC Fund for International Development) on Prevention of Transfusion-Transmitted HIV/AIDS and Hepatitis Infections. The overall goal of the project is to prevent transfusion-transmitted HIV/AIDS and hepatitis infections in Bangladesh, Bhutan, Nepal and Pakistan through universal coverage and quality-assured testing of all donated blood. As a part of this project, a study was conducted by SBTP to develop the country strategy for blood donation screening for TTIs. The Pakistan Medical and Research Council (PMRC) collaborated in this study with SBTP. The study conducted a systematic analysis of the current blood bank laboratory capacities in the public and private sectors and developed a strategy for TTI screening in the Pakistani context. The document developed is imperative to guide and support the establishment and implementation of an appropriate, effective and reliable blood screening strategies which are uniform and standardized across the country. There are many challenges associated with executing the proposed strategy, ranging from structural (policy, law) to operational (staff training, developing quality assurance protocols) and technical. The Government of Pakistan is committed to the objective of blood safety and will provide resources for the validation of national testing strategies on a regular basis over time. It should be recognized, however, that all every screening strategy will have limitations and that absolute safety, in terms of freedom from infection risk, cannot be guaranteed.

The Programme would like to appreciate the support being provided by the WHO/OFID Joint Programme for the prevention of TTIs in Pakistan. The Programme also acknowledges the PMRC in completing this assignment. The technical advice and recommendations of the provincial blood programmes, blood transfusion authorities and other key stakeholders has been pivotal to finalize the report. The SBTP team deserves a special metjkone for their tireless efforts in the completion of this task especially Mr. Sajid Hussain Shah and Mr. Usman Waheed. It is expected that implementation of the country strategy thus developed will go a long way in promoting blood safety in Pakistan.

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Safe Blood Transfusion Programme
Government of Pakistan
EXECUTIVE SUMMARY

Almost 100 million blood donations are donated annually, of which more than (taken) half are collected in developed countries, where less than one sixth of the world’s population resides. Less than half of these transfusions are for the remaining 5.8 billion inhabitants of the planet. Blood transfusion, while being a life-saving procedure on one hand, may sometimes be associated with risks which cause adverse reactions, and these may be acute or delayed, or may sometimes be responsible for the transmission of infection from an infected donor to the recipient. These Transfusion Transmitted Infections (TTIs) are mainly HIV, HBV, HCV and syphilis, but in Pakistan, malaria, dengue, and in some cases CMV and toxoplasmosis have also been documented to be of important as a TTI.

The population in Pakistan is coming close to 200 million, but unfortunately, the healthcare sector in Pakistan is very poorly documented. Data on blood donations, transfusion and screening is scanty, and where available, not very reliable. However, it is estimated that more that 1.5 million pints of blood are donated each year in Pakistan, of which 25% is from voluntary donors, 65% from replacement donors and about 10% from professional donors. UNAIDS estimates that only half of the 1.5 million transfusions are actually screened in Pakistan.

In Pakistan, a number of seroprevalence studies for HIV, HBV and HCV have shown a very low prevalence of HIV, but is rather high for HBV being between 3% to 7% and HCV, between 2-6% in these transfusions. As part of this study, 35 blood banks were selected randomly from all over Pakistan and questioned to find their blood banking practices and facilities. The results show a great need to encourage voluntary blood donors throughout the country.

This report also studied the prevalence of TTIs in 9 centres in Pakistan, which reported the results of their screening. The results were very noteworthy. Out of 149,648 samples screened, 4.66% screened positive for HCV, 4.3% for HBV, 1.01% for Syphilis and 0.02% for HIV.

Algorithms have been developed specially for use in Pakistan, according to the situation in blood banks locally.

It is hoped that this document will form a backbone to improve the overall screening for each TTI in Pakistan, and therefore each chapter has been written to be a stand-alone document in itself.
INTRODUCTION

Blood transfusion is the commonest form of organ transplantation performed globally. It is a life-saving intervention, which saves millions of lives all over the world every year.

Almost 108 million blood donations are donated annually worldwide, of which more taken half are collected in developed countries, where less than one sixth of the world’s population resides. Of the 108 million blood donations collected globally, approximately half of these are collected in the high-income countries, home to 18% of the world’s population. Family replacement donors and paid or professional donors account for 37% of all donations in developing countries, 26% in transitional countries and only 2% in developed countries. In 2008 alone, 30 countries reported collecting almost 1 million paid donations. Blood donation rate in high-income countries is 36.8 donations per 1000 population; 11.7 donations in middle-income and 3.9 donations in low-income countries.

Data on blood donations, transfusion and screening in Pakistan is scanty and not very reliable. It is estimated that more that 3 million units of blood are donated each year of which 15% is from voluntary donors and remaining from replacement donors.

There are approximately 290 blood banks in government institutions all over the country and about 1500 small and medium sized blood banks in NGO and private sector, which provide more than 60% of the blood transfusions per year.

Blood transfusion, while being a life-saving procedure on one hand, may sometimes be associated with risks which cause adverse reactions, and these may be acute or delayed, or may sometimes be responsible for the transmission of infection from an infected donor to the recipient. Such infections are known as Transfusion Transmissible Infections (TTI). The World Health Organization recommends screening for four TTIs viz. HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis, as mandatory.

WHO Global Database Report for 2012 showed that the prevalence of TTI in 297 public and private sector blood banks surveyed was: HIV (7.01%), HBV (3.76%), HCV (4.8%), Syphilis (1.16%) and Malaria (1.78%).

Screening for TTIs to exclude blood donations at risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible. Effective screening for evidence of the presence of the most common and dangerous TTIs can reduce the risk of transmission to very low levels.

Blood transfusion services should therefore establish efficient systems to ensure that all donated blood is correctly screened for specific TTIs and that only non-reactive blood and blood components are released for clinical and manufacturing use. The adoption of screening strategies appropriate to the needs, infrastructure and resources of each country can contribute significantly to improvements in blood safety.
CHAPTER 1

1. TRANSFUSION TRANSMISSIBLE INFECTIONS (TTI)

1.1 Pathogens present in blood and plasma

Human blood can contain a number of infectious agents. However, not all blood borne pathogens can be transmitted by plasma for transfusion or by plasma derivatives.

Historically, clinical use of blood and blood components has been associated with transmission of blood-borne viruses (HBV, HCV, HIV, Syphilis, Malaria) 19.

1.1.1 Viral Hepatitis

Hepatitis was the first documented transfusion-transmitted disease. Many of the current practices for diminishing risk in transfusion medicine are based on the experiences of controlling the transmission of hepatitis. Hepatitis viruses which infect the liver include hepatitis B and C. Before the late 1970’s, the risk of transmitting hepatitis by transfusion was very high because of blood collection from prisoners and paid donors and the lack of sensitive serological tests. Between 1965 and 1972, approximately 1 in 60 units of blood transmitted hepatitis in developed countries. The change to an all-volunteer blood supply and the introduction of a third generation test for HBsAg in the mid 1970’s led to a marked reduction in transfusion transmitted hepatitis B infection. The risk decreased even further with the implementation of ALT and anti-HBc tests in 1987 and 1988 as surrogate markers for hepatitis C. Nucleic acid testing for Hepatitis B virus was introduced in 2009. Today the risk of transmitting hepatitis B is estimated to be 1 case per 352,000 units and the window period has been reduced to 5-8 days in developed countries.

In the late 1970’s, approximately 10% of patients who were transfused with multiple units of red cells became infected with hepatitis C. The introduction of more stringent donor eligibility criteria and both serological and nucleic acid tests hepatitis C virus antibody and RNA has reduced the risk of transmission to about 1 infection per 2,000,000 units transfused.

The natural history of transfusion transmitted HCV is similar to that of HCV acquired through other modes of transmission. Approximately 50% of patients will develop chronic elevations of liver enzymes and 10% of these will develop cirrhosis.

1.1.1.1 Hepatitis B Virus (HBV)

Hepatitis B virus (HBV) is transmitted through parenteral and sexual exposure. The mean incubation time is 90 days with a range of 30 to 180 days. Donor blood is routinely tested for Hepatitis B surface Antigen (HBsAg) as a marker of infection.
In a population based study conducted by the Pakistan Medical Research Council (PMRC) in 2008, the prevalence of Hepatitis B infection in Pakistan was 2.5%. Out of a total of 47,043 persons screened, 2.5% were in Sindh, 2.4% in Punjab, 1.3% in Khyber Pakhtunkhwa and 4.3% in Balochistan 20.

Agent:

Hepatitis B virus (HBV) is an enveloped DNA virus. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes.

Transmissibility:

While HBV is present in the bloodstream, the levels of the virus itself are variable. In recently infected individuals, viral DNA is normally present, although not always at high levels. Chronically infected individuals may either be infectious (viral DNA present) or non-infectious (viral DNA absent) and viraemia would generally be expected to be very low or absent entirely. Screening for hepatitis B surface antigen (HBsAg) indicates infection with HBV, but does not in itself distinguish between recent and chronic infections.

All HBsAg positive donations should be considered to be at high risk of transmitting HBV and should not be released for transfusion. Even in the absence of HBsAg in donors, certain blood donors can still harbor the HBV DNA in their blood. Addition of hepatitis B Core Antibody tests in the TTI panel will help to identify HBsAg negative blood donors.

Even when HBsAg screening test is negative, the blood may still contain HBV (window period).

1.1.1.2 Hepatitis C Virus (HCV)

Hepatitis C, formerly known as non-A, non-B hepatitis, was discovered in the late 1980s. Since 1990, all blood donations in developed countries, have been screened for it. Acute hepatitis C virus (HCV) is a relatively mild infection, and most people are unaware they have become infected; however, HCV becomes chronic in 80 percent of those infected. While the rate of new HCV infections is falling rapidly due to behavior changes and blood screening, HCV is an important source of serious chronic liver disease, which often develops decades after the initial exposure to the virus.

According to PMRC Survey 2008, the prevalence of Hepatitis C infection in Pakistan was 4.9% (5% in Sindh, 6.7% in Punjab, 1.1% in NWFP and 1.5% in Balochistan) 21.

Antibody screening was started in 1990, and the test has undergone significant improvement since. In the United States, after more than 10 years of testing for HCV, the risk of HCV transmission through transfusion is less than 1 per 1,000,000-screened units of blood, but similar statistics for Pakistan are not yet available.
Agent:

Hepatitis C virus (HCV) is a member of the flavivirus group and is an enveloped RNA virus. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes, resulting in a similar picture to that seen with HBV infection. Seroconversion has been seen in numbers of individuals who have resolved their infections. The loss of circulating antibody may leave no readily detectable evidence of previous infection. At present, only testing for hepatitis C antibody is available (recently tests for hepatitis C Antigen have been developed and available). The antibody to the hepatitis C virus appears 54 to 192 days in a person's Blood after infection.

Transmissibility:

While HCV is present in the bloodstream, the levels of the virus itself are variable. In recently infected individuals, virus is normally present. However, only around 70% of chronically infected individuals are viraemic and the length of time that viraemia persists is not fully understood. Nonetheless, it is expected that most HCV infected donations would contain virus and thus be infectious.

Screening for both HCV antigen and antibody does not in itself distinguish between recent and chronic infection. The distinction is, however, not relevant to the screening of blood for transfusion and all HCV antigen-antibody reactive donations should be considered to be at high risk of transmission of HCV and should not be used for clinical or manufacturing use.

Even when HCV antibody screening test is negative, the blood may still contain HCV but antibody not yet developed (window period).

1.1.2 Human Immunodeficiency Virus (HIV)

In 1982 the first cases of AIDS transmitted from blood or blood components were reported, but little was known about the infection at that time. By 1983 radical changes began to occur in the donor criteria to exclude those at high risk for transmission of HIV. The testing of blood products for HIV started in 1985. It was a test to detect the presence of the antibody directed against HIV, rather than a direct test for HIV.

In developed countries, transmission of HIV through transfusion has been almost completely eradicated since blood banks began interviewing donors about at-risk behaviors and a blood test became available in early 1985. Unfortunately, testing for HIV infection is still not being carried out on each and every donation in most developing countries, including Pakistan. The prevalence of HIV infection in general population in Pakistan is 0.01%.

Agent:

The human immunodeficiency virus (HIV) is a retrovirus, an enveloped RNA virus, which is transmissible by the sexual and parenteral route. It is found in blood and other
Once in the bloodstream, the virus primarily infects and replicates in lymphocytes. The viral nucleic acid persists by integrating into the host cell DNA. A number of different groups and subtypes (clades) have been identified with some significant antigenic differences; HIV-1 and HIV-2 are the two major distinct virus types and there is significant cross-reactivity between them. HIV-1 is now endemic in many parts of the world, although its incidence and prevalence is low in some regions. HIV-1 group M is responsible for more than 99% of the infections worldwide, whereas the prevalence of HIV-2 is mainly restricted to countries in West Africa and India. In Pakistan, the major type found is HIV-1. The appearance of antibody marks the onset and persistence of infection, but not immunity.

**Transmissibility:**

As HIV can be present in the bloodstream in high concentrations and is stable at the temperatures at which blood and individual blood components are stored, the virus may be present in any donated blood from an HIV-infected individual. Infectivity estimates for the transfusion of infected blood products are much higher (around 95%) than for other modes of HIV transmission owing to the much larger viral dose per exposure than for other routes.

Even when HIV antibody screening test is negative, the blood may still contain HIV Virus but antibody not yet developed (window period).

### 1.1.3 Syphilis

**Agent:**

Syphilis is caused by the spirochaete bacterium Treponema pallidum. It is transmissible by sexual and parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the bacteria spread throughout the body. A primary lesion, chancre, usually occurs about three weeks after exposure, although the duration may be shorter in cases of transfusion-transmitted infection where the organism enters the bloodstream directly. Syphilis is endemic in many parts of the world. In the 2008 WHO Global Survey in Pakistan, out of 963,496 blood donors 0.2% were found positive for Syphilis.

**Transmissibility:**

While T. pallidum may be found in the bloodstream, levels are variable. In addition, the treponemes are heat-sensitive; storage of blood (2°-8°C) for more than 72 hours results in irreparable damage to the organism such that it is no longer infectious. Thus, although clearly potentially infectious, the risk of transmission through the transfusion of blood and blood components stored below 2°-8°C is very low.

Platelet concentrates, stored at higher temperatures (22°-24°C), present a significantly higher risk of transmitting syphilis, if not screened. Thus, although the risk of transmission of syphilis from unscreened donations is variable, the screening test is nonetheless considered essential.
1.1.4 Malaria

Agent:

Malaria is caused by parasites of one of the four Plasmodium species. Two of them P. falciparum and P. vivax are prevalent in Pakistan. Malaria is primarily transmitted to humans through the bite of the female anopheles mosquito. Malaria has gradually spread from endemic to non-endemic regions where it had previously been eradicated.

Malaria is endemic in Pakistan and constitutes a national health priority. The provinces of Balochistan, Sindh and Khyber Pakhtunkhwa and the Federally Administered Tribal Areas have the highest malaria burden. Districts and agencies bordering Afghanistan and Islamic Republic of Iran account for 37% of the malaria burden with an annual parasite incidence (API) exceeding 4.5/1000 population per year. Moreover, there has been a growing risk of Plasmodium falciparum malaria incidence in areas where previously P. vivax was predominant.

In malaria endemic areas, it is not feasible to reject donors who have had malaria previously. In some malarial endemic areas, it is the policy to give curative antimalarial drugs followed by prophylactic drugs for 3 weeks to all recipients of blood.

Transmissibility:

Although primarily transmitted by mosquitoes, malaria is readily transmitted by blood transfusion through donations collected from asymptomatic, parasitaemic donors. The parasite is released into the bloodstream during its lifecycle and will therefore be present in blood donated by infected individuals. The parasites are stable in plasma and whole blood for at least 18 days when stored at +4°C and for extended periods in frozen state.

1.1.4.1 Bacterial Contamination of Blood Products

This is another less often observed risk disorder directly associated with Blood transfusion. It is increasingly rare but a very serious complication of blood transfusion. Most commonly associated with contamination during blood collection or during handling of blood products, such as preparation of platelet pools, and on occasion, associated with bacterial infection of the donor, it is sometimes recognizable by obvious changes in the appearance of the Blood product. Studies indicate that the rate of contamination of blood products by bacterial pathogens may be significant.

Transfusion of bacterially contaminated blood can cause fever, shock, collapse and death.

Blood must always be examined for sign of contamination at the time of use (when collected from the blood bank and at the patient’s bed side. When grossly contaminated, blood appears haemolyzed and dark in color or may be clotted.
CHAPTER 2

2. NATIONAL TESTING STRATEGIES FOR TRANSFUSION TRANSMITTED INFECTIONS

The healthcare system in Pakistan faces many challenges, which are unique to this country. Therefore, it is essential that National Testing Strategies should be specifically formulated to cater for every step in blood transfusion system which may compromise the provision of infection free blood to a recipient.

As per recommendations of the World Health Organization, it is essential to screen blood and blood components for transfusion for the following five infections:

i. Hepatitis B
ii. Hepatitis C
iii. HIV/AIDS
iv. Syphilis
v. Malaria

The selection of appropriate assays is a critical part of the screening programme. Reliable results depend on the consistent use of well-validated and effective assays. A number of factors need to be considered in selecting the most appropriate assays. In general, a balance has to be found between screening needs and the resources available, including finances, staff and their expertise, equipment, consumables and disposables.

Each screening system has its advantages and limitations that should be taken into consideration when selecting assays. Some limitations include:

a. The length of time following infection before the screening test becomes reactive (window period). This is an important point and must be emphasized in verbal screening, so that proper and effective screening strategies can be planned.

b. Higher the sensitivity of the testing system, higher the rate of false positives which may result in the wastage of donations and deferral of donors.

c. Higher the specificity of the testing system, lower the rate of false negatives that reduce the transmission of TTIs.

d. In blood transfusion services, selection of screening kits should have highest sensitivity and specificity. WHO recommended specifications are given below under each TTI.

2.1 Hepatitis B Virus (HBV)

The main markers for hepatitis B are detected in the serum (serological markers). These are:

a. Hepatitis B surface antigen (a marker of infection)

b. Hepatitis B core antibody, in some situations (a marker of exposure)
These are assayed by various laboratory techniques, some of which are relatively simple, inexpensive and rapid such as Immuno Chromatography Test (ICT).

Some require complex equipment such as:
   a. Enzyme Linked Immuno Sorbent Assay (ELISA)
   b. Chemiluminescent Microparticle Immuno Assay CMIA which is a type of Chemiluminescence Immuno Assay (CMIA)

Detection of components of infected cells, like viral nucleic acid, e.g. HBV DNA, can also be detected, by techniques like:
   a. Polymerase Chain Reaction (PCR) which can be;
      i. Qualitative
      ii. Quantitative

Recommendations:

The purpose of screening is to minimize the risk of HBV infection by blood transfusion.

1. WHO recommends that screening should be performed using EIA/CMIA with:
   a. Sensitivity = 100%
   b. Specificity > 98%
   c. The test system should be approved by FDA/EMEA/WHO for blood donor screening

2. In remote areas of the country where basic amenities are not available and blood bank are not fully equipped, screening should be done using a Rapid kit with following characteristics:
   a. Sensitivity = 100%
   b. Specificity > 98%
   c. Inter-reader variability < 5%
   d. Invalid rates of < 5%

3. In urban areas, for blood banks with lower workload (less than 20 blood donors a day), it is recommended that blood should still be screened by EIA/CMIA. This can be achieved through:
   a. Alternate day screening of blood donations
   b. Outsourcing
   c. Pooling of resources of two or more blood banks to perform screening together to make it cost effective

2.2 Hepatitis C Virus (HCV)

Screening:

Antibodies to Hepatitis C Virus (HCV antibodies), become detectable approximately 30 to 60 days after exposure.
The methods used to identify the presence of HCV exposure in blood donors is the presence of anti HCV antibodies. However, recently nucleic acid testing and HCV antigen test have also become available. Addition of these tests to the five WHO recommended mandatory tests for blood screening will improve the safety.

Anti HCV antibodies are detected using the following assay techniques:
   a. Chemiluminescent Microparticle Immuno Assay (CMIA)
   b. Enzyme Linked Immuno Sorbent Assay (ELISA/EIA)
   c. Immuno Chromatography Test (ICT)

HCV viral antigen (HCV antigen) appears between 0 and 20 days after the appearance of viral RNA and is detected by using Chemiluminescent Microparticle Immuno Assay (CMIA). Now Combo Test of HCV antigen/antibodies is available based on CMIA technology. The HCV RNA can be detected by Polymerase Chain Reaction (PCR).

Since the prevalence of Hepatitis B & C in Pakistan is much higher than that of HIV and Syphilis, therefore, it is recommended to screen for hepatitis first. If a blood donation is found positive for either Hepatitis B or C, then no further testing is advised, and the sample should be properly discarded.

Recommendations:

1. WHO recommends that screening should be performed using ELISA/CMIA with:
   a. Sensitivity = 100%
   b. Specificity > 98%
   c. The test system should be approved by FDA/EMEA/WHO for blood donor screening
   d. Results which are up to three times the upper limit of the cutoff (grey zone) may be advised to get confirmation on RIBA test. HCV antibodies positive samples may or may not have HCV RNA if tested, therefore, it is not recommended to test for HCV RNA in blood donors for confirmation of HCV antibodies
   e. For blood banks who opt for NAT testing should follow the AABB/WHO guidelines

2. In remote areas of the country where basic amenities are not available and blood bank are not fully equipped, screening should be done using a Rapid kit with following characteristics:
   a. Sensitivity ≥ 98%
   b. Specificity > 97%
   c. Inter-reader variability < 5%
   d. Invalid rates of < 5%

3. In urban areas, for blood banks with lower workload (less than 20 blood donors a day), it is recommended that blood should still be screened by EIA/CMIA. This can be achieved through:
   a. Alternate day screening of blood donations
   b. Outsourcing
   c. Pooling of resources of two or more blood banks to perform screening together to make it cost effective
2.3 **Human Immunodeficiency Virus (HIV)**

**Screening:**

The methods used to identify the presence of HIV detect serological markers, as well as viral components, like viral nucleic acid.

1. **Serological markers, include:**
   i. Anti HIV-1 and antiHIV-2
   ii. HIV p24 antigen (p24 Ag)

2. **Viral nucleic acid: HIV RNA**

Now Combo Test of HIV antigen/antibodies is available based on CMIA and Rapid (ICT) methods.

The assay techniques for detecting these analytes include:

1. **Enzyme Linked Immunosorbent Assay (ELISA or EIA)**
2. **Rapid Tests (only be performed in laboratories with small throughput, in remote areas or emergency situations) like Immuno Chromatographic Tests (ICT)**
3. **Chemiluminescent Microparticle Immuno Assay (CMIA)**

**Recommendations:**

1. WHO recommends that screening should be performed using ELISA/CMIA with:
   a. Sensitivity 100%
   b. Specificity > 98%

2. In remote areas of the country where basic amenities are not available and blood bank are not fully equipped, screening should be done using a Rapid kit with following characteristics:
   a. Sensitivity ≥ 99%
   b. Specificity > 98%
   c. Inter-reader variability < 5%
   d. Invalid rates of < 5%

2.4 **Syphilis**

In Pakistan, the prevalence of syphilis infection has been documented to be much lower than in Western countries. Hence it is recommended to screen using highly sensitive and specific tests for treponemal antibodies, which include either Treponema pallidum Haemagglutination Assays (TPHA) or enzyme immunoassays (ELISA), so that the already small amount of syphilis present in the community, especially the donor pool, can easily be detected.

If these are unavailable, then non-specific assays such as Venereal Diseases Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) tests can help to identify those individuals who may have been more recently infected but it is not recommended for blood screening.
Recommendations:

1. WHO recommends that screening should be performed using ELISA/TPHA with:
   a. Sensitivity 100%
   b. Specificity > 98%

2. In remote areas of the country where basic amenities are not available and blood bank are not fully equipped, screening should be done using a Rapid kit with following characteristics:
   a. Sensitivity ≥ 98%
   b. Specificity > 97%
   c. Inter-reader variability < 5%
   d. Invalid rates of < 5%

3. In urban areas, for blood banks with lower workload (less than 20 blood donors a day), it is recommended that blood should still be screened by ELISA/TPHA. This can be achieved through:
   a. Alternate day screening of blood donations
   b. Outsourcing
   c. Pooling of resources of two or more blood banks to perform screening together to make it cost effective

2.5 Malaria

Pakistan is a high endemic country for malaria. Hence, after a careful history of recent high grade fever, direct detection of parasite by thick film is often used to identify parasitaemic donations.

Antigen detection tests by Immuno Chromatography Test (ICT) may be useful in detecting asymptomatic donors.

Recommendations:

1. Donor selection criteria should be developed to identify and collect blood from donors at the lowest risk of infection, both during the malaria season and also during rest of the year, as well as for high and low malaria endemic areas.

2. WHO specific recommendations for donor selection in malaria endemic countries

3. All donations should be screened for parasitaemia using thick blood films or malarial antigen using a highly sensitive enzyme immunoassay/immuno chromatographic test method.

4. Transfusion should be followed by the administration of appropriate and effective malarial prophylaxis to all recipients or at least to those recipients at risk of significant disease as a result of transfusion transmitted malaria.
CHAPTER 3

3.1 Standards for Performance of Testing

Standards for performance of testing will vary according to the level of functioning of the blood bank.

3.1.1 Referral or Reference Laboratories, and Regional Blood Banks

The expertise is required at the level of the referral or reference laboratories, and of the regional blood banks. A qualified person (blood transfusion specialist, haematologist or infectious disease specialist), should be the head of the laboratory, supported with a graduate medical laboratory technologist leading the team of laboratory technologists or technicians.

Standard Operating Procedures (SOPs) should be developed for testing of all TTIs with periodic updates. Quality Assurance procedures should be implemented and all activities should be documented.

3.1.2 Blood Banks in larger cities and towns

A haematology & blood banking trained medical laboratory technician under the supervision of a specialist should be entrusted the responsibility of screening of transfusions for TTIs, following the SOPs developed by the referral laboratories.

3.1.3 Peripheral Blood Banks

Blood Banks in peripheral areas should be able to provide reliable, quality assured blood screening, following the minimum requirements for blood testing. A laboratory technician should be responsible for blood screening, and should be trained and updated periodically in the regional blood bank.

3.2 Quality Assurance Systems within which the screening is to be performed

Quality Assurance includes all the procedures involved from the selection of donors, collection of blood samples, adequate storage, relevant screening with proper quality control, reliable reporting, and culminating in the judicious use of the transfusion to the patient. Detailed and cross checked documentation is an essential part of any satisfactory Quality Assurance system.

The steps involved in proper quality maintenance in the laboratory procedures themselves are known as Quality Control (QC).

3.2.1 Quality Control
There are two essential components of any quality control system viz. Internal and External.

3.2.2 Internal Control

The internal assay control is almost always provided as a component of any diagnostic or screening kit and will always be a part of the testing process. On the other hand, internal controls are produced in-house. In some kits, these controls may be provided as a separate material, but will still be used with each test.

i. Each diagnostic test run must include appropriate set of assay controls plus whatever QC samples are required/available
ii. The assay controls for each test run must yield results that confirm to the manufacturer’s criteria for acceptability and validity of the run
iii. All test kits must be used before the expiration date to ensure valid results
iv. Physical parameters of the test such as incubation time and temperature must be followed to ensure proper performance

Run a negative and a positive control (and weakly positive whenever possible) at the following times:

- Once weekly or fortnightly, preferably at the beginning of the week
- When a new operator (a trained staff member who has not been doing testing for a while, or a newly trained operator) is performing testing
- When beginning the use of diagnostic test kits or reagents with a new lot number
- Whenever a new shipment of test kits is received
- If test kits are exposed to environmental conditions that fall outside the range needed for stability as defined by the manufacturer, especially after prolonged periods of electricity failure, severely adverse weather conditions or any other factor which may directly or indirectly affect the performance of the diagnostic test

3.2.3 External Control

The blood bank/laboratory should take part in the external quality control programme. Emphasis should be to make sure that the feedback of the submitted results be analyzed and any problem identified. All “out with consensus” results in the EQC should be checked and appropriate measures taken to rectify the problem.

3.3 Screening Kits Storage and Laboratory Conditions

Most laboratory diagnostic kits should be stored according to manufacturer’s instructions. Most of the quality assured test kits available in the market require storage at temperatures ranging between 2° to 8° C and all the appropriate measures should be taken to ensure the compliance.

Uninterrupted Power Supply (UPS) systems or stand-by generators and voltage stabilizers should be available to ensure continuous uninterrupted power supply at all times.

3.3.1 Transportation of blood and blood products
Transportation at ambient temperatures is not acceptable for short periods of time and in moderate climates. In climates with extremes of hot or cold, test kits and reagents should be transported under fully controlled conditions at specified temperatures, such as between +2°C and +8°C. However, when evaluating test kits before procurement, it is essential to evaluate them over a range of temperatures and weather conditions, to be able to guide the end-user laboratories of possible pitfalls in their performance.

3.4 **Data Management**

It is imperative that, in addition to electronic data storage, a manual record of patient and tests data are maintained, so that in the event of a power failure, records can still be retrieved.

3.5 **Strategies for emergency situations when large amount of blood is needed as in disasters**

In any good blood transfusion centre, a detailed history of any past medical problems, and, if required, a thorough physical examination, will help to guide towards the presence of an infectious, and possible blood transmittable, disease.

In emergency situations in which blood and blood components are needed urgently, but are not readily available from blood inventory, screening with rapid/simple, single-use assays could be used to obtain results quickly and enable blood to be released for clinical use in consultation with the prescribing clinician.

Wherever possible, however, the blood sample should be retested as soon as possible using an ELISA/CMIA in order to check the validity of the test results.

3.6 **The definition and interpretation of reactive and non-reactive tests**

Automated assay systems, especially ELISA, which can provide a colorimetric reading of the absorbance or transmittance of a color generated by the test or the generation of luminescence, can qualitatively provide a result as being either positive, negative or equivocal based on the absorbance values of the positive, negative and the cut-off control.

Diagnostic and screening test kits usually provide clear cut instructions on how each test result should be interpreted, but it is the duty of the supervisor of the Quality Assurance programme, to ensure that the person(s) performing the test are proficient in the interpretation of the results, of even ambiguous tests. Even a task as apparently simple as reading a positive or negative agglutination should not be overlooked, and proper training and guidance for the technicians and operators of test must be provided and followed up periodically.

3.7 **Pooling for Serological Assays**
The pooling of samples for serology testing is not recommended for a blood screening programme.

3.8 Parallel versus Serial (Sequential) testing in Pakistan

Testing algorithms may involve serial (also called Sequential) or parallel testing. ELISA-based algorithms are almost always serial in nature, while rapid test algorithms can be either.

Serial testing: In blood transfusion services, the National Testing Strategies state a single test strategy to screen donated blood. If the sample is found positive, no further testing is done and the sample is discarded while negative samples are further tested for other TTIs.

Parallel testing: In parallel testing, in blood transfusion services environment, a blood sample is screened for all the TTIs simultaneously. This saves time, but is not cost effective.

In Pakistan, according to the National Survey conducted by the Pakistan Medical Research Council the prevalence of Hepatitis C infection in the general public was 4.9%, of Hepatitis B (HBsAg) 2.5%, of syphilis between 0.5 to 0.67% and of HIV less than 0.01%.

Based on National Prevalence data on prevalent TTIs, it is suggested that the blood donation should be tested in the following order:

1. Hepatitis C
2. Hepatitis B
3. Syphilis
4. HIV
5. Malaria

Samples which are negative for HCV should then be tested for HBV and so on.

One disadvantage of this strategy is that donors with co-infections (i.e. with more than one infection) will not be identified and cannot, therefore, be notified and counselled about these additional infections as part of the duty of care towards blood donors.

3.9 Linked testing strategy

In linked testing strategy, it is the duty of the concerned blood bank to inform all blood donors that are found positive for any TTIs so that the patient could seek treatment and the system could reduce further transmission of these blood borne diseases.
CHAPTER 4

4. TESTING FOR TTI

4.1 Blood Screening and Diagnostic Testing

Screening for TTIs and diagnostic testing are carried out by the same methods and assays, however, there is a difference in the reasons for the testing, the population being tested, the interpretation of the results and the subsequent actions. So while screening assays and tests must be highly sensitive to pick out even the slightest infection, a diagnostic test must be highly specific to eliminate the chances of false positive or false negative results.

All blood samples are potentially high-risk samples.

4.2 Blood Donors and Blood Screening

Screening of donated blood for TTIs is one of the most important strategies for blood safety and availability. The first line of defence in providing a safe blood supply and minimizing the risk of transfusion-transmitted infection is to collect blood from well-selected, voluntary non-remunerated blood donors from low-risk populations, particularly those who donate regularly. The prevalence of TTIs in voluntary non-remunerated blood donors is generally much lower than among family/replacement and paid donors. Each country should establish and promote voluntary blood donor programmes which provide donor information and education and develop stringent national criteria for blood donor selection and deferral to exclude prospective donors at the risk of TTIs.

4.3 Selection Criteria

Assay specific factors include:
- Assay presentation
- Clarity of instructions
- Ease of use
- Assay characteristics, including sensitivity and specificity
- Sample volume
- Sample and reagent addition monitoring
- Robustness
- Assay reproducibility and precision
- Number of tests per assay
- Kit size
- Total assay time
Laboratory specific factors include:

- Number of samples to be tested
- Staff levels
- Staff competence
- Available equipment
- Level of laboratory quality system

Logistics that need to be taken into consideration include:

- Vendor selection and validation
- Price
- Procurement system
- Availability and reliability of the supply of test kits and reagents
- Shelf-life of test kits and reagents
- Infrastructure: e.g. controlled storage conditions and uninterrupted power supply
- Technical support for trouble-shooting
- Equipment maintenance, servicing and repair

4.4 Evaluation of Assays

Assays produced by the major international diagnostics companies are generally well-designed and are normally evaluated scientifically, both by the manufacturers themselves and by independent laboratories, prior to release onto the market. WHO operates a prequalification of diagnostics programme, which provides technical information and advice on the quality of currently available HIV/AIDS, malaria and hepatitis B and C test kits and assay systems with the aim of increasing access to affordable diagnostic technologies of assured quality that are appropriate for use in resource-limited settings.

Data published in kit package inserts and the scientific literature also provide useful information guiding selection of vendors, testing platforms and specific assays. However, well-planned and documented assay evaluations prior to their procurement are essential to ensure that the most appropriate selections are made from the available options. Assay evaluations are required to determine scientifically the most suitable assays for use in particular situations.

Evaluations should be carried out in at least one major facility, but some blood transfusion services may not have the necessary resources, expertise, experience and, importantly, panels of samples required. In such situations, the evaluations should be undertaken on behalf of the blood transfusion service, and in close conjunction with it, by an appropriate laboratory, such as the national reference laboratory. If none is available, the evaluation data required should be obtained from a blood transfusion service or reference laboratory in another country with similar demography, infection incidence and prevalence and BTS requirements, preferably in the same region. Reference should also be made to information available from laboratories elsewhere in the region or globally.
The evaluation process normally consists of performing each assay under consideration against selected panels of samples that will challenge the assay and deliver statistically valid results. The panels are generally comprised of:

True positive samples and true negative samples in which the sensitivity and specificity respectively are determined:
- Samples collected during seroconversion
- Low-level positive samples: for example, samples from very early or very late in the course of infection
- Samples covering a range of different genotypes and/or serotypes with emphasis on local samples
- Known non-specifically reacting samples or potentially cross-reactive samples: i.e. samples from patients not infected with the target infection, but with a range of clinically relevant conditions such as hyper-gammaglobulinaemia, other infections or autoimmune disease.

The overall size of the panels will be determined by local availability but, generally, the more samples tested, the more useful and reliable is the information generated. It is particularly important to include as many examples of locally-acquired infections as possible, especially samples from blood donors found previously reactive and confirmed to be infected. Analysis of the results will identify the assay that gives the best overall performance against all samples tested. It is therefore important that the panels are as broad as possible and that overall performance is assessed in the context of the planned use of the assay.

Each country should determine the minimum sensitivity and specificity levels required for each assay. Evaluation should be conducted on sufficient numbers of known antibody positive and negative samples to ensure that evaluation results are statistically significant. It is recommended that the minimum evaluated sensitivity and specificity levels of all assays used for blood screening should be as high as possible and preferably not less than 99.5%.

4.4.1 Monitoring Assay Performance

In blood screening, assay performance should be continually monitored in order to identify any changes in performance that are occurring and that, without correction, might ultimately lead to a failure in either the assay runs or the detection of low-level true positive samples. Performance is usually assured by monitoring one or more parameters that can reasonably be expected to change relatively quickly as a result of any change in the performance or use of the assay (the assay or the operator/system performing the assay). These parameters include:
- Quality control sample results
- Assay control values
- Repeat reactivity

The use of appropriate quality control (QC) samples included with every batch of tests performed will rapidly generate useful and reliable data for monitoring. In this context, a batch of tests can be any defined block of tests; for example, a single microplate is a
batch of tests and at least one external QC sample could be included on each plate. External quality controls do not substitute for internal (kit) controls.

QC samples are normally well-characterized samples, individual or pooled that are calibrated against international standards, where possible, and are diluted in an appropriate matrix. These samples may be used as external go–no–go controls, in which case the QC sample(s) has to be reactive for the assay run to be valid. If QC samples are not available, tracking the assay control values may be used as an alternative for assessing the consistency of performance.

In all cases where quantitative values are used, such as EIA optical density (OD) values, the results should be normalized to allow comparison between different runs and, to a certain degree, between different assays. The normalized OD value is calculated as follows:

- Non-competitive EIAs: divide the sample OD value by the cut-off OD value
- Competitive assays: divide the cut-off OD value by the sample OD value

The ratio generated can then be directly compared to the ratios generated by any other runs of the assay, including different manufacturers’ lots. The analysis is less objective in situations where assay results are qualitative, such as in the use of particle agglutination assays. However, the QC sample can be used to determine whether the results of the assay run are valid. Where it is not, the assay run should be repeated.

4.5 Use of Automation for Performing Assays

The use of automation is a major consideration for blood transfusion services that perform a large number of screening tests. While all EIAs need a basic level of automation (automated plate washers and readers), highly sophisticated automated screening systems are available that can perform all aspects of an immunoassay from sampling through to the final analysis of the results. These systems perform immunoassays from any major manufacturer and are referred to as “open” systems; they are generally microplate-based and the equipment and assays are not linked. Dedicated systems, known as “closed” systems, are fully automated and use only specific, dedicated assays with all the necessary reagents and controls produced by or in collaboration with the equipment manufacturer.

Depending on the number of donation samples to be screened each day and the resources available, the use of a fully automated system can offer substantial advantages in terms of quality, especially if the system handles the samples as well as performing all the steps of the assay. Automated systems generally offer a high level of consistency and reproducibility in assay performance and can also help to reduce operator errors. However, they have specific additional requirements, including special staff training needs, regular and effective maintenance and calibration and may involve higher capital and running costs. Open and closed systems each have their advantages and disadvantages but, in general, an open system offers greater flexibility and may be more cost-effective, although the technical input and skill required from the user is often greater.
As in the selection of assay types, the overall workload is a major factor in determining whether automation is appropriate. Automated systems are particularly useful where large numbers of samples are screened regularly. At lower workload levels, where EIAs are performed, at the very least automated plate washers and plate readers are essential.

4.6 New Assays and Technologies

New blood safety technologies are constantly becoming available which may offer new opportunities to blood screening programmes. Although it is important to be aware of scientific and technological developments, these may or may not offer any advantages or significant improvements over current practice. In the context of screening donated blood, the use of a new technology is generally an advantage only if the technology currently in use is failing to identify infected donations or if the new technology offers significant cost savings and efficiency benefits without reducing the overall effectiveness of the current screening programme.

Before any new technology is introduced into a blood screening programme, it should be fully investigated and systematically evaluated. Even if there is a potential advantage, the feasibility of implementing a new technology should be fully considered, including the requirements for infrastructure, financing, staffing levels, training and quality systems. Since the overall costs of implementation may far outweigh any potential benefit in terms of increased blood safety, a cost-benefit analysis should be performed and found to be favorable.
ALGORITHM for Testing in Laboratories
WITHOUT Well-Established Quality Systems

History of malaria fever or Dengue like symptoms in last 6 months

NEGATIVE → COLLECT BLOOD DONATION

SCREEN for HBsAg & HCV

NEGATIVE → SCREEN for HIV & Syphilis

NEGATIVE → RELEASE BLOOD FOR TRANSFUSION / STORAGE

POSITIVE → DEFER Blood Donation until 6 months from last episode of fever

POSITIVE → DISCARD BLOOD SAMPLE

POSITIVE → DISCARD BLOOD SAMPLE
This Algorithm represents, in outline, a COMBINATION of BOTH Options for Blood Screening

**History of malaria fever or Dengue like symptoms within the previous 6 months**

**POSITIVE**
- DEFER Blood donation until 6 months after last episode of fever

**NO** history of malaria or dengue

**Take Blood Donation**

**Non Reactive (A1-ve)**

**Perform initial Screening test (A1)**

**Initial reactive (A1+ve)**

**Option 1**
(No/limited quality system)
- DISCARD the donation and derived Blood components

**NEGATIVE in both repeat tests (A1+ve, A2-ve, A3-ve)**

**NO further confirmatory testing required**
- Release Donation and Derived blood components
- Accept donor for future blood donations

**Option 2**
(Effective Quality System)
- Repeat test in duplicate using same sample and same assay (A2, A3)

**REACTIVE in both repeat tests (A1+ve, A2+ve, A3-ve) or (A1+ve, A2+ve, A3+ve)**
- DISCARD the donation and derived blood components

**Send for CONFIRMATORY TESTING in accordance with National Testing Strategy**

**KEY:** A=Assay, A1=Assay No.1, A2, A3 = 2\textsuperscript{nd} & 3\textsuperscript{rd} Repeat Assays
- A+ve = Reactive result in A
- A-ve = Non-reactive result in A

Model Algorithm for Blood Donor Management based on screening and confirmatory testing.

- **History of malaria fever or Dengue like symptoms within the previous 6 months**
  - **POSITIVE**
  - DEFER Blood donation until 6 months after last episode of fever

- **NO history of malaria or dengue**
  - **Take Blood Donation**

- **Non Reactive (A1-ve)**
  - Perform initial Screening test (A1)
  - **Initial reactive (A1+ve)**
  - **Option 1**
    - (No/limited quality system)
    - DISCARD the donation and derived Blood components
    - **NEGATIVE in both repeat tests** (A1+ve, A2-ve, A3-ve)
    - **NO further confirmatory testing required**
    - Release Donation and Derived blood components
    - Accept donor for future blood donations
  - **Option 2**
    - (Effective quality system)
    - Repeat test in duplicate using same sample and same assay (A2, A3)
    - **REACTIVE in both repeat tests** (A1+ve, A2+ve, A3-ve) or (A1+ve, A2+ve, A3+ve)
    - DISCARD the donation and derived blood components
    - Perform CONFIRMATORY TESTING in accordance with National Testing Strategy

- **CONFIRMED NEGATIVE**
  - Inform and counsel donor and defer until screen non-reactive

- **INCONCLUSIVE**
  - Likely non-specific reactivity
  - Inform and counsel donor, defer and follow-up for further investigations

- **CONFIRMED POSITIVE**
  - Donor infected
  - Notify and counsel donor. Defer and refer for treatment

**KEY:** A=Assay, A1=Assay No.1, A2, A3 = 2nd & 3rd Repeat Assays
A+ve = Reactive result in A. A-ve = Non-reactive result in A

CHAPTER 5

5. MANAGING POSITIVE BLOOD DONATIONS

5.1 Quarantine of Blood and Blood Components Prior to Release or Discard

A quarantine system should be established and put in place for the physical segregation of all unscreened donations and their blood components until screening for infection markers has been completed and the suitability of donations for therapeutic use has been determined. A proper documented system should be in place to ensure that screened and unscreened units are stored in separate blood storage equipment to prevent the issue of unscreened units. All reactive or positive donations and all components derived from these donations should be labeled “Not for Transfusion” and segregated for discard or non-clinical use.

The BTS should ensure that separate blood storage equipment is clearly designated for:
- Unscreened units
- Reactive/positive units
- Unresolved/indeterminate units
- Units suitable for clinical use: i.e. available blood stock
- Units pending disposal (however, these may be combined with Reactive Units)

There should be a fully documented system that identifies the current location and eventual fate of all blood and blood components, whether destined for clinical use (transfusion or component separation) or disposal. The BTS should also have documented policies and procedures to deal with the emergency release of blood components prior to all screening being completed.

Reactive or positive units of blood or plasma are valuable resources for quality control samples and panels, evaluations and validations, and for research purposes. Blood screening laboratories can provide blood or plasma to be used as reagents to institutions involved in research or to quality assessment schemes for the production of proficiency panels.

5.1.1 Release of Blood and Blood Components

Only blood and blood components from donations that are non-reactive for all markers screened for should be released for clinical or manufacturing use. When all the required blood screening tests have been performed, the results have been checked and any other required checks have been made, formal release procedures can be undertaken to release quarantined units and physically move the released blood stock from one location to another.

The BTS should have appropriate systems for labeling the blood and blood components as “Ready for Clinical Use”. The label on each blood unit should contain the relevant
details of the donation and the tests carried out on the donation. When this has been carried out, the screening process is considered to be complete.

All reactive units should be removed from the quarantined stock and stored separately and securely until further handling.

5.1.2 Long-Term Storage of Serum and Plasma Samples received from Donation

The long-term archiving of donation serum and/or plasma samples can be very useful for a BTS in facilitating the investigation of adverse transfusion events and TTIs or the evaluation of new screening assays or reagents. However, archiving should be considered only if adequate and suitable resources are available, including sufficient space and efficient paper-based or software-based warehousing systems to manage sample retrieval.

A number of crucial issues should be considered before building a sample archive. These include:
1. System for the identification and history of each sample in the archive related to its use and length of time of storage
2. Type of storage containers required
3. Specified temperature at which samples are to be stored
4. Volume of samples to be archived
5. Criteria and documentation of the reasons for the recovery of an archive sample

5.2 Managing Blood Donors

In addition to collecting and screening a unit of blood, the BTS has several other functions ranging from motivation of donors, to the management of blood donors. This is an essential part of the activities of every blood transfusion service. Donors are the source of the blood and blood components that are processed and released for clinical or manufacturing use. Accordingly, they should be managed in a way that ensures high standards of care and assures them that the BTS cares for their health, safety and well-being.

It is possible to identify infected donors (or donors with non-specific reactivity or inconclusive results), through blood screening and confirmatory testing. Even if only limited facilities and resources are available, the blood transfusion service has a duty of care to donors, their families and the general population to ensure that infected individuals are referred for appropriate counselling, treatment and further management as they may infect other individuals if they are not aware of their status, or appropriately counseled about their disease. The BTS and relevant authorities should have a clear policy and systems for communicating with these donors and informing them of their status in order to minimize any risk of further transmission. Donors who test negative for TTIs should be encouraged to donate regularly and lead low-risk lifestyles.

5.2.1 Deferral of Blood Donors
Based on the results of blood screening and repeat testing, a blood donor may be either confirmed positive, or may have an initial reactive but repeat negative blood test, or may be inconclusive, for any particular TTI, especially HIV. They are all to be dealt with separately.

**Confirmed positive donors:**

Donors who are confirmed positive should be deferred from blood donation, notified of their infection status, counselled and referred for clinical management as soon as possible.

**Repeat Reactive But Confirmed Negative Donors:**

The handling of repeat reactive donors with non-specific reactivity is a critical part of a screening programme because the selection of suitable screening assays and the use of an appropriate screening algorithm can minimize the unnecessary deferral of donors and loss of donations. Donors showing repeated reactive results on screening and negative results on confirmatory testing should be informed, reassured, counselled and temporarily deferred until non-reactive on follow-up using the same screening assay or a different assay. If negative, they can again be accepted as blood donors.

**Inconclusive donors:**

Donors with inconclusive results present challenges to blood transfusion services and screening laboratories as their management is less clear than with confirmed positive or confirmed negative donors. It is important to decide whether they can be retained on the donor panel or are to be deferred. It is advisable to inform, counsel and defer inconclusive donors temporarily, usually for up to six months. If they screen non-reactive and confirmed negative on follow-up, they can be accepted as blood donors in the future.

5.2.2  Post-donation counseling

Informing a donor that he or she is confirmed positive for an infection clearly poses sensitive issues which need to be addressed and handled very carefully. Donors need to be counseled on the results and the actions that should subsequently be taken. It may be necessary, where feasible, for the BTS to appoint specialist donor counseling staff and provide referrals to agencies that provide further counseling, treatment and care. If applicable, the BTS should request the donors’ own physicians to communicate with them.

Informing donors of non-specific reactivity is highly problematical and should be undertaken with care because this reactivity often varies and usually does not have any impact on the actual health of the individuals. Clear policies on the handling of non-specifically reactive donors are essential. The permanent deferral of these donors is sometimes considered to be unnecessary, but may be unavoidable unless policies and procedures are in place that recognize variable non-specific reactivity and facilitate the appropriate management of such donors.
Post-donation counseling of donors can provide information on the possible routes of infection and the effectiveness of donor education and donor selection criteria, including why the donor decided to donate, whether they already knew they were infected and whether donor education materials give sufficient information about risk behavior. This kind of information aids in understanding patterns of infection in seemingly “healthy” individuals and can be used to ensure that donor information and education materials are clear and unambiguous. It can also be used to improve donor selection criteria and the donor selection process.
CHAPTER 6

6. PROGRAMMATIC SYSTEMS IN BLOOD SCREENING

6.1 Management at Programmatic Level

6.1.1 Organization and Management

It is essential that Blood Transfusion Services should be coordinated and controlled at a national level. This is a prerequisite for an effective, efficient and sustainable national blood screening programme. It is also required for the uniform application of national standards and procedures across an entire country. Coordination is essential to maintain continuity in operations and consistency in performance in all facilities in which screening is performed, including blood centres and hospital-based services. Each screening facility requires a specific and sufficient budget, a suitable infrastructure, with reliable water and power supplies, well-maintained equipment and efficient transportation and telecommunications systems, and above all, well-trained and motivated staff.

Thus, greater efficiency and safety can be achieved by bringing together key blood screening activities into a network of strategically located central and/or regional blood centres with well-trained staff, suitable equipment and efficient procurement and supply systems.

By facilitating economies of scale, this enables overall costs to be minimized without compromising quality. Conversely, the screening of blood in multiple small centres usually leads to the wastage of precious resources and a lack of uniform standards.

In countries like Pakistan, which have largely hospital-based blood services, national health authorities should assess the need and feasibility of consolidating screening activities at national and/or regional levels so that the national screening programme can be implemented more efficiently and cost-effectively. This would require a periodic situation analysis through the identification and mapping of all existing facilities that screen blood donations and an assessment of their organizational structure, infrastructure, technical and human resources.

From this, a needs assessment can be carried out to identify requirements and priority interventions to strengthen TTI screening of donated blood. This will enable the development of national and regional operational plans involving all relevant stakeholders for strengthening and, if appropriate, reorganizing the structure and network of facilities for blood screening. Plans should include a monitoring and evaluation mechanism, with a baseline, targets and indicators in order to measure progress and impact in all facilities in which TTI screening of donated blood is performed.
6.2 Reference Laboratory(s)

Most countries have at least one well-established laboratory with the relevant expertise and experience that could be designated as a reference laboratory. A national public health/reference laboratory is generally suitable for this work. Alternatively, the role of the reference laboratory may be delegated to a blood transfusion service laboratory if it has suitable facilities, adequate resources and an effective quality system. An assessment of requirements for the strengthening of the reference laboratory may be needed to ensure its capacity to support the blood screening programme.

In Pakistan, the situation is unique, since health (and thus blood transfusion services) has been provincialized. Most of the provinces in Pakistan have well-equipped, and well-staffed laboratories which can serve as Reference Laboratories for a particular province, and can provide Referral Services to neighboring provinces which may not have such facilities.

The role of the reference laboratory should include, but not be limited to:

i. Evaluation and selection of assay systems and equipment
ii. Confirmatory testing on donations which screened reactive/positive for blood donor management
iii. Provision of quality control samples
iv. Facilitation of Internal Quality Assessment in the BTS
v. Organization of External Quality Assessment schemes
vi. Periodic Training Workshops of the BTS Technical and Non-Technical staff

6.3 Financial and Human Resources

Transfusion Transmitted Infections (or TTIs) are an unnecessary burden and pressure on the healthcare system of a country. Investment in blood safety measures to prevent transfusion-transmitted infection is more cost-effective than allowing the further spread of TTIs. Every country, especially resource scarce countries like Pakistan, should ensure that sufficient and sustained resources are available for an effective and comprehensive blood screening programme that ensures the high quality screening of all donations for TTIs.

In order to make optimal use of limited healthcare resources, the screening programme should ensure a balance between the application and implementation of good scientific principles and the best use of the resources available. The implementation of new systems for screening is best undertaken in a stepwise fashion with appropriate resources allocated for establishing functional quality systems.

A sufficient number of qualified and trained staff should be available to perform the laboratory activities associated with blood screening, including the implementation of quality control and assurance systems. In-service training programmes should be established and reviewed at appropriate intervals to define areas where further training or re-training is necessary. The competency of all staff to perform their roles to the required standards should be assessed on a regular basis, and appropriate measures taken accordingly to ensure the best performance of the technical and support staff.
Blood transfusion services should work with national health, public health and education authorities to ensure that education and training institutions provide suitable opportunities for qualifications and training. Measures should be adopted to provide opportunities for career recruitment and progression and to retain experienced staff in order to ensure that laboratories function effectively.

6.4 Laboratory Quality Systems

The overall effectiveness of the blood screening programme and its potential to minimize the transmission of infection through transfusion can only be ensured by the implementation of effective quality systems.

Quality systems should not be limited to laboratories only, but should encompass all activities of the blood transfusion service to ensure that all donations are screened correctly and handled appropriately before and after laboratory testing. The implementation of quality standards will ensure the safety and clinical efficacy of blood and blood products for patients as well as protecting the health and safety of staff.

6.5 Procurement and Supply of Assays and Reagents

Proper and regular procurement and continuity in the supply of the assays, reagents and consumables required for testing depends on reliable procurement and supply systems. Frequent variations in assays and reagents could affect the quality system as they would each require evaluation and validation, and appropriate documentation and training before their use. Interruptions to supplies of assays and reagents may result in the temporary inability of screening facilities to screen for TTIs and having to issue unscreened blood for transfusion. This is equally dangerous for the transmittance of diseases of both high and low prevalence in a community, and if a disease frequency is low, should not give a sense of false security, leading to avoidance of screening for that disease.

A national procurement system will require the development of specifications for equipment, test kits, reagents and consumables and assessment of the quantity and types required. The implementation of centralized bulk procurement with an efficient distribution system is likely to provide significant cost savings, simplify stock management and enable an uninterrupted supply of assays and reagents to be maintained. WHO and other technical agencies operate procurement services to increase access to affordable assays of assured quality that are appropriate for use in resource-limited settings.

The blood transfusion service should have an efficient supply chain management system in place to monitor expiry dates of test kits and reagents and manage stocks in order to maintain an uninterrupted supply. The system should include procedures to ensure the traceability of the batch numbers of all test kits and reagents and their manufacturers. Regular liaison with suppliers is essential to ensure that they are fully aware of the requirements for test kits and reagents, including usage rates and the
required frequency of supply. This should enable suppliers to ensure that stocks are always available for delivery, when required, and in addition to have system for replacement of near-expiry or expired test kits, to ensure maximum efficiency, and cost savings.

6.6 Regulatory Mechanisms

As per WHO recommendation, since it is essential that each country should establish regulatory mechanisms that perform oversight functions for the activities of the blood transfusion service, including blood screening, the Government of Pakistan established the Safe Blood Transfusion Programme (SBTP) for Pakistan.

The SBTP possesses the expertise and competence in blood transfusion activities to assess the BTS against appropriate national and international standards, as they become applicable. As required, these assessments may be formalized as a system of inspection, licensing, certification and/or accreditation and may involve not only the BTS, but also transfusion-related activities at hospital level. An effective oversight system gives confidence in the blood transfusion service to all stakeholders.
CHAPTER 7

7. QUALITY SYSTEMS IN BLOOD SCREENING

7.1 Quality in the Laboratory and Blood Bank

7.1.1 The Elements of Quality Systems

Quality systems are crucial for the overall effectiveness of all aspects of the screening programmes and in assuring the quality, safety and efficacy of all blood and blood products.

The key elements of a quality system for blood screening include organizational management, quality standards, documentation, traceability, training, assessment and maintenance and calibration. All screening tests should be performed in accordance with defined quality requirements, and all blood donations and blood components prepared from them should be handled appropriately before, during and after laboratory testing. It is the responsibility of the blood transfusion service as well as individual laboratories to implement these standards consistently.

A quality system in a laboratory defines all the processes and procedures that should be put in place to ensure effective blood screening. Its implementation minimizes errors and ensures that:

i. Appropriate tests are performed on the correct samples
ii. Accurate and reliable results are obtained
iii. Only reliably screened, non-reactive blood and blood components are released for transfusion or manufacturing use
iv. Screened blood and blood components are available in the blood inventory at all times. This is especially important in emergency situations

7.1.2 Organizational Management within the Blood Bank/Screening Laboratory

The support of senior management is required for developing a quality policy and quality system in each screening facility. A Quality Assurance Manager should be designated in all facilities in which screening is performed. Management should ensure that responsibility, authority, accountability and job descriptions are clearly defined and communicated within the organization.

7.1.3 The Objectives of the Quality Assurance System

i. To ensure quality of the overall testing process, starting from donor selection till the final use of the blood bag or component
ii. To detect and reduce errors and their sources
iii. To improve consistency between testing sites, and between various testing protocols
iv. To help contain costs

7.1.4 Quality Control (QC)

Quality Control strictly refers to the measures that must be included during each assay to verify that the particular screening or diagnostic laboratory test is working properly. It essentially refers to the daily monitoring of precision and accuracy of an ongoing routine screening or testing programme. Quality control may be *internal* when positive, negative and cut-off controls provided with the diagnostic test kit are used. It is *external* when known positive and negative control sera, usually procured from commercial sources, are tested along with a batch of specimens.

7.1.5 Quality Assurance (QA)

The term Quality Assurance is more comprehensive and refers to an entire range of activities and procedures in the screening or testing laboratory to ensure consistent accuracy and reliability of the service starting from the selection of the donor, proper collection and labeling of the blood sample, its storage, screening, and recording of results, and ending with the judicious use of the donation for clinical use or component manufacture.

7.1.6 External Quality Assessment (EQAS)

For objective assessment of laboratory performance, each laboratory should participate in external quality assessment (EQA) in which sets of samples for testing are provided regularly by an external laboratory. The quality of performance and service of a screening and/or testing laboratory is assessed by an independent external agency. This may be National (NEQAS), regional or international, or may be linked with that of a particular country offering such services, like Australia, United Kingdom etc.

The results are then submitted back to the external laboratory. Analysis of these results provides useful information on the performance of assays, as well as of each laboratory participating in EQA.

7.1.7 Basic Elements of a Quality Assurance Programme

A successful Quality Assurance Programme must be established to ensure reliability of screening and testing so that safe blood and blood components, free from Transfusion Transmitted Infections can be provided.

7.1.8 Standard Operating Procedures

Standard Operating Procedures, commonly called SOPs, are a set of procedures which must be established for each laboratory, or a set of laboratories which share similar working conditions and circumstances. SOPs must be prepared for each procedure to be followed in a Blood Transfusion Service, starting from donor selection, blood labeling and storage, to screening and testing procedures.
These must be updated periodically, and each and every step must be documented. Proper documentation is an essential component of any successful Quality Assurance Programme. The original source of the procedure and all subsequent changes must be documented and be made available to the testing staff. All worksheets and testing records including computer records must be kept up to date.

7.1.9 Staff training and skill

The educational background, training and expertise of the laboratory personnel is one of the most critical factors for the success of a QA programme. Continuing education (CE) of all laboratory workers, ranging from the senior-most to the junior-most person working in the laboratory, should be an essential component of any QA activity. Individual laboratory personnel should be evaluated to identify areas for improvement.

All staff should be fully trained to perform blood screening to the required standards. Initial and ongoing training should be provided to ensure that the knowledge and competence needed are maintained and further developed, including the ability to perform basic troubleshooting if problems are encountered. Formal training programmes for laboratory managers and technical staff should be established and reviewed at appropriate intervals.

The WHO distance learning materials, Safe Blood and Blood Products, provide a useful basis for training, particularly the Introductory Module: Guidelines and Principles for Safe Blood Transfusion Practice (50) and Module 2: Screening for HIV and Other Infectious Agents (51) 48, 49.

All training should be conducted in accordance with a national training plan and curricula should be reviewed regularly. Staff should be assessed on a regular basis on their knowledge of policies and their competency in the performance of procedures. Accurate training and competency assessment records should be maintained for each member of staff which will also be useful in assessing ongoing training requirements.

The management should assess the need for changes to the quality system when deficiencies and opportunities for improvement are identified. Management reviews should specify the actions and resources required to improve the effectiveness of the quality system.

7.1.10 Standards for Quality Systems

Blood screening laboratories should have appropriate quality standards, based on national standards, to ensure process control and valid results. Globally recognized international standards could also be adopted by BTSs to ensure that there is a consistent approach to quality throughout all their activities and to assure the overall safety and efficacy of the blood and blood products prepared for therapeutic use. The standards should take into account relevant existing legislation or other national requirements.
7.1.11 Documentation

Comprehensive, accurate and well catalogued documentation forms the backbone of a successful and efficient Quality Assurance system. A complete set of appropriate documents, including the quality policy, quality manual and standard operating procedures, forms and datasheets should be developed and kept up-to-date. In places where electronic record maintenance may be a problem, or may be likely to loss, manual records should be maintained as a backup. These documents should be used to guide every process, procedure and task to ensure consistency, traceability and accuracy. All processes carried out by the laboratory should be documented and the records kept for traceability.

Records include donor information, screening test results, quality control results, batches of test kits and expiry dates. Completed testing forms provide the records of the screening process. There should be a document management system in place for the safe storage, retrieval, archiving and disposal of documents. This system should also ensure confidentiality of records.

For the records of donors whose test results are reactive, inconclusive or positive should be marked or flagged to prevent further donations or for further action to be taken, such as follow-up for further investigation or recall for future donations.

7.1.12 Traceability

Traceability is a critical part of the quality system in a blood transfusion service. All activities and actions associated with the handling, testing and processing of each donation should be recorded completely and fully linked to the donation, the donor, the fate of the donation and the patient. A fully documented audit trail should be available to demonstrate that each donation has, in fact, been tested and handled correctly and that all test results are valid. To provide this evidence, records and other documents should be stored for a defined period of time; this should be determined nationally in accordance with any relevant existing legislation or other national requirements.

7.1.13 Assessment

Ongoing monitoring and assessment, using appropriate parameters, are integral parts of a quality system. In a blood screening programme, assessment may be at two broad levels: at national levels to assess the effectiveness of the programme and individual facility level to assess the effectiveness of blood screening.

Data generated at national level can be used to assess the achievement of expected outcomes and to collect information on national indicators. For example, the percentage of donated blood units screened for TTIs in a quality-assured manner is one of the key blood safety indicators used by WHO (52) and the United National General Assembly Special Session on HIV/AIDS 50, 51.

At the screening facility level, the control of assay performance is the first step in ensuring reproducibility and reliability. Laboratories should record daily quality control
data and analyze these data for trends so that any corrective action required can be taken early to maintain the optimum performance of the screening process.

All activities in screening laboratories should be reviewed on a regular basis through self-inspection and internal and external audits. Assessments should pertain to all areas of the screening process and should be carried out using approved procedures to identify areas for improvement and demonstrate that quality systems are being implemented adequately.

A national haemovigilance system should also be established which includes monitoring, investigation and reporting of TTIs in donors and patients.

7.1.14 Maintenance and Calibration

Maintenance and care of equipment in Pakistan is a very important issue which needs careful attention. It has been observed that while equipment is frequently purchased under grants or governmental programmes, the infrastructure, including finances for its repair and maintenance are frequently lacking. In addition to routine maintenance, all sensitive laboratory equipment requires frequent calibration to ensure reliable and reproducible results.

A preventive maintenance programme is essential to ensure that equipment is well-maintained and any potential problems are detected and corrected prior to the machine's breakdown and subsequent period of inactivity (down-time). All equipment used for blood screening should be maintained and calibrated regularly and correctly. This also includes equipment like storage refrigerators, and blood banks to ensure their temperature and humidity control. In general terms, maintenance can be divided into:

i. Maintenance performed by users
   ii. Maintenance requiring professional service personnel

For each item of equipment, daily records should be kept of all the work performed from start-up to shut-down. Daily user maintenance and preventive maintenance, at appropriate intervals, should be carried out in accordance with the manufacturer's instructions. All maintenance activities should be planned and completed on schedule and should be fully recorded. Error records should also be maintained for all equipment.

All equipment and instruments that measure specific parameters should be calibrated and validated at set intervals, in accordance with a planned schedule, to ensure that it provides reliable results. Records should be maintained of all calibration. Only equipment and instruments that have been calibrated to perform volumetric procedures should be used in procedures requiring the aspiration and dispensing of a specified volume.
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