Screening Donated Blood for Transfusion-Transmissible Infections in Nepal

National Guidelines

2013
Foreword

Blood transfusion is an essential component of health services in Nepal. Used correctly, blood and blood products save life and improve health, whereas inadequately tested blood donations put recipients at an increased risk of life-threatening transfusion-transmitted infections. Quality-assured screening of all donated blood for transfusion-transmissible infections (TTIs), including HIV, hepatitis B, hepatitis C and syphilis, is one of the strategies recommended by the World Health Organization (WHO) for the provision of safe blood and blood products and is a core component of every national blood program. The aim of the National Blood Program in Nepal is also that all patients receive blood transfusions that have been adequately tested for TTIs and systems have therefore been established to ensure that all donated blood is correctly screened for TTIs.

Dr Geeta Shakya, Director, National Public Health Laboratory (NPHL), and Dr Manita Rajkarnikar, Director, Central Blood Transfusion Service Centre, played a leading role in the preparation of these guidelines, with the support of local experts, Mr Purushottam Paudel and Mr Anil Maharjan. The guidelines were reviewed by members of the National Steering Committee (NSC) and National Technical Advisory Committee for Blood Safety (NTAC), and technical experts from the WHO Blood Transfusion Safety unit. I thank them all for their contribution and congratulate them on producing this guide, which I trust will be helpful, informative and will be implemented by all blood transfusion service centres in the country.

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Preface

Blood transfusion is a life-saving intervention that has an essential role in patient management within the health care system. The preparation of these National Guidelines on Screening Donated Blood for Transfusion-Transmissible Infections in Nepal was based on the guiding principles of the National Blood Policy and the National Guidelines on Management of Blood Transfusion Services in Nepal. It was recognized that national guidelines on TTI screening were needed to provide uniformity in screening strategies and in the quality and effectiveness of the blood screening process in blood transfusion service centres (BTSCs) countrywide. This guide was developed to provide directives for blood centres in Nepal to ensure safe blood supplies through effective blood screening to minimize the risk of transmission of blood-borne infections through transfusion. The guidelines address national screening strategies and algorithms, appropriate selection of test kits and reagents, the management of reactive blood donors and the safe disposal of reactive blood units.

The technical and financial support provided by the World Health Organization (WHO) and the OPEC Fund for International Development (OFID) played a key role in the successful completion of this task. The consultants involved in the draft development and everyone who provided input into the endeavour are gratefully acknowledged. The members of the National Technical Advisory Committee for Blood Safety and WHO-Nepal BTS unit team are also thanked for refining and editing this guide. I trust that these guidelines will be adopted by all blood transfusion service centres in the country so that donated blood is screened for transfusion-transmissible infections in a quality-assured manner.

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**Acronyms**

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<td>Blood Donors Association of Nepal</td>
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<td>BTS</td>
<td>Blood transfusion service</td>
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<td>Blood transfusion service centre</td>
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<td>CBTSC</td>
<td>Central Blood Transfusion Service Centre</td>
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<td>DBTSC</td>
<td>District blood transfusion service centre</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DOHS</td>
<td>Department of Health Services</td>
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<td>EIA</td>
<td>Enzyme immunoassay</td>
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<td>Enzyme linked immunosorbent assay</td>
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<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
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<td>Hepatitis C virus</td>
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<td>Human immunodeficiency virus</td>
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<td>HTLV</td>
<td>Human T-cell lymphotropic virus</td>
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<td>IAs</td>
<td>Immunoassays</td>
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<td>MOHP</td>
<td>Ministry of Health and Population</td>
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<td>NAT</td>
<td>Nucleic acid amplification technologies</td>
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<td>NBP</td>
<td>National Blood Program</td>
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<td>NCASC</td>
<td>National Centre for Aids and STD Control</td>
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<td>NPHL</td>
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<td>NRCS</td>
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<td>National Steering Committee for Blood Transfusion Services</td>
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<td>National Technical Advisory Committee for Blood Transfusion Services</td>
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<td>OFID</td>
<td>OPEC Fund for International Development</td>
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<td>PA</td>
<td>Particle agglutination</td>
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<td>QC</td>
<td>Quality control</td>
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<td>RBTSC</td>
<td>Regional blood transfusion service centre</td>
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<td>RDT</td>
<td>Rapid diagnostic test</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>RPR</td>
<td>Rapid plasma reagin</td>
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<td>SOP</td>
<td>Standard operating procedures</td>
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<td>TPHA</td>
<td>Treponema pallidum haemagglutination assay</td>
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<td>TTI</td>
<td>Transfusion-transmissible infection</td>
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<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
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<td>VNRRBD</td>
<td>Voluntary non-remunerated blood donors</td>
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1 Introduction

1.1 Context
Millions of lives are saved each year because of safe blood and blood products. Transfusion has a particular impact on women (as a consequence of pregnancy-related complications), children (malnutrition, malaria and life-threatening anaemia), and those requiring transfusion during surgery, or as a result of blood-borne diseases or trauma. The Nepal Red Cross Society (NRCS), through its blood transfusion service centres (BTSC) located in various parts of the country, is responsible for the 24-hour availability of safe blood for transfusion. Currently, the National Blood Program operates 86 blood centres in 62 of the 75 districts in Nepal. Of these, one functions as the NRCS Central Blood Transfusion Service Centre (CBTSC), four as NRCS regional blood transfusion service centres (RBTSC) and 21 as NRCS district blood transfusion service centres (DBTSC), delivering a fully-fledged blood transfusion service. Thirty-one centres operate as emergency service units and 28 as hospital-based units.

1.2 Blood donation by low-risk populations
The collection of blood from voluntary non-remunerated blood donors (VNRBD) from low-risk populations, particularly those who donate regularly, is one of the strategies adopted to provide a safe blood supply and minimize the risk of transfusion-transmitted infection. The prevalence of transfusion-transmissible infections (TTIs) in VNRBD is generally much lower than among family/replacement and paid donors. An impressive 85% of all blood in Nepal is supplied through voluntary non-remunerated blood donation, largely as a result of donation campaigns organized by voluntary donor organizations. Approximately 92% of blood donations collected by the Nepal Red Cross Society in Kathmandu, Nepal, are from VNRBD. Blood collection from paid donors has been banned in Nepal since 1982. However, family replacement donation is still practised. The voluntary blood donor program in Nepal provides donor information and education, national criteria for blood donor selection and deferral criteria to exclude prospective donors at risk of TTIs. The recruitment of 100% VNRBD is the primary goal in order to minimize the risk of TTIs and reduce the discard of donated blood, and leads to improved efficiency and use of resources.

1.3 National Guidelines on Screening Donated Blood for Transfusion-Transmissible Infections
The provision of safe and efficacious blood and blood components for transfusion or manufacturing purposes involves a number of processes, from the selection of blood donors and the collection, processing and screening of blood, to the testing of patient samples, the issue of compatible blood and its administration to the patient. There is a risk of error at each stage of the process in this “transfusion chain”. A failure at any stage can have serious implications for the recipients of blood and blood products. Thus, while blood transfusion can be life-saving, there are associated risks, particularly the transmission of blood-borne infections.

Screening for transfusion-transmissible infections 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. Quality-assured screening for 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 use and manufacturing purposes.

A National Blood Policy was developed and approved by the Government of Nepal in 1993 for the operation of the National Blood Program (NBP). This policy was revised and updated in 2006 and 2012. The revised blood policy mandates the screening of all blood donations for HIV hepatitis B (HBV), hepatitis C (HCV) and syphilis for the provision of safe blood and blood components.

**Constraints and challenges**

A large array of assay systems with varying sensitivities and specificities are available on the market for the screening of donated blood for TTIs. In the absence of a system of assay evaluation, a wide variety of test kits from different manufacturers are used in BTSCs in Nepal. Although all units of donated blood are screened for TTIs, challenges remain in the implementation of quality-assured screening due to the lack of national standards, screening strategies, limited resources such as inadequate infrastructure, shortage of trained staff and a lack of coordination amongst BTSCs.

In 2008, the National Public Health Laboratory (NPHL) under the Ministry of Health & Population/Department of Health Services (MoHP/DoHS) developed *National Guidelines on the Management of Blood Transfusion Services in Nepal*. These guidelines did not sufficiently address the need for screening strategies, algorithms and the use of test kits. In the absence of specific national guidelines, various test kits with different levels of sensitivity and specificity have been in use at blood centres throughout the country. At present, BTSC are advised to use WHO recommended kits until the country program is able to evaluate test kits and recommend the most appropriate for use in the country. It was therefore recognized that there was a need to develop national guidelines on screening for TTIs for the adoption of screening strategies appropriate to the requirements, infrastructure and resources of Nepal.

These *National Guidelines on Screening Donated Blood for Transfusion-Transmissible Infections in Nepal* describe the strategy for the following:

- Screening donated blood, using algorithms that define the actual tests to be used in each screening facility
- Quality-assured screening of all donated blood for TTIs, including HIV, hepatitis B, hepatitis C and syphilis, including standards, training, documentation and assessment
- Evaluation and selection of test kits
- Management of blood that is reactive for HIV, HBV, HCV or syphilis.

These screening guidelines specify the tests required on donated blood units, screening strategies, algorithms, test assays and quality control measures to be adopted for quality-assured screening of donated blood for TTIs in Nepal.

**Aim and objectives**

The aim of these guidelines is to strengthen and improve the blood screening program in Nepal.
The general objective of these guidelines is to guide BTSCs in Nepal in the establishment of a reliable blood screening program to detect TTIs and prevent or minimize the risk of transmission of infection to recipients of blood transfusion.

The specific objectives of these guidelines are:

- To clearly identify and list those TTIs for which all donated units of blood should be screened
- To provide a detailed screening strategy and algorithms to be used with selected test kits and reagents
- To provide guidance on the selection and evaluation of assays
- To provide guidance on the management of positive or reactive blood donors
- To provide guidance on biosafety and waste disposal.

**Guideline development process**

The National Public Health Laboratory initiated the development of guidelines on the screening of donated blood for TTIs by assigning two local experts, Mr Anil Maharjan, Quality Officer, CBTSC, and Mr Purushottam Poudel, Senior Medical Technologist, National Technical Advisory Committee (NTAC). Meetings were organized to discuss the aspects that the guidelines should address and to gain technical input from NTAC members during the preparation of the draft guidelines. The first draft was discussed with concerned stakeholders in a one-day workshop on 26 December 2010. Participants included representatives of MoHP/DoHS, NPHL, NRCS, NTAC, NSC and technical staff from blood transfusion service centres (central, regional, district and hospital-based centres). Workshop participants discussed the draft guidelines and provided suggestions and comments to improve them. The issues raised in discussion during the workshop were addressed in the final version, with the incorporation of recommendations suggested by the experts. The finalized guidelines were approved by the National Technical Advisory Committee and endorsed by the National Steering Committee.
2 National Policy on Blood Screening and Regulatory Mechanism

The National Blood Policy specifies, as minimum screening requirements for TTIs, that all blood donations should be screened for the following infections:

- HIV-1 and HIV-2
- Hepatitis B
- Hepatitis C
- Syphilis.

Screening for malaria and other diseases may be undertaken, as recommended by the National Steering Committee for Blood Safety.

The Ministry of Health and Population is responsible for ensuring that relevant policies, standards, systems and infrastructure are in place for the screening of all blood and blood components for TTIs prior to their release for clinical use. The BTS legislative framework has been developed and the National Guidelines are based on these regulations, which apply to all BTSCs in Nepal.
3  Organization and Management

3.1  Blood transfusion services
The Government of Nepal has mandated the Nepal Red Cross Society to organize the collection, processing, testing and distribution of blood and blood products through its central, regional and district BTSCs; emergency and hospital-based centres receive technical support from NRCS to perform these functions. The National Public Health Laboratory has been identified as the focal point for blood safety on behalf of the Ministry of Health and Population. A coordinated effort by both organizations is essential for the efficient coordination of blood transfusion services at national level and a well-organized blood screening program within a quality system for the provision of safe and adequate blood supplies.

3.2  Reference laboratory
The National Public Health Laboratory, as a reference laboratory for transfusion-transmissible infections, has the following roles:

- Evaluation and selection of assay systems and equipment
- Confirmatory testing on screen reactive donations for blood donor management
- Provision of quality control samples
- Organization of an external quality assessment scheme.

The NRCS Central Blood Transfusion Service Centre will be developed as a reference laboratory for the investigation of transfusion-related reactions.

3.3  Human and financial resources
BTSCs should ensure that sufficient qualified and trained staff members are available to perform blood screening in the laboratory. All staff involved in blood screening should be trained to perform their functions against nationally required standards. Refresher training on quality-assured screening of donated blood should also be in place for BTS staff.

Blood transfusion services will operate using the following financial resources:

- Government grants, bilateral and multilateral resources
- Contributions by the Nepal Red Cross Society
- Service charges from clients
- Contributions from national and international organizations
- Personal contributions from individual donors
- Resources from income-generating activities.

The budget required for BTS operations will be assessed in respect of the physical infrastructure, and requirements for capital expenditure and consumables. Costing will be not-for-profit and based on cost-recovery. The Ministry of Health and Population will facilitate the management of the necessary resources and allocate an annual budget for the operation of blood transfusion services.
4 National Screening Strategy

These guidelines define the national screening strategy for the following:

- TTI screening requirements and markers of infection to be targeted during screening for each transfusion-transmissible infection
- Approach to blood screening and screening algorithm
- Assays to be used for each marker of infection
- Standards for the performance of testing, including assay performance characteristics
- Quality systems within which the screening is to be performed
- Blood screening in specific situations: e.g. in remote areas with low workloads and limited facilities, when equipment is lacking, where there is no electricity, or in emergencies when blood is urgently needed
- Interpretation of the results of screening tests
- Procedures for the quarantine and release or discard of blood and blood components
- Release of non-reactive units of blood and blood components
- Confirmatory testing to distinguish between true reactivity and non-specific reactivity for donor management
- Subsequent actions to be taken for blood donors whose blood tests are repeat reactive
- Long-term storage of donation serum/plasma samples
- Safe disposal of reactive/positive units of blood and blood components.

4.1 TTI screening requirements

The objective of blood screening is to detect markers of infection in order to prevent the release of infected blood for clinical use or manufacturing. Laboratory screening for TTIs should be performed on blood samples collected at the time of donation.

1. All donations are to be screened for HIV-1 and HIV-2, hepatitis B, hepatitis C and syphilis.

2. Screening is to be performed using highly sensitive and specific assays that have been specifically evaluated and validated for blood screening.

3. Quality-assured screening of all donations using serology should be in place before additional technologies such as nucleic acid amplification testing are considered.

4. Only blood and blood components from donations that are non-reactive in all screening tests for all markers may be released for clinical use.

5. All screen reactive units should be clearly marked, removed from the quarantined stock and stored separately until they are disposed of safely or kept for quality assurance or research purposes, in accordance with national policies.

6. All tests on blood samples should be performed and recorded, in accordance with standardized procedures, in laboratories that are properly equipped to undertake them.
4.1.1 Human immunodeficiency virus agent

HIV is a retrovirus, an enveloped RNA virus. It is transmissible by the parenteral route. It is found in blood and other body fluids. Once in the bloodstream, the virus infects and replicates in lymphocytes. A number of different groups and subtypes (clades) have been identified; HIV-1 and HIV-2 are the two major distinct virus types and there is significant cross-reactivity between them. HIV-1 is 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 infections worldwide, whereas the prevalence of HIV-2 is mainly restricted to countries in West Africa and India. Additionally, a few infections with HIV group O and group N have been observed in Africa.

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 transmitted through blood donated by 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.

Markers to be used for HIV screening of blood donations

The methods used to identify the presence of HIV should target the following markers:

- Serological markers:
  - Anti-HIV-1 against gp 41, including group O, + anti-HIV-2 against gp 36
  - HIV p24 antigen (p24 Ag)

- Viral nucleic acid: HIV RNA.

An assay capable of detecting both HIV-1 and HIV-2 subtypes should be selected. Screening donations for both antigen and antibody will identify the vast majority of donations from infected donors. Antibody is detectable approximately three weeks after infection and antigen approximately six days after antigen is first detected. Viral RNA can be detected approximately 7–11 days after infection, while HIV p24 antigen may appear from 3–10 days after viral RNA; its detection can further reduce the serological window period by 3–7 days before antibody detection.

Quality control of anti-HIV-1 and anti-HIV-2 screening

Each batch of anti-HIV-1 and anti-HIV-2 tests, and/or HIV p24 tests, should conform to the minimum criteria for specificity and sensitivity.

In addition to the test kit manufacturer’s controls, quality control measures should be performed to demonstrate acceptable sensitivity of the test method.

No series of tests should be considered acceptable unless the test kit manufacturer’s instructions are followed and test results in all quality control samples within the test batch have satisfied the stipulated criteria.

4.1.2 Hepatitis B virus agent

HBV is a member of the hepadnavirus group and is an enveloped DNA virus. HBV is transmissible by the parenteral route. It may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes. HBV is endemic globally and hyper-
endemic in some parts of the world. It is difficult to determine the total number of cases of transfusion-transmitted HBV globally.

**Transmissibility**

While HBV is present in the bloodstream, the levels of the virus may be variable. In recently infected individuals, viral DNA is normally present, although not always at high levels. Screening for hepatitis B surface antigen (HBsAg) indicates infection with HBV. However, it does not in itself distinguish between recent and chronic infections. The distinction between acute and chronic infection is not relevant to blood screening. All HBsAg positive donations should be considered to be at high risk of transmitting HBV and should not be released for transfusion. In general, the earlier in life that HBV is acquired, the more likely the individual is to develop chronic infection. Such infection has a higher probability of progressing to cirrhosis and hepatocellular carcinoma.

**Markers to be used for HBV screening in blood donations**

The serology of HBV is complex. A number of different serological markers develop during the course of infection, including hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc). In addition, HBV DNA can be detected in the majority of cases. Low DNA levels may be detected in HBsAg negative phases of infection and the viraemia may be transient.

The methods used to identify the presence of HBV should employ the following screening targets:

- Serological marker: hepatitis B surface antigen
- Viral nucleic acid: hepatitis B virus DNA.

**Hepatitis B surface antigen**

HBsAg normally appears within three weeks of the first appearance of HBV DNA and levels rise rapidly. It can thus be detected by most of the highly sensitive HBsAg assays available.

**Viral nucleic acid: hepatitis B virus DNA**

The detection of HBV DNA further reduces the risk of HBV transmission through the transfusion of infected blood donated during the acute window period: i.e. when the results of HBsAg assays are negative, but HBV DNA is positive.

**Quality control of HBsAg screening**

Each batch of HBsAg tests should conform to the minimum criteria for specificity and sensitivity. In addition to the test kit manufacturer’s controls, the local working standard must be included in each series of tests to demonstrate acceptable sensitivity of the test method. No series of tests should be considered acceptable unless the test kit manufacturer’s instructions are followed and test results in all quality control samples within the test batch have satisfied the stipulated criteria.

**4.1.3 Hepatitis C virus agent**

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. Seroreversion has been seen in numbers of individuals who have resolved their infections.
Transmissibility
While HCV is present in the bloodstream, the levels of the virus itself are variable. All HCV antigen-antibody reactive donations should be considered at high risk of transmission of HCV and should not be used clinically or for manufacturing.

Markers to be used for HCV screening in blood donations
The methods used to identify the presence of HCV should employ the following screening targets:

- Serological markers:
  - HCV antibody
  - HCV antigen
- Viral nucleic acid: HCV RNA.

HCV antibody and antigen
HCV antibody becomes detectable approximately 30–60 days after infection. Viral antigen normally appears 0–20 days after viral RNA first appears. Antibody generated can be detected between 10–40 days after antigen is first detected.

Viral nucleic acid: HCV RNA
Viral RNA is normally detectable within a few weeks of infection and persists for 6–8 weeks prior to antibody seroconversion. The detection of HCV RNA may further reduce the risk of HCV transmission through the transfusion of infected blood donated during the window period of antigen and antibody assays.

Quality control of anti-HCV screening
Each test kit batch of anti-HCV should conform to the minimum criteria for specificity and sensitivity.

In addition to the test kit manufacturer’s controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method.

No series of tests should be considered acceptable unless kit manufacturer’s instructions are followed and test results in all quality control samples within the test batch have satisfied the criteria stipulated.

4.1.4 Syphilis
Syphilis is caused by a spirochete bacterium, Treponema pallidum. It is transmissible by the 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, a 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.

Transmissibility
While T. pallidum may be found in the bloodstream, levels are variable. In acute primary syphilis, bacteraemia is often short-lived. The treponemes are relatively fragile, in particular being heat-sensitive. Storage below +20°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 +20°C is very low. However, blood components stored at higher temperatures (above +20°C), such as platelet concentrates, or those not stored at lower temperatures, such as blood collected and used within 48 hours, present a higher risk of transmitting syphilis. Thus, screening for syphilis is mandatory as most blood transfusion services provide some blood components that are either stored above +20°C or are not stored below +20°C for sufficient time to kill organisms that may be present.

Markers to be used for syphilis screening in blood donations

The methods used to identify the presence of syphilis should employ the following screening targets:

- Specific treponemal antibodies:
  - Enzyme immunoassay
  - *T. pallidum* haemagglutination assay (TPHA).
- Non-specific markers of syphilis: antibody to lipoidal antigen (reagin)
  - Venereal Diseases Research Laboratory (VDRL)
  - Rapid plasma reagin (RPR).

Quality control of syphilis screening

Each syphilis screening run should conform to the minimum criteria for specificity and sensitivity.

In addition to the test kit manufacturer’s controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method.

No series of tests should be considered acceptable unless kit manufacturer’s instructions are followed and test results in all quality control samples within the test batch have satisfied the criteria stipulated.

4.1.5 TTIs for which universal screening is recommended in some countries or for which selective screening is recommended

Infections such as malaria, Chagas disease and the human T-cell lymphotropic viruses I/II (HTLV) may present a greater risk in certain regions and countries. In Nepal, malaria is endemic in certain districts. The screening of blood for malaria can be made mandatory on the recommendation of the National Steering Committee.

4.1.6 Emerging and re-emerging infections

Reports of emerging or re-emerging infections and their transmission through transfusion appear regularly in the scientific literature. Examples include variant Creutzfeldt Jakob disease, West Nile virus, babesiosis, dengue and chikungunya. Dengue has been identified as an emerging infection in certain districts of Nepal since 2006.

Laboratory screening for any potential or known TTI, other than the four mandatory infections, will be considered only if:

- There is a proven risk of transmission of infection to recipients
- The transmission carries a significant disease risk
- An appropriate screening assay is available.
When there is a *proven* risk of transfusion-associated transmission but no appropriate screening assays are available, donor selection criteria will be developed to identify and defer potentially infected donors for an appropriate period of time.

When there is a *theoretical* risk of transfusion-associated transmission and no appropriate screening assays are available, donor selection criteria may be developed to identify and defer potentially infected donors for an appropriate period of time.

### 4.2 Approach to blood screening

A single approach to screening donated blood should be adopted irrespective of the level of the blood transfusion service centre. The aim is to detect all donations that are or may be infected, prevent their use for transfusion and minimize the wastage of blood due to false positive results.

#### 4.2.1 Screening algorithm

The following screening algorithm (Figure 1) facilitates consistent decisions by BTS personnel on the release of screened blood and blood components for transfusion.

1. Use a single assay (A1) (validated and specified by the National Steering Committee for the specific TTI) and test each blood sample following standard operating procedures.

2. If a result is non-reactive (A1–), the blood unit can be released for transfusion.

3. If a blood sample is initially reactive for a TTI (A1+), immediately separate the blood donation and all blood components derived from it. Repeat the test in duplicate using the same assay (A1) and sample, and also using a sample from the tubing attached to the blood bag. This requires careful attention to ensure that:
   - The correct sample is retrieved for repeat testing
   - The results are carefully verified.

4. If both the repeat screening tests are clearly non-reactive, the donation may be considered to be non-reactive and the blood and any derived components could be released for transfusion.

5. If one or both of the repeat screening tests are reactive, the blood and any derived components must immediately be segregated and should not be used for transfusion purpose.

6. When a donated blood is found reactive in the repeat screening test, remove the donor from the active panel and refer to sections 4.10 and 4.11 on diagnostic confirmation and donor management.
4.2.2 Recording and reporting of results
The laboratory report should indicate the result of every test, preferably by a system that provides positive sample identification. Each test result should be recorded either by using a computerized system, or manually, and the results must be documented in detail.

4.3 Screening assays and assay selection
4.3.1 Screening assays
Various types of assay are available for blood screening. The assays most commonly used are designed to detect antibody, antigen or the nucleic acid of the infectious agent. Common assays in use are:
- Immunoassays (IA):
  - Enzyme immunoassays (EIA)
  - Haemagglutination (HA)/particle agglutination (PA) assays
  - Rapid/simple single-use assays: i.e. rapid diagnostic tests (RDT)
- Nucleic acid amplification technology (NAT) assays.
Immunoassays

Immunoassays are assay systems available in several formats that may be used to detect antibody, antigen or a combination of the two. Generally, the simplest antibody detection assays are based on the use of immobilized antigen which captures any specific antibody present in the test sample (indirect IA). Commonly used antigen detection assays are based on the use of immobilized antibody to capture pathogen-specific antigens present in the sample.

Enzyme immunoassays (EIA)

Enzyme immunoassays are currently the most commonly used assays for screening donated blood for TTIs. EIAs are designed to detect immune complexes formed, with colour generation. EIAs with high sensitivity will generally detect the required target markers of infection if they have been properly evaluated for blood screening. EIA are suitable for screening large numbers of samples and require a range of specific equipment.

Particle agglutination assays (PA)

Particle agglutination assays detect the presence of specific antibody or antigen in a test sample through the agglutination of particles coated with complementary specific antigen or antibody respectively. Agglutination assays, mainly antibody assays, use a range of particles including red cells (haemagglutination) and inert particles such as gelatine or latex. In a manual system, tests are read visually. PAs are suitable for screening large numbers of samples, also by automation.

Rapid/simple single-use assays: rapid diagnostic tests (RDT)

Many rapid tests are based on a form of immune-chromatography in which the added sample flows down an inert strip and reacts with previously immobilized reagents. The sample can be serum, plasma or even whole blood in some cases. A positive reaction is visualized as a dot or a band appearing on the device strip. Most of these assays also include a control dot or band that is used to validate the results of each individual device, irrespective of the specific test result. Rapid tests are provided in simple-to-use formats that generally require no additional reagents except those supplied in the test kit. They are read visually and give a qualitative result within minutes. They are not suitable for screening large numbers of samples.

Nucleic acid amplification technology (NAT) assays

NAT assays detect the presence of viral nucleic acid, DNA or RNA, in blood samples. The amplification step enables the detection of low levels of virus in the original sample. The presence of specific nucleic acid indicates the presence of the virus itself and that the donation is likely to be infectious.

4.3.2 Assay selection

Assay selection will be based on the result of assay evaluation. Assays will be evaluated by the designated reference laboratory, the National Public Health Laboratory, on the recommendation of NTAC. A list of assays for use for TTI screening recommended by NTAC and evaluated by NPHL will be made available for BTSCs. The sensitivity and specificity of recommended assays should be the highest possible, preferably 100% but not less than 99.5%.
The assays evaluated by NPHL on the recommendation of NTAC should be used for TTI screening and confirmatory tests by all blood transfusion service centres.

All centres meeting the following conditions should use EIA assays for TTI screening:
- Central Blood Transfusion Service Centre, regional blood transfusion service centres, all academic organization-based, hospital-based and district blood transfusion service centres with 25–35 donations/samples per day for testing
- Trained laboratory technician/medical technologist available to conduct EIA
- Availability of adequate financial resources, consumables and equipment.

A centre should use rapid diagnostic tests in the following conditions:
- Less than 25 donations/samples per day for testing
- Limited number of trained laboratory assistants/technicians available
- Limited infrastructure such as physical facilities and equipment
- Need for emergency testing and release of blood units.

Table 1: Summary of recommendations: screening for transfusion-transmissible infections

<table>
<thead>
<tr>
<th>TTI</th>
<th>Serological marker</th>
<th>Assays</th>
<th>Level of BTSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 + HIV-2</td>
<td>Anti-HIV-1 against gp41, including group O, + anti-HIV-2 against gp 36</td>
<td>EIA, RDT, NAT*</td>
<td>Central and regional centres, and selected district and hospital-based centres (with large collections) should adopt EIA</td>
</tr>
<tr>
<td></td>
<td>HIV p24 antigen (p24 Ag)</td>
<td></td>
<td>RDT should be used only by district, emergency and hospital-based centres and by others only when emergency testing is required</td>
</tr>
<tr>
<td></td>
<td>Viral nucleic acid*: HIV RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Hepatitis B surface antigen</td>
<td>EIA, RDT, NAT*</td>
<td>Central and regional centres, and selected district and hospital-based centres (with large collections) should adopt EIA</td>
</tr>
<tr>
<td></td>
<td>Viral nucleic acid*: HBV DNA</td>
<td></td>
<td>RDT should be used only by district, emergency and hospital-based centres and by others only when emergency testing is required</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>HCV antibody</td>
<td>EIA, RDT, NAT*</td>
<td>Central and regional centres, and selected district and hospital-based centres (with large collections) should adopt EIA</td>
</tr>
<tr>
<td></td>
<td>HCV antigen</td>
<td></td>
<td>RDT should be used only by district, emergency and hospital-based centres and by others only when emergency testing is required</td>
</tr>
<tr>
<td></td>
<td>Viral nucleic acid*: HCV RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td>IgG and IgM antibodies against T. pallidum</td>
<td>EIA/TPHA, RDT, RPR</td>
<td>Central and regional centres, and selected district and hospital-based centres (with large collections) should adopt EIA</td>
</tr>
<tr>
<td></td>
<td>Reagin antibodies</td>
<td></td>
<td>RDT should be used only by district, emergency and hospital-based centres and by others only when emergency testing is required</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RPR should be used only by district, emergency and hospital-based centres and by others only when emergency testing is required</td>
</tr>
</tbody>
</table>

*NAT has not yet started and will be developed by NPHL, the reference laboratory
4.3.3 Evaluation and validation of assay systems

NPHL will evaluate TTI screening assays available on the market. Initial stage evaluation will be based on published literature on the assays. These assays will be evaluated based on various factors such as assay presentation, clarity of instructions, ease of use, assay sensitivity and specificity, total assay time, assay reproducibility and precision, and number of tests per assay. Based on a paper evaluation conducted by NPHL, a list of assays for TTI screening will be shortlisted and will be further validated using:

- True positive samples and true negative samples
- Low-level positive samples: e.g. samples collected during very early or very late course of infection.

The size of the panels will be determined by local availability and as decided by NTAC. 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%. Analysis of the results will identify the assay that gives the best overall performance against all samples tested. Selected assays need to be approved by NTAC as being suitable for the detection of markers identified above in each blood donation.

4.4 Standards for performance of testing, including assay performance characteristics

Assay performance should be continually monitored to identify any changes in performance which might ultimately lead to a failure in either the assay runs or the detection of low-level true positive samples. Performance should be measured by monitoring the following parameters:

- Quality control samples (local controls with known results)
- Repeat reactivity.

Appropriate, clearly characterized quality control (QC) samples should be included with every batch of tests. This will generate useful and reliable data for assay monitoring. In this context, at least one external QC sample should be included in every batch of tests. External quality controls do not substitute for internal (kit) controls.

4.5 Quality systems within which screening is to be performed

Effective quality systems must be in place to ensure the safety of blood and blood components, as follows:

- Blood donations, components and their laboratory samples are correctly identified
- Donations can be linked to their donors
- Donor samples are suitably stored under appropriate conditions of time and temperature to preserve the properties for which they will be tested
- Tests are appropriately performed and controlled using validated procedures and the results are recorded
- Test results, and other relevant test information, are archived
- If there is a deviation from the kit manufacturer’s instructions, the variation must be validated to ensure it meets the required specificity and sensitivity criteria.
Quality control units
The CBTSC and each regional level BTSC should set up a separate unit for quality control. The quality control unit in-charge at the respective centre will be responsible for the quality assurance of TTI screening tests performed. The QC unit should test all reactive and 10% (or as directed by NTAC) of randomly selected non-reactive donations using the same assay used in the initial screening tests.

District level and other hospital-based centres that do not have adequate resources for setting up separate QC units should send all positive/discrepant and 10% of screen negative samples to CBTSC for confirmation, as practised currently. All discrepant samples from CBTSC and RBTSCs should be sent to NPHL for further verification.

Additionally, testing sites must ensure that the expected standard of performance of assays is being achieved by using appropriate batch pre-acceptance testing and monitoring of test results on defined quality control samples.

4.6 Blood screening in specific situations
In emergency situations in which blood and blood components are needed urgently but are not readily available from blood inventory, or when ELISA equipment is unavailable, not functioning or affected by power shortages, BTSCs may perform rapid diagnostic tests using recommended assays.

4.7 Interpretation of results of screening tests
Interpretation of the results of screening tests should be based on the test kit/assay manufacturer’s instructions.

4.8 Release of non-reactive units of blood and blood components
Only blood and blood components from donations that are screen negative for all markers should be released for transfusion/clinical use, in accordance with the screening algorithm (Figure 1). Units should be formally released from the “quarantine block” into the “ready-to-issue block” only when all required blood screening tests have been performed, results checked and all other required checks made. Each BTSC should have an appropriate system for labelling blood and blood components as ready for clinical use. The label on each blood unit should contain 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.

4.9 Quarantine of blood and blood components prior to release or discard
Each BTSC should have a quarantine system for the physical segregation of all unscreened donations and their blood components until screening for TTIs has been completed. Screened and unscreened blood units should be 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 labelled “Not for Transfusion” and removed from the quarantined stock and
stored separately and securely until further handling. The BTSC should ensure that separate blood storage equipment or cold room is clearly designated for:

- Unscreened units
- Reactive/positive units
- Unresolved/indeterminate units
- Units suitable for clinical use: i.e. available blood stock.

Documentation should be in place to identify the current location and eventual fate of all blood and blood components, whether destined for clinical use or disposal.

4.10 Confirmatory testing to distinguish between true reactivity and non-specific reactivity for donor management

If a donation is reactive for any of the mandatory tests, in one or both of the repeat screening tests, the blood and any derived components must be labelled *Not for Transfusion*. Test samples from the donor/donation must undergo confirmatory testing for the purpose of diagnostic and donor management. For diagnostic confirmation, the donor should be referred to the reference laboratory, the National Public Health Laboratory. The algorithm below should be used for diagnostic purposes only.

**Figure 2: Algorithm for diagnostic confirmation of initially reactive cases**

\[\begin{align*}
A_1 &= \text{Assay 1} \\
A_2 &= \text{Assay 2} \\
A_1+ &= \text{Reactive result in } A_1 \\
A_2+ &= \text{Reactive result in } A_2 \\
A_2- &= \text{Non-reactive result in } A_2
\end{align*}\]
4.11 Subsequent actions to be taken for blood donors whose TTI tests are repeat reactive

When a repeat screening test at a BTSC is reactive, blood or components derived from that donation must not be used for transfusion; the donor’s record must be flagged. The donor should be removed from the active donor panel and informed accordingly. No further material from the donor should be used for clinical purposes until the donor has been returned to the active panel.

A sample from the donation or the donor must be sent for confirmatory testing.

- If the sample is positive on confirmatory testing, the donor must be informed, counselled and permanently deferred as a donor
- If the specimen or donor is negative on confirmatory testing, donor reinstatement may be considered. In such a case, the donor may be returned to the active panel as being eligible for future donations. The next donation may be used only if a negative result is obtained in the screening test.

4.12 Long-term storage of donation serum/plasma samples

The long-term archiving of donations (serum/plasma samples) may be useful for the BTS in facilitating the investigation of adverse transfusion events, transfusion-transmitted infections and the validation of new screening assays or reagents. Archiving should be considered only if adequate and suitable resources are available, including sufficient storage space, equipment and efficient systems (manual or electronic) to manage sample retrieval.

The following issues should be considered before building a sample archive:

- System for the identification and history of each sample in the archive related to its use and length of time of storage
- Types of storage containers required
- Specified temperature at which samples are to be stored
- Volume of samples to be archived
- Criteria and documentation of the reasons for the recovery of an archived sample.

4.13 Safe disposal of reactive units of blood and blood components

One of the most important aspects of blood transfusion practice is the safe disposal of TTI reactive blood and blood components. If not disposed of correctly, laboratory staff, other hospital staff or the general public could be exposed to infectious materials and become infected. It is therefore a public health concern to ensure that there are strict procedures for the disposal of TTI reactive units and that these procedures are followed at all times. All infectious waste, including reactive donations and components derived from them, should be autoclaved before disposal. The materials should be placed in appropriately secured containers and autoclaved under minimum conditions of +121°C for 30 minutes. The waste should then be disposed of by secured land fill.

Laboratory waste that is not infectious, such as packing material and waste paper, may be kept separate from infected waste and disposed of along with general waste. This reduces the load of waste for specialized disposal.
Personnel involved in transfusion services must routinely follow universal safety precautions during all laboratory procedures.
5 Procurement and Supply of Test Kits and Reagents

Continuity in the supply of assays, reagents and consumables required for testing depends on a reliable procurement and supply system. National guidelines on procurement and supply chain management of BTS equipment, test kits and other consumables, have been developed and should be followed by all blood transfusion service centres.
6 Storage, Transportation and Inventory of Test Kits and Reagents

All test kits and reagents should be stored and transported under controlled conditions. Blood transfusion service centres should ensure that reliable cold chain systems are in place in each screening laboratory to assure compliance at all times. Test kits and reagents should always be transported and stored in accordance with the manufacturers' instructions.

All test procedures should be documented and an inventory maintained of test kits and reagents in stock.

Procedures should ensure the traceability of the manufacturer and batch number of all test kits and reagents.

Test equipment should be regularly validated, calibrated and maintained, and appropriate records for these activities should be made and retained.

Appropriate reactivity with control samples must be demonstrated with every series/batch of tests. A series/batch of tests is defined as the number of tests set up at the same time, under the same conditions and processed in a similar manner. Where a microplate format is used for microbiological testing, each plate constitutes a series, even if only a few wells are used.
## Annex

### Participants in Guidelines Finalization Workshop, 26 December 2010, National Public Health Laboratory, Teku

<table>
<thead>
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<th>Name</th>
<th>Designation</th>
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