Annex 4

Guidelines on estimation of residual risk of HIV, HBV or HCV infections via cellular blood components and plasma

Guidelines published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA.

Abbreviations

anti-HBc	antibodies to hepatitis B core protein
anti-HBs	antibodies to hepatitis B surface antigen
CE	*Conformité Européenne* (conforms to European requirements)
CLIA	chemiluminescence immunoassay
EIA	enzyme immunoassay
FDA	Food and Drug Administration
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
ID-NAT	individual donation nucleic acid amplification technique
IDI	interdonation interval
IU	International Unit(s)
IVD	in vitro diagnostic
MP-NAT	minipool nucleic acid amplification technique
NAT	nucleic acid amplification technique
OBI	occult hepatitis B infection
P	probability
PCR	polymerase chain reaction
PDMP	plasma-derived medicinal product
RDT	rapid diagnostic test
RR	residual risk (used in mathematical formulae)
vDWP	viraemic phase of the diagnostic window period

1. Introduction

The course that a viral infection may take in an individual and the different phases of viral infections are described in the following sections – together with the advantages and limitations of using different blood-screening assays for the different infection phases. Blood-screening assays are differentiated by distinct categories. The residual risk of missing viral infections using any screening assay is mainly due to the viraemic phase of the diagnostic window period (vDWP) for each assay – the mean size of which varies between different assay categories. Another component of the residual risk is the virus epidemiology of the donor population (consisting of repeat and first-time donors) with the rate of new infections (incidence) in donors determining the probability of window-period donations. The residual risk per donation from the repeat-donor subpopulation may be used to extrapolate the respective risk for the first-time donor subpopulation, for which incidence data are often unavailable. The residual risk affects recipients of non-pathogen-inactivated blood components to whom viruses may be transmitted. It also determines the potential viral load of plasma pools used for the manufacturing of plasma-derived medicinal products (PDMPs); this potential contamination level needs to be assessed against the viral inactivation or reduction strategies in the manufacturing process.

2. Purpose and scope

These WHO Guidelines provide advice on estimating the residual risk of human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV) being present in cellular blood components and plasma. This estimation has implications for the safety of non- (or incompletely) pathogen-inactivated blood components or plasma products. There are large differences in the prevalence and incidence of viral infections in blood donors around the world. The impact of such epidemiological differences on blood safety needs to be assessed together with the sensitivity of the testing strategy applied. Such assessments may be used to guide strategic decisions on the choice of assays to detect virus-positive blood donations and as a basis for cost–benefit analysis of the different testing scenarios most suitable in the region. The factors influencing the risk of virus transmission by blood components are described, as well as simple mathematical formulae to calculate its probability. These estimates may also be used to counsel recipients on the risks of transfusion. Similarly, the probability and potential level of viral contamination of plasma pools used for the manufacture of PDMPs can be calculated. The infectivity risk of plasma products can then be estimated in relation to the inactivation and reduction capacity of the manufacturing process.

Currently, recovered plasma from whole blood donations is often not used for plasma fractionation because of perceived potential virus risks and quality concerns. This is true for (but not limited to) many blood establishments in low- and middle-income countries, where specific data (for example, on interdonation periods of individual donors) are often not available due to a lack of computerized systems. These WHO Guidelines therefore aim to enable the approximate estimation of residual risks based on limited data, while recognizing that more precise models have been published in the scientific literature. Nevertheless, it is hoped that this document can help in rationalizing decision-making on the use of plasma units for fractionation.

Since the performance of screening assays is one of the key elements in minimizing the residual risk of blood components and guaranteeing the safety of plasma products, these WHO Guidelines also contain advice on the assessment of in vitro diagnostics (IVDs) in studies using specimen panels from the region (Appendix 1). Such targeted performance evaluations for new assays may be performed prior to the acceptance of a new blood-screening assay in a country.

3. Terminology

The definitions given below apply to the terms as used in these WHO Guidelines. These terms may have different meanings in other contexts.

Analytical sensitivity: the smallest amount of the target marker that can be precisely detected by an IVD assay; it may be expressed as the limit of detection and is often determined by testing limiting dilutions of a biological reference preparation.

Apheresis: the process by which one or more blood components are selectively obtained from a donor by withdrawing whole blood, separating it by centrifugation and/or filtration into its components, and returning those not required to the donor. The term "plasmapheresis" is used for a procedure dedicated specifically to the collection of plasma.

Blood collection: a procedure whereby a single donation of blood is collected in a sterile receptacle containing anticoagulant and/or stabilizing solution, under conditions designed to minimize microbiological contamination, cellular damage and/or coagulation activation.

Blood component: a constituent of blood that can be used directly or after further processing for therapeutic applications. The main therapeutic blood components are red blood cell concentrates, platelet concentrates, plasma for transfusion and cryoprecipitate.

Blood establishment: any structure, facility or body that is responsible for any aspect of the collection, testing, processing, storage, release and/or distribution of human blood or blood components when intended for transfusion

or further industrial manufacturing. It encompasses the terms "blood bank", "blood centre", "blood transfusion unit", "blood service" and "blood transfusion service". The definition of this term may differ between legislations.

Blood product: any therapeutic substance derived from human blood, including whole blood, blood components and PDMPs.

Diagnostic sensitivity: the probability that an assay gives a positive result in human specimens containing the target marker (that is, being true positive).

Diagnostic window period: the time interval from infection to the time point when a blood specimen from that infected person first yields a positive result in a diagnostic or screening assay for that agent (for example, for specific antibodies); in the context of residual risk this is often simply called the "diagnostic window" or "window period". The diagnostic window period consists of two phases – the first period of viral replication in the target tissue without presence in peripheral blood is called the eclipse period; the eclipse period is then followed by the ramp-up phase during which the virus concentration increases exponentially in the blood (viraemic phase). Blood components prepared from a blood donation made during the viraemic phase of the diagnostic window period (vDWP) (the potentially infectious window period) can transmit infection to the transfusion recipient, or the respective plasma may contaminate the plasma pool used for manufacturing PDMPs.

Donor: a person in defined good health conditions who voluntarily donates blood or blood components.

First-time (tested) donor: a donor whose blood or plasma is tested for the first time for infectious disease markers in a blood establishment.

Fractionation: the (large-scale) process by which plasma is separated into individual protein fractions that are further purified for medicinal use. The term "fractionation" is usually used to describe a sequence of processes, including plasma protein separation steps (typically precipitation and/or chromatography) and purification steps (typically ion-exchange or affinity chromatography). These steps may also contribute to the inactivation or removal of bloodborne infectious agents (most specifically viruses and, possibly, prions).

Hepatitis B virus (HBV): an enveloped double-stranded DNA virus; causative agent of hepatitis B.

Hepatitis C virus (HCV): an enveloped single-stranded RNA virus; causative agent of hepatitis C.

Human immunodeficiency virus (HIV): an enveloped diploid single-stranded RNA virus; causative agent of acquired immunodeficiency syndrome (AIDS).

Incidence: the number of newly acquired infections per unit of time in a defined population.

NAT conversion: the time period during which specific nucleic acids (for example, viral nucleic acids after a recent virus infection) become detectable by a nucleic acid amplification technique.

Nucleic acid amplification technique (NAT): a testing method to detect the presence of a targeted area of a defined nucleic acid sequence (for example, viral genome) using amplification techniques such as polymerase chain reaction (PCR) or transcription mediated amplification (TMA).

Plasma: the liquid portion remaining after separation of the cellular elements from blood – collected in a receptacle containing an anticoagulant, or separated by the continuous filtration or centrifugation of anticoagulated blood.

Plasma for fractionation: plasma (from whole blood or apheresis) used for the production of PDMPs.

Plasma for transfusion: plasma (from whole blood or apheresis) used for direct infusion into patients without a prior fractionation step. It can be subjected to treatment for inactivating a broad range of pathogens.

Plasma-derived medicinal products (PDMPs): a range of medicinal products obtained by the fractionation of human plasma. Also called plasma derivatives, plasma products or fractionated plasma products.

Plasmapheresis: see "Apheresis" above.

Prevalence: the proportion of past infections identified over a specified period in a defined population.

Recovered plasma: plasma recovered from a whole blood donation and used for transfusion or for fractionation into PDMPs.

Repeat donor: a person who has donated blood/plasma previously in the blood establishment. The definition of this term may differ between legislations.

Sensitivity: see "Analytical sensitivity" and "Diagnostic sensitivity" above.

Seroconversion: the time period during which specific antibodies develop (for example, after a recent virus infection) and become detectable in the blood; this term is sometimes also used for the time period during which viral antigens, such as hepatitis B surface antigen (HBsAg), or viral nucleic acids become detectable in the blood after recent infection. See also "NAT conversion" above.

Source plasma: plasma obtained by apheresis for further fractionation into PDMPs.

Viraemic phase of the diagnostic window period (vDWP): the part of the diagnostic window period during which viruses are present in the blood; the beginning of the viraemic phase is defined by the putative presence of one virus particle in a blood component (20 mL plasma for packed red blood cells) and can be extrapolated using viral replication kinetics (viral doubling time).

Window period: see "Diagnostic window period" above.

4. Course of HIV, HBV and HCV infections

The course of infection in humans differs for HIV, HBV and HCV depending on the biological features of the virus and on the individual immunological response to the infection. In principle, chronically persistent virus infections can be distinguished from infection courses leading to clearance of the virus. Both courses have in common an acute phase which is associated with viral replication, detectable viraemia and sometimes with clinical symptoms. A chronically persisting infection without viral clearance almost always occurs with HIV, frequently with HCV and sometimes with HBV.

4.1 Acute infection

The acute viraemic phase of infection is followed by the humoral and cellular immune responses, resulting in seroconversion and potential clearance of the virus. For some infections the immunity also protects against reinfection. The acute viraemic phase of virus infection in blood donors may be detected by antigen assays or, more sensitively, by assays based upon the nucleic acid amplification technique (NAT). Antibody assays are not useful for the detection of acute infections, but have long been used for the detection of persistent infection (HIV, HCV). Usually there is an overlap of immunoglobulin detection (for example, of immunoglobulin M) and the declining phase of viraemia.

For HBV, both acute resolving and chronic persistent infection courses occur. The frequencies of either are dependent upon different factors (such as the age of the individual becoming infected). It has been estimated that in 70% of HBV-infected donors hepatitis B surface antigen (HBsAg) may be detected transiently in blood, 5% develop chronic HBV infection with continuous antigenaemia and 25% do not show detectable antigenaemia. In principle the marker HBV DNA follows the same transient pattern as HBsAg but the median length of viraemia detection is longer. The transient nature of these HBV blood-screening markers requires the use of an adjustment factor when calculating rates of new infections (*1*).

4.2 Chronic persistent infection

HIV causes persistent infection in almost all infected individuals, while HCV infection becomes chronic in approximately 70% of cases (*2*). A minority of HBV-infected adults (around 5%) become chronic carriers, depending on the age and immune status of the infected subjects. These chronic infections of HIV, HBV and HCV are usually lifelong active infections associated with viral replication characterized by continuous or reappearing (undulating) phases of viraemia, despite the presence of specific antibodies.

Persistent viraemic infections are usually detectable by both serology and NAT-based assays. An exception is HBV where low-level HBV-DNA-positive carriers (HBsAg negative; antibodies to hepatitis B core protein (anti-HBc) positive) have been associated with so-called occult hepatitis B infection (OBI) (*3*, *4*). In some low-prevalence countries the potential OBI transmission risk has been greatly reduced by the introduction of anti-HBc testing. However, in large parts of the world where HBV is endemic, screening for this marker would lead to the loss of an unacceptable proportion of donors. Blood components from donors with OBI have transmitted HBV at a low frequency (approximately 3%), while the presence of detectable levels of antibody against HBsAg (anti-HBs) has been found to protect against infection, with few exceptions (*5–9*). The OBI-associated risk for HBV transmission via cellular blood components may be reduced by sensitive NAT-based screening assays. The OBI-associated input of HBV into plasma pools used for the manufacture of PDMPs appears negligible when compared to the potential viral loads in diagnostic window period donations.

5. Residual risk origins

Predominantly, the residual risk of HIV, HBV or HCV infections in blood or plasma donations is defined as the probability of collecting a donation from an asymptomatic viraemic donor infected with one of these bloodborne viruses, and this not being detected by the routine screening assays.

Such an undetected blood donation may transmit the infection to a recipient if the blood components are not pathogen inactivated. If the pathogen inactivation and removal capacity of the production process is not sufficient an infectious unit of plasma may also contaminate a manufacturing plasma pool and pose a risk to the recipients of the plasma-derived products.

The non-detection of virus infection in blood or plasma donors may be caused by assay failures or by donors being in the diagnostic window period.

5.1 Assay failures

Assay failures in blood screening can occur due to viral variants escaping detection (for example by oligonucleotide mismatches in NAT-based methods, monoclonal antibodies not detecting the antigen of a mutant virus, or recombinant antigens/peptides not detecting antiviral antibodies) (*10–12*). The contribution of assay failures to the residual risk is considered negligible for "state-of-the-art" assays and will not be factored into the residual risk calculation suggested by these Guidelines. Nevertheless, it is important to continuously survey the quality features of screening assays and to identify potential causes of false test results. Post-marketing surveillance of assay safety,

quality and performance is a mechanism for detecting, investigating and acting on any issues and failures identified, and for addressing the need for continuous assay improvement (*13*).

Another potential root cause of assay failure is an inadequate quality management system in place within the testing laboratory. Quality assurance aspects include: (a) participation in external quality assessment (proficiency testing and on-site supervision); (b) the conduct of process (quality) control; (c) maintaining adequate documentation (through standard operating procedures) and record-keeping (testing logbooks, registers); (d) maintaining proper inventory and purchasing systems; (e) equipment maintenance; (f) safe facilities; (g) appropriate organization; and (h) measures to ensure adequately trained and competent testing personnel.

5.2 Diagnostic window periods

Historically, the phase elapsing between the time point of infection and the point of first detectability of the viral marker by the screening assay has been called the diagnostic window period. All types of screening assays are associated with a diagnostic window, the length of which is dependent upon the screening marker, the screening assay category, the sensitivity of the assay used and the replication kinetics of the virus during early infection.

The diagnostic window of HIV, HBV and HCV infections begins with the eclipse phase during which the virus is not yet detectable in blood (even by highly sensitive NAT-based assays). This non-viraemic phase is followed by the viraemic ramp-up phase during which virus concentration in the plasma increases in an exponential fashion. For each of the three bloodborne viruses covered in these Guidelines (HIV, HBV and HCV) a specific constant replication rate is apparent until a peak or plateau phase of maximal viral concentration is reached.

In the context of blood safety, the viraemic phase within the diagnostic window period is relevant. The start of the potentially infectious window period during the early ramp-up phase of viraemia can be defined as the point at which one virus particle is present in a blood component. A generally accepted worst-case assumption for cellular components is to define the start of the infectious window period as the point at which the concentration reaches one virus particle in 20 mL of plasma (the volume co-transfused with a red blood cell unit suspended in additive solution) (*14*). Viral replication characteristics in the early phase of infection are rather consistent among recently infected individuals. This phenomenon results in characteristic doubling times of plasma viral concentration for HIV, HBV and HCV. By knowing the viral replication kinetics of HIV, HBV or HCV in the early infection phase, along with the diagnostic sensitivity of the screening assay, the length of the viraemic phase can be extrapolated for each screening assay.

5.2.1   **HIV**

HIV replicates with an average doubling time of 20 hours (0.83 days) to reach a peak level of viraemia of up to 10^7 IU HIV RNA/mL (*15*). This virus concentration decreases in parallel with the development of specific antibodies detectable by anti-HIV assays. The currently most sensitive antigen assays can detect HIV p24 antigen at a level corresponding to 10^4 IU HIV RNA/mL. Most HIV antigen-antibody combination ("combo") assays are less sensitive in their detection of p24 antigen when compared to antigen assays – with the corresponding HIV RNA concentration for detection by state-of-the-art combo assays being around 10^5 IU/mL (*15*, *16*). Attention should be paid to donors having taken early antiretroviral treatment or pre-exposure antiretroviral treatment which could reverse seroconversion and lower viral load (*17*).

5.2.2   **HBV**

The replication rate of HBV in the early infection phase as determined by the increase in viraemia is significantly lower when compared to HIV or HCV, with an HBV average doubling time of 2.6 days (*18*, *19*). HBV viraemia in the early infection phase is detected earlier by NAT-based assays than by HBsAg assays. In the absence of NAT-based assays the use of HBsAg assays with a high analytical sensitivity is key for the detection of early infection.

5.2.3   **HCV**

For HCV an average doubling time of 10.8 hours (0.45 days) during the ramp-up phase has been determined, followed by an anti-HCV-negative plateau phase of several weeks characterized by high-level viraemia of up to 10^8 IU HCV RNA/mL (*20*, *21*). HCV core antigen appears to be detectable by core antigen assays during the major part of this anti-HCV-negative phase, namely the entire plateau phase and the last part of the ramp-up phase. Similar to HIV, the antigen detection efficiency of current HCV combo assays is less than that of the antigen assays. Combo assays have an overall detection rate of approximately 40% of anti-HCV-negative window period specimens, and preferentially detect those with virus concentrations above 10^6 IU HCV RNA/mL (*22*).

6. Screening assay categories and diagnostic window periods

6.1   Screening assay categories

In these Guidelines screening assays are discussed according to the following categories:

- NAT-based

- antigen
- combo
- antibody
- rapid diagnostic test (RDT).

While antibody assays are designed to detect both recent and chronic persistent infections, the additional benefit of antigen or viral genome detection lies mainly in further reducing the diagnostic window period. The length of the diagnostic window period varies greatly between the different assay categories.

6.1.1 NAT-based assays

NAT-based assays detect viral nucleic acids after in vitro amplification of a target region of the viral genome. Such assays are performed on individual donations (ID-NAT) or in small minipools of donations (MP-NAT). A true infection may not be detectable by NAT-based assays if the concentration of viral genomes is below the detection limit of the assay. Without virus-enrichment steps (for example, ultracentrifugation) in pooled specimens the length of the window period increases with the minipool size and is shortest with ID-NAT. At low virus concentrations in the early ramp-up phase of the window period the amount of virus in a defined volume follows a Poisson distribution, with higher virus concentrations associated with increasing detection probabilities by NAT-based assay. The concentration range between a 5% and a 95% probability of detection may be 100-fold, and this complicates the estimation of window-period reduction that can be achieved by the use of NAT-based assays. In these WHO Guidelines the three-fold concentration of the 95% detection probability has been taken as worst-case assumption for reliable NAT detection for estimating virus concentration in a potentially contaminated plasma pool (Table A4.1; normal font). However, NAT-based assay window periods may be significantly shorter at the lower bound of uncertainty range. The vDWP corresponding to the 50% NAT-detection probability is considered a more accurate estimate for virus transmission risk by blood components without pathogen inactivation (Table A4.1; bold italic font) (*23*, *24*).

6.1.2 Antigen assays

Antigen assays have been optimized for the detection of viral proteins (antigens), which are part of the virus particle, such as viral capsids (for example, HIV p24 or HCV core) or virus envelopes; or are subviral particles (for example, HBsAg). For recently infected individuals, non-reactive test results using antigen assays are caused by an absence of viral proteins, the presence of mutated antigen or the presence of antigens with concentrations below the detection limit of the assay.

6.1.3   Combo assays

Combo assays are designed to simultaneously detect specific antibodies and viral proteins; non-reactive combo assay test results for a true infection may be caused by the absence (or too low a concentration) of antibodies and/or viral antigens in the test sample, or by hidden epitopes in the immune complexes. The antigen-detection potency of combo assays is often lower than that of assays optimized for exclusive antigen detection.

6.1.4   Antibody assays

Antibody assays report infection through the detection of specific antibodies against the pathogen; for recently infected individuals, non-reactive test results using antibody assays can be caused by the absence of specific antibodies, an antibody concentration that is insufficient for obtaining a signal in the immunoassay or low binding strength (avidity) of antibodies. The design of the antibody assay determines its sensitivity and capacity to detect low-avidity antibodies.

6.1.5   RDTs

RDTs are diagnostic devices of simple design, often based on immunochromatographic (lateral flow) or immunofiltration (flow-through) technologies. RDTs do not require complex equipment and provide the test result within a short time (15–30 minutes). Although often not claimed by the manufacturer a suitable for use in blood screening, these devices are sometimes used for blood-safety testing in resource-limited settings or in emergency situations. RDT technology is associated with a lower sensitivity than that of more sophisticated immunoassays developed specifically for blood screening (*25*, *26*).

6.2   Diagnostic window periods

NAT-based assays are generally able to detect a recent infection sooner than antigen assays, followed by combo assays and antibody assays. These differential capacities for detecting recent infections result in different lengths of the diagnostic window period for different assay categories. Within each of the assay categories, individual assays from different manufacturers may have different sensitivities. These differences sometimes result in overlapping diagnostic sensitivities in detecting early infection when less sensitive assays of one category are compared with the more sensitive assays in another category. For example, currently the most sensitive HIV1/2 antibody assay provides a shorter diagnostic window period than the least sensitive HIV1/2 combo assay. This is true both for assays prequalified by WHO and for CE-marked assays. Furthermore, assays may

have differing sensitivities for different viral genotypes and/or for viral subtypes. The vast majority of commercial seroconversion panels used for diagnostic sensitivity studies originate from regular plasma donors, and mainly represent viral genotypes and subtypes prevalent in Europe and the United States (namely HIV subtype B, HCV genotypes 1–3 and HBV genotype A). However, the sensitivity of assays observed with these seroconversion panels may not always be representative for early infection with viral genotypes prevalent elsewhere in the world (*27*). Further details on this and other considerations in the evaluation of new blood-screening assays are provided in Appendix 1.

Mean estimates of the length of the vDWP for so-called state-of-the-art assays are presented by assay category in Table A4.1. These estimates should be used for risk calculation unless more detailed information is available on the sensitivity and corresponding window period of the assay used for blood screening. Hence, if comparative data obtained with multiple seroconversion panels indicate that the sensitivity of a specific assay is clearly different from the mean value shown in Table A4.1, the more accurate data for this assay should be used for the estimation of residual risk.

Table A4.1
Length of the vDWP for different assay categories (days)[a]

	ID-NAT[b]	MP16-NAT[b,c]	Antigen EIA/CLIA[d]	Combo EIA/CLIA[d]	Antibody EIA/CLIA[d]	Antigen RDT[e]	Combo RDT[e]	Antibody RDT[e]
HIV	8 *4*	11 *7*	14	16	21	–	20	28
HBV	27 *17*	37 *27*	42	–	–	55	–	–
HCV	5 *3*	7 *5*	9	38	60	–	–	80

[a] **vDWP:** defined here as the period with a virus concentration of ≥ 1 virus particle in 20 mL plasma. 1 virus particle has been assumed to correspond to 1 (HCV, HBV) or 2 (HIV) viral genome copies. 1 IU HCV RNA has been assumed to correspond to 4 genome copies HCV RNA; 1 IU HBV DNA to 5 genome copies HBV DNA; and 1 IU HIV-1 RNA to 0.5 genome copies HIV-1 RNA.

[b] **NAT-based assays:** to date, only a limited number of NAT-based assays claiming blood screening as an intended use have been CE-marked or approved by the United States Food and Drug Administration (FDA). For a worst-case scenario, diagnostic window periods of less sensitive NAT-based assay versions have been taken as examples in Table A4.1.

Plasma pool contamination: for estimating the maximal virus concentration in a contaminated plasma pool the three-fold concentration of the 95% detection probability has been taken as a worst-case assumption for reliable and consistent ("100%") NAT detection. This approach is analogous to the determination of the whole system failure rate in the European Commission's common technical specifications for in vitro diagnostic medical devices (28). The respective sizes of the vDWPs in days are indicated in normal font.

Transmission risk by non-pathogen-inactivated blood components: the Poisson distribution property of analyte detection by NAT-based assay is considered suitable for more accurate estimation of virus transmission risk by blood components without pathogen inactivation. NAT-based assay window periods may be significantly shorter at lower bound of uncertainty range. The probability of 50% detection in the early ramp-up phase of viraemia may be taken as the basis for the respective vDWPs (indicated in bold italic font) (23, 24).

[c] **MP16-NAT** = MP-NAT of 16 donations.

[d] **EIA/CLIA:** for these assay types United States FDA-approved, CE-marked and/or WHO-prequalified assays of medium sensitivity have been selected as examples (20, 22, 25, 26, 29, 30).

[e] **RDT:** there is a wide range of sensitivity among different RDT assays; values for medium-sensitivity RDTs have been used for Table A4.1 (25, 26).

7. Virus concentrations during diagnostic window period

For risk modelling of plasma pool contamination the maximal virus concentrations that can be found during the respective window period are relevant. Viral loads in viraemic plasma units undetected by screening assays define the extent of initial contamination of the plasma pool. Other parameters for calculation of potential contamination of plasma pools are the number of viraemic donations expected per pool and the individual plasma unit volume relative to the pool size. The maximal viral loads of window-period donations are listed in Table A4.2 as worst-case scenarios for each of the different assay categories correspondingly shown in Table A4.1.

Table A4.2
Maximal concentration of viral genomes in the vDWP (IU/mL)[a]

	ID-NAT	MP16-NAT	Antigen EIA/CLIA	Combo EIA/CLIA	Antibody EIA/CLIA	Antigen RDT	Combo RDT	Antibody RDT
HIV	150	2400	2×10^4	10^5	10^7		10^7	10^7
HBV	24	384	10^3		3×10^4			
HCV	30	480	10^4	5×10^6	10^8			10^8

[a] IU/mL = International Units per millilitre

8. Confirmation of reactive screening results

The residual risk estimations rely on reactive screening assay results representing true infection events. Initially reactive screening results obtained by antibody or antigen tests should be checked by repeat testing in duplicate in the same assay. Even when reactivity is repeatedly obtained in the routine screening assay, the test result should still be checked by a confirmation strategy (*31*).

Confirmation strategies may include the use of more specific assays (for example, HIV Western blot or immunoblot, HCV immunoblot and HBsAg neutralization assay) or another screening or diagnostic assay for the same marker, but of different design.

NAT results should be checked by testing an independent aliquot of the donation to exclude contamination and/or by testing replicates to overcome potential Poisson distribution of the analyte present at low concentration. Follow-up investigations of the donor may further assist in differentiating false-positive from true-positive test results.

Only reactive screening test results subsequently confirmed as true positive should be taken for the estimation of residual risk. If no confirmation is performed, residual risk estimations based on reactive test results represent a worst-case scenario and may considerably overestimate the risks.

9. Virus epidemiology of donor populations

Donor populations consist of first-time donors (individuals donating for the first time) and repeat donors (donors with previous donation(s) having tested negative). Blood systems aim towards having an established population of repeat donors undergoing constant selection for absence of infectious markers.

9.1 First-time donors

Positive screening test results in first-time donors may be an indication of infections that occurred either a longer time ago (prevalent infections) or more recently (incident infections). Prevalent infections in first-time donors are expected to be easily detected by high-quality screening assay(s) without assay failures; in contrast, incident infections represent the major contribution to the residual risk of window-period infections. Making the distinction between prevalent and incident infections will require more detailed investigation – recently infected donors may be identified by NAT-only or antigen-only positive results. Furthermore, for antibody-positive donors, modified antibody assays ("detuned" or "recency" assays) can be used to determine the antibody binding strength (avidity). As antibody avidity increases with maturation of the humoral immune response it is possible to differentiate first-time donors with more recent (incident) infections (low-avidity antibodies) from donors with past (prevalent) infections (high-avidity antibodies) and thus determine the specific incidence of infection in this subpopulation (*14*, *32*). If results from these investigations are not available for a specific first-time donor population, the incidence of infection in these donors can be derived from the rate among repeat donors by applying an adjustment factor. A number of scientific studies on HIV, HBV and HCV infections in different donor populations have investigated their incidence among both first-time and repeat donors. Although some of these studies found a two- to three-fold higher rate of recent infections among first-time donors (compared to the corresponding repeat donors) other studies have not found such a difference between the two donor subpopulations (*33–38*). In the absence of incidence data specific to the first-time donor population, one option is to assume a three-fold higher incidence of virus infections as the worst-case scenario for this subpopulation when compared to the corresponding repeat-donor subpopulation of the same blood establishment. This factor will be referred to as the "first-time donor incidence adjustment factor".

First-time donor incidence (and corresponding adjustment factor) does not have to be calculated for blood establishments in which newly registered donors are routinely tested for bloodborne infections prior to their first donation of blood or blood components.

9.2   Repeat donors

For repeat donors any confirmed positive screening test result indicates a new infection, which is likely to have occurred during the interdonation interval (IDI) – defined as the time period between the most recent donation (which in this case will have tested positive) and the previous donation (which will have tested negative). However, it is also possible that the previous donation (tested negative) was drawn during the diagnostic window period of the screening assay. The relative frequency of this possibility depends on the length of the IDI, with shorter IDIs increasing the probability of a vDWP donation that tested negative in the screening assay.

10. Estimation of incidence and window period modelling of risks

10.1   Incidence

The rate of new infections of repeat donors (incidence) is defined as the number of NAT conversions or seroconversions (number of infected donors) divided by the total number of person years of observation of all donors during the study period (*14*, *39*, *40*). Determining the person years of observation requires a computer system that records the follow-up periods for each individual donation. This kind of information management system is often not available in resource-limited blood establishments.

For the purpose of these Guidelines, both the estimation of incidence and the estimation of the residual risk per blood donation are derived from data from the repeat-donor population for the period of one calendar year (365 days). Incidence is calculated by dividing the number of newly infected repeat donors by the total number of repeat donors, usually expressed as the number of new infection cases per 100 000 repeat donors. If one calendar year is taken as the observation period then the incidence is expressed as per 100 000 person years. This simplification assumes that each repeat donor has been followed for one year during the calendar year and that differences in follow-up periods for individual donors will average out at one person year of observation per donor.

In low-incidence regions the number of positive donors may show strong year-to-year variation. For these situations longer periods may be chosen for the calculation of residual risks.

Screening-positive donations that were excluded for other reasons (for example, donor self-exclusion) may be excluded from the calculation (adjusted incidence).

Formula 1: Incidence (per 100 000 person years)

$$\text{Incidence} = \frac{\text{number of repeat donors tested positive during one year}}{\text{total number of repeat donors in the year}} \times 100\,000$$

10.2 Residual risk per blood donation in repeat donors

For calculating the probability that a blood donation has been collected during the vDWP different factors are involved:

- the rate of new infections (incidence) in the repeat-donor population
- the length of the vDWP for the assay used (Table A4.1).

The residual risk of a blood donation from a repeat donor having been collected during the vDWP of the screening assay used can be calculated as follows:

Formula 2: Residual risk (RR) per donation

$$\text{RR per donation} = \text{vDWP} \times \text{incidence}$$

RR is usually expressed as per million donations (for which one has to multiply the RR figure calculated above by 1 million.

Formula 2 can be directly used to calculate RR for HIV and HCV infections in repeat donors; for HBV infections RR calculated by this formula has to be multiplied by an HBV incidence adjustment factor.

10.2.1 HBV incidence adjustment factor

An adjustment factor of ≥ 1 is necessary because HBV (sero)conversions in repeat donors may be missed due to the transient nature of viraemia and antigenaemia in HBV infections that resolve after the acute phase. Such a transient infection course is seen in adults for the majority of HBV infections (95%) while 5% become chronic carriers. The probability of missing transiently detectable HBsAg or HBV DNA in repeat donors by respective screening assays depends on the length of the IDIs and on assay sensitivity. The donation frequency of repeat donors (average number of donations per repeat donor) determines the average length of the IDI. The average IDI (in days) can be calculated by dividing the observation period of one calendar year (365 days) by the average number of donations per repeat donor. For each assay category

a mean detection period for the transient HBV marker (HBsAg or HBV DNA) can be factored into the adjustment. Further contributions to the adjustment factor originate from HBV infections without detectable antigenaemia (assumed to be 25%; transiently picked up by sensitive HBV NAT-based assays) (*1*). The scientific literature provides several different estimates for the length of transient antigenaemia (*1*, *19*, *41*). The differences observed between the underlying studies may be explained by different infection routes, different inoculum, different HBV genotypes, and HBsAg or HBV-DNA assays of different sensitivity.

The lengths of the HBV marker detection periods have been estimated from the available data for the different assay categories and are listed in Table A4.3.

Table A4.3
HBV DNA and HBsAg detection period (days) for different assay categories

NAT ID	MP16-NAT	HBsAg EIA/CLIA	HBsAg RDT
90	70	60	44

The probability *P* (in %) of detection by HBsAg assays (Table A4.3) may be calculated as:

$$P = 70\% \times \frac{\text{HBsAg detection period}}{\text{IDI}} + 5\%$$

The probability *P* (in %) of detection by NAT-based testing (Table A4.3) may be calculated as:

$$P = 95\% \times \frac{\text{HBV DNA detection period}}{\text{IDI}} + 5\%$$

The HBV incidence adjustment factor is calculated as 100/*P*. For results where *P* ≥ 100% no adjustment is necessary.

To determine the RR per donation for HBV infection, the figure obtained for HBV using Formula 2 in section 10.2 above is then multiplied by the adjustment factor for the specific assay category used.

10.2.2 Adjustment for IDIs

The incidence/window period modelling of residual risk, as described above, assumes that donation behaviour with regard to donation timing and frequency is the same for both infected and non-infected donors. However, evidence can be found in the scientific literature indicating that seroconverting or

NAT-converting donors sometimes delay their return to blood donation, and therefore have larger average IDIs than non-infected donors, resulting in a lower residual risk (*42*). Mathematical models are available to reflect this difference in donor behaviour (*43*). For high-incidence settings (that is, settings in which a higher number of repeat donors have tested positive (seroconverters or NAT converters) for HIV, HBV or HCV infection) the harmonic mean of individual IDIs (in days) of the converting repeat donors (that is, the period between the last negative donation and the first positive donation after infection with the respective virus) may be compared with the mean IDI of non-infected repeat donors (*36*). Respective functions for calculating mean or harmonic mean values are part of commonly used statistical software (for example, Excel). The residual risk calculation may then include the IDI adjustment factor S.

$$S = \frac{\text{mean IDI of all donors}}{\text{harmonic mean IDI of converters for virus X}}$$

If, however, only a few acute infections are found it is advised to take the average IDI of all repeat donors.

10.2.3   First-time donor incidence adjustment factor

In the absence of specific incidence data for first-time donors, a three-fold higher residual risk may be assumed for blood donations from such donors when compared to repeat donors of the same donor population.

Accordingly, the residual risk of a blood donation from a first-time donor having been collected during the vDWP of the screening assay may be assumed to be three-fold higher than the risk calculated for a blood donation obtained from the corresponding repeat donors of the same blood establishment.

11. Residual risks

The approach to residual risk estimation proposed by these Guidelines requires less detailed data on individual donors when compared to other models published in the scientific literature. A recent comparison of seven different models for estimating HIV incidence was performed by simulating donor populations with different donation frequencies combined with different incidence rates (*44*). The approach proposed by these Guidelines was retrospectively included in the same simulation scenarios. In summary, this exercise revealed a slight overestimation of incidence (by up to 20%) in the scenarios with low donation frequency. This finding confirms the validity of the approach proposed in these Guidelines and is in line with the worst-case scenarios chosen for the different parameters, for example: (a) the proposed lengths of the vDWP (Table A4.1);

(b) the assumption of one virus particle in 20 mL plasma being infectious; or (c) the use of the maximal viral concentration for all vDWP donations for the calculation of potential plasma pool contamination (Table A4.2).

11.1 Infection of recipients of non-pathogen-inactivated blood components

The actual infection risk in recipients of non-pathogen-inactivated blood components is dependent on factors such as the amount of intact viruses transmitted, the presence of potentially neutralizing antibodies in the donation or recipient, virus properties and recipient immunological factors (*30*). Using worst-case scenarios, the probability of viraemic donations escaping screening can be estimated using Formula 2 in section 10.2 above. For whole blood donations, different blood components (red cells, platelets and plasma) may be obtained from the same donation and transfused to recipients, each contributing to the residual risk. The amount of plasma in the blood component, the probability of non-detection by the screening assay(s) and the infectivity of the virus after storage of the blood component are all important factors influencing the infection risk but are beyond the scope of these Guidelines (*24*, *30*).

11.2 Contamination of plasma pools

Plasma prepared from whole blood donations (recovered plasma) or obtained by plasmapheresis may be used as source material for plasma-derived products manufactured from plasma pools (such as immunoglobulins, albumin and clotting factors). These pools may be contaminated with HIV, HBV or HCV as a result of the inclusion of plasma units originating from window-period donations not detected by the screening assays. The extent of potential plasma-pool contamination depends upon a number of factors:

- the expected probability of obtaining donations during the vDWP of the screening assay used;
- the (maximal) amount of virus contamination in vDWP plasma units;
- the volume of contaminated plasma unit(s) relative to pool size.

The proportion of viraemic plasma units is estimated by the residual risk calculation. The (maximal) level of virus contamination in respective plasma units can be calculated from the individual plasma volume and its virus concentration. For these calculations, the maximal viral load of window-period donations (shown above in Table A4.2 for the different assay categories) should be taken as the worst-case scenario, even though only a minority of window-period plasma units will reach the maximal viral load.

Authors and acknowledgements

References

1. Korelitz JJ, Busch MP, Kleinman SH, Williams AE, Gilcher RO, Ownby HE et al. A method for estimating hepatitis B virus incidence rates in volunteer blood donors. Transfusion. 1997;37(6):634–40 (https://www.researchgate.net/publication/14027062_A_method_for_estimating_hepatitis_B_virus_incidence_rates_in_volunteer_blood_donors_National_Heart_Lung_and_Blood_Institute_Retrovirus_Epidemiology_Donor_Study, accessed 18 December 2016).

2. Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection. Updated version April 2016. Geneva: World Health Organization; 2016 (http://apps.who.int/iris/bitstream/10665/205035/1/9789241549615_eng.pdf?ua=1, accessed 18 December 2016).

3. Hollinger FB. Hepatitis B virus infection and transfusion medicine: science and the occult. Transfusion. 2008;48(5):1001–26.

4. Allain JP. Occult hepatitis B virus infection: implications in transfusion. Vox Sang. 2004;86(2):83–91 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/15023176/, accessed 18 December 2016).

5. Seed CR, Maloney R, Kiely P, Bell B, Keller AJ, Pink J. Infectivity of blood components from donors with occult hepatitis B infection – results from an Australian lookback programme. Vox Sang. 2015;108(2):113–22 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/25234417, accessed 18 December 2016).

6. Allain JP, Mihaljevic I, Gonzalez-Fraile MI, Gubbe K, Holm-Harritshøj L, Garcia JM et al. Infectivity of blood products from donors with occult hepatitis B virus infection. Transfusion. 2013;53(7):1405–15 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/23362802, accessed 18 December 2016).

7. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H et al. Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. Transfusion. 2007;47(7):1197–205 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/17581154, accessed 18 December 2016).

8. Levicnik-Stezinar S, Rahne-Potokar U, Candotti D, Lelie N, Allain JP. Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients. J Hepatol. 2008;48(6):1022–5 (https://www.researchgate.net/publication/5417215_Anti-HBs_positive_occult_hepatitis_B_virus_carrier_blood_infectious_in_two_transfusion_recipients, accessed 18 December 2016).

9. Spreafico M, Berzuini A, Foglieni B, Candotti D, Raffaele L, Guarnori I et al. Poor efficacy of nucleic acid testing in identifying occult HBV infection and consequences for safety of blood supply in Italy. J Hepatol. 2015;63(5):1068–76 (abstract: https://www.researchgate.net/publication/279300519_Poor_efficacy_of_nucleic_acid_testing_in_identifying_occult_HBV_infection_and_consequences_for_safety_of_blood_supply_in_Italy, accessed 18 December 2016).

10. Humpe A, Legler TJ, Nübling CM, Riggert J, Unger G, Wolf C et al. Hepatitis C virus transmission through quarantine fresh-frozen plasma. Thromb Haemost. 2000;84(5):784–8 (abstract: https://www.researchgate.net/publication/12200759_Hepatitis_C_virus_transmission_through_quarantine_fresh-frozen_plasma, accessed 18 December 2016).

11. Schmidt M, Korn K, Nübling CM, Chudy M, Kress J, Horst HA et al. First transmission of human immunodeficiency virus Type 1 by a cellular blood product after mandatory nucleic acid screening in Germany. Transfusion. 2009;49(9):1836–44 (abstract: https://www.researchgate.net/publication/24438716_First_transmission_of_HIV-1_by_a_cellular_blood_product_after_mandatory_NAT_blood_screening_in_Germany, accessed 19 December 2016).

12. Chudy M, Weber-Schehl M, Pichl L, Jork C, Kress J, Heiden M et al. Blood screening nucleic acid amplification tests for human immunodeficiency virus Type 1 may require two different amplification targets. Transfusion. 2012;52(2):431–9 (abstract: https://www.researchgate.net/

publication/51541171_Blood_screening_nucleic_acid_amplification_tests_for_human_ immunodeficiency_virus_Type_1_may_require_two_different_amplification_targets, accessed 19 December 2016).

13. Post-market surveillance for in vitro diagnostics (IVDs) [website]. Geneva: World Health Organization (http://www.who.int/diagnostics_laboratory/postmarket/en/, accessed 19 December 2016).

14. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion. 2005;45(2):254–64 (https://www.researchgate.net/publication/8070296_A_ new_strategy_for_estimating_risk_of_transfusion-transmitted_viral_infections_based_on_ rates_of_detection_of_recently_infected_donors, accessed 19 December 2016).

15. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS. 2003;17(13):1871–9 (https://www.researchgate.net/ publication/271733355_Dynamics_of_HIV_viremia_and_antibody_seroconversion_in_plasma_ donors, accessed 20 December 2016).

16. Consolidated guidelines on HIV testing services. Geneva: World Health Organization; 2015 (http://apps.who.int/iris/bitstream/10665/179870/1/9789241508926_eng.pdf?ua=1&ua=1, accessed 20 December 2016).

17. de Souza MS, Pinyakorn S, Akapirat S, Pattanachaiwit S, Fletcher JLK, Chomchey N et al. Initiation of antiretroviral therapy during acute HIV-1 infection leads to a high rate of nonreactive HIV serology. Clin Infect Dis. 2016;63(4):555–61 (https://www.researchgate.net/ publication/304070497_Initiation_of_Antiretroviral_Therapy_during_Acute_HIV-1_Infection_ leads_to_A_High_Rate_of_Non-Reactive_HIV_Serology, accessed 20 December 2016).

18. Biswas R, Tabor E, Hsia CC, Wright DJ, Laycock ME, Fiebig EW et al. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion. 2003;43(6):788–98 (https://www.researchgate.net/publication/10750139_Comparative_sensitivity_of_HBVNATs_ and_HBsAg_assays_for_detection_of_acute_HBV_infection, accessed 20 December 2016).

19. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K. Hepatitis B NAT virus-positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang. 2005;88(2):77–86. Erratum in Vox Sang. 2005;88(3):223 (https://www.ncbi.nlm.nih.gov/pubmed/15720604/, accessed 20 December 2016).

20. Nübling CM, Unger G, Chudy M, Raia S, Löwer J. Sensitivity of HCV core antigen and HCV RNA detection in the early infection phase. Transfusion. 2002;42(8):1037–45 (abstract: https://www. researchgate.net/publication/11076628_Sensitivity_of_HCV_core_antigen_and_HCV_RNA_in_ early_infection_phase, accessed 20 December 2016).

21. Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C et al. Dynamics of viremia in early hepatitis C virus infection. Transfusion. 2005;45(6):994–1002 (https://www.researchgate. net/publication/7807301_Dynamics_of_viremia_in_early_hepatitis_C_virus_infection, accessed 20 December 2016).

22. Laperche S, Nübling CM, Stramer SL, Brojer E, Grabarczyk P, Yoshizawa H et al. Sensitivity of hepatitis C virus core antigen and antibody combination assays in a global panel of window period samples. Transfusion. 2015;55(10):2489–98 (https://www.researchgate.net/ publication/277412688_Sensitivity_of_hepatitis_C_virus_core_antigen_and_antibody_ combination_assays_in_a_global_panel_of_window_period_samples_HCV_Core_Antigen_ Detection_in_Window_Period, accessed 20 December 2016).

23. Weusten JJ, van Drimmelen HA, Lelie N. Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. Transfusion. 2002;42(5):537–48 (abstract: https://www.researchgate.net/publication/11291469_Mathematic_modeling_of_the_risk_of_HBV_HCV_and_HIV_transmission_by_window-phase_donations_not_detected_by_NAT, accessed 20 December 2016).

24. Weusten J, Vermeulen M, van Drimmelen H, Lelie N. Refinement of a viral transmission risk model for blood donations in seroconversion window phase screened by nucleic acid testing in different pool sizes and repeat test algorithms. Transfusion. 2011;51(1):203–15 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/20707858, accessed 20 December 2016).

25. Scheiblauer H, El-Nageh M, Diaz S, Nick S, Zeichhardt H, Grunert H-P et al. Performance evaluation of 70 hepatitis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes. Vox Sang. 2010;98(3p2):403–14 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2860763/, accessed 20 December 2016).

26. Scheiblauer H, El-Nageh M, Nick S, Fields H, Prince A, Diaz S. Evaluation of the performance of 44 assays used in countries with limited resources for the detection of antibodies to hepatitis C virus. Transfusion. 2006;46(5):708–18 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/16686838, accessed 20 December 2016).

27. Apetrei C, Loussert-Ajaka I, Descamps D, Damond F, Saragosti S, Brun-Vézinet F et al. Lack of screening test sensitivity during HIV-1 non-subtype B seroconversions. AIDS. 1996;10(14):F57–60 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/8970678, accessed 20 December 2016).

28. 2009/108/EC: Commission Decision of 3 February 2009 amending Decision 2002/364/EC on common technical specifications for in vitro-diagnostic medical devices. European Commission. Official Journal of the European Union. 2009;L39/34–L39/49 (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2009.039.01.0034.01.ENG&toc=OJ:L:2009:039:TOC, accessed 18 December 2016).

29. Assal A, Barlet V, Deschaseaux M, Dupont I, Gallian P, Guitton C et al. Sensitivity of two hepatitis B virus, hepatitis C virus (HCV), and human immunodeficiency virus (HIV) nucleic acid test systems relative to hepatitis B surface antigen, anti-HCV, anti-HIV, and p24/anti-HIV combination assays in seroconversion panels. Transfusion. 2009;49(2):301–10 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/19389212, accessed 20 December 2016).

30. Kleinman SH, Lelie N, Busch MP. Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion. Transfusion. 2009;49(11):2454–89 (available at: https://www.researchgate.net/publication/26743554_Infectivity_of_human_immunodeficiency_virus-1_hepatitis_C_virus_and_hepatitis_B_virus_and_risk_of_transmission_by_transfusion, accessed 20 December 2016).

31. Screening donated blood for transfusion-transmissible infections. Recommendations. Geneva: World Health Organization; 2010 (http://www.who.int/bloodsafety/ScreeningDonatedBloodforTransfusion.pdf, accessed 20 December 2016).

32. Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. Euro Surveill. 2008;13(36):4–10 (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18966, accessed 20 December 2016).

33. Schreiber GB, Glynn SA, Busch MP, Sharma UK, Wright DJ, Kleinman SH. Incidence rates of viral infections among repeat donors: are frequent donors safer? Transfusion. 2001;41(6):730–5 (https://www.researchgate.net/publication/11939481_Incidence_rates_of_viral_infections_among_repeat_donors_Are_frequent_donors_safer, accessed 20 December 2016).

34. Zou S, Stramer SL, Dodd RY. Donor testing and risk: current prevalence, incidence, and residual risk of transfusion-transmissible agents in US allogeneic donations. Transfus Med Rev. 2012;26(2):119–28 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/21871776, accessed 20 December 2016).

35. O'Brien SF, Yi QL, Fan W, Scalia V, Fearon MA, Allain JP. Current incidence and residual risk of HIV, HBV and HCV at Canadian Blood Services. Vox Sang. 2012;103(1):83–6 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/22289147, accessed 20 December 2016).

36. Bruhn R, Lelie N, Custer B, Busch M, Kleinman S. Prevalence of human immunodeficiency virus RNA and antibody in first-time, lapsed, and repeat blood donations across five international regions and relative efficacy of alternative screening scenarios. Transfusion. 2013;53(10 Pt 2): 2399–412 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/23782054, accessed 20 December 2016).

37. Bruhn R, Lelie N, Busch M, Kleinman S. Relative efficacy of nucleic acid amplification testing and serologic screening in preventing hepatitis C virus transmission risk in seven international regions. Transfusion. 2015;55(6):1195–205 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/25727549/, accessed 20 December 2016).

38. Offergeld R, Ritter S, Hamouda O. HIV, HCV, HBV and syphilis surveillance among blood donors in Germany 2008–2010. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2012;55(8):907–13 (article in German; English abstract: https://www.ncbi.nlm.nih.gov/ pubmed/22842883, accessed 20 December 2016).

39. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. N Engl J Med. 1996;334:1685–90 (http://www.nejm.org/doi/full/10.1056/ NEJM199606273342601#t=article, accessed 20 December 2016).

40. Glynn SA, Kleinman SH, Wright DJ, Busch MP. International application of the incidence rate/window period model. Transfusion. 2002;42(8):966–72 (https://www.researchgate. net/publication/11076616_NHLBI_Retrovirus_Epidemiology_Donor_Study_International_ application_of_the_incidence_ratewindow_period_model, accessed 20 December 2016).

41. Yoshikawa A, Gotanda Y, Minegishi K, Taira R, Hino S, Tadokoro K et al. Lengths of hepatitis B viremia and antigenemia in blood donors: preliminary evidence of occult (hepatitis B surface antigen-negative) infection in the acute stage. Transfusion. 2007;47(7):1162–71 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/17581150, accessed 20 December 2016).

42. Schreiber GB, Glynn SA, Satten GA, Kong F, Wright D, Busch MP et al. HIV seroconverting donors delay their return: screening test implications. Transfusion. 2002;42(4):414–21 (https://www. researchgate.net/publication/11299285_HIV_seroconverting_donors_delay_their_return_ screening_test_implications, accessed 20 December 2016).

43. an der Heiden M, Ritter S, Hamouda O, Offergeld R. Estimating the residual risk for HIV, HCV and HBV in different types of platelet concentrates in Germany. Vox Sang. 2015;108(2):123–30 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/25335096, accessed 20 December 2016).

44. Brambilla DJ, Busch MP, Dodd RY, Glynn SA, Kleinman SH. A comparison of methods for estimating the incidence of human immunodeficiency virus infection in repeat blood donors. Transfusion. 2017;57(3pt2):823–31 (abstract: https://www.ncbi.nlm.nih.gov/pubmed/27910095, accessed 20 April 2017).

Appendix 1

Evaluation of new blood-screening assays

Depending on the legal structure in a country, a regulatory body or the national blood system itself may be responsible for decisions on the acceptability of new blood-screening assays. It is recommended that previous assessments of quality features of the assay performed by experienced regulatory authorities (for example, United States FDA approval, European CE certification, and Australian Therapeutic Goods Administration (TGA) or Health Canada marketing authorizations) or by the WHO Prequalification Programme for IVDs should be taken into account. Previous assessments by such stringent regulatory bodies will have included the review of analytical and clinical performance data submitted by the manufacturer, and of the manufacturer's quality management system and batch-to-batch consistency – and in the case of WHO prequalification, an independent performance evaluation.

As a result, a country's assessment of manufacturer documentation, with a focus on the specific regional situation and needs, may be sufficient for assays already approved elsewhere under stringent regulation.

If local regulation requires a performance evaluation of new assays (for example, by a national reference laboratory) prior to their implementation, it is recommended that the evaluation focuses on essential assay features through a targeted performance evaluation.

Assessment of documents

Documents provided by the IVD manufacturer may be assessed, with a special focus placed on the specific regional situation and needs. Such a focus may include assessing whether or not the stability studies performed by the manufacturer cover the regional environmental conditions (for example, with regard to temperature and humidity) or whether the Instructions for Use are appropriate for the target users.

In addition, performance evaluation studies documented by the IVD manufacturer may be reviewed to evaluate the extent of representation of specimens reflecting the regional situation (for example, with regard to viral genotypes or variants) or to assess potential interference with the test result by other regionally more prevalent infections.

Targeted performance evaluation of new assays used for blood screening

If laboratory testing of a new IVD is a component of the national or regional evaluation and approval scheme, it is advisable not to repeat evaluation elements already performed by other bodies, but to focus instead on regionally important quality aspects. This would involve, for example, a focused assessment of performance data with respect to viral variants or genotypes prevalent in the region.

Well-characterized specimen panels representing the regional epidemiological situation with regard to viral variants/genotypes of HIV, HBV or HCV may be helpful for comparative independent evaluation of new assays. A comparative database obtained using a number of assays may then be the scientific basis for the definition of acceptance criteria for new assays and for the identification of less suitable assays.

The preconditions for the suitability of such panels are the inclusion of specimens differentiating between different assays (for example, low-positive specimens or positive specimens previously tested discrepantly by different assays) and the availability of sufficient volumes to allow a number of evaluations to obtain comparative data. The recommended size of such a panel strongly depends on its composition, with more critical panel members (for example, low-positive or early infection specimens) able to differentiate between assays being more important than a high number of strong positive specimens. Panels used for this type of exercise typically comprise 20–50 members collected from different phases of the infection. A strategy for the replacement of panel members should be in place.

Furthermore, WHO offers through its IVD standardization programme a range of biological reference preparations that may be useful in the confirmation of basic assay features. WHO International Standards (expressed in IU) are available for the confirmation of analytical sensitivity, while WHO Reference Panels representing the major viral genotypes could be used to check genotype-detection efficiency.

These WHO reference preparations are usually lyophilized to facilitate worldwide shipping and are listed in the WHO online catalogue (http://www.who.int/bloodproducts/catalogue/en/). They can be obtained from the WHO Collaborating Centres which act as WHO custodians in this field – namely, the National Institute for Biological Standards and Control (NIBSC), England, or the Paul-Ehrlich-Institut (PEI), Germany.

Table A4A1.1 summarizes the most important WHO reference preparations currently available in the field of blood screening.

Table A4A1.1
WHO reference preparations in the field of blood screening

Marker	Preparation	Details	Custodian
anti-HIV-1/2	1st International Reference Panel Lyophilized No unitage	HIV-1 subtypes A, B, C, CRF01_AE; group O; HIV-2	NIBSC
HIV-1 p24	1st International Reference Reagent Lyophilized 1000 IU/ampoule	–	NIBSC
HIV-1 RNA	3rd International Standard Lyophilized 185 000 IU/mL	–	NIBSC
	1st International Reference Panel HIV-1 circulating recombinant forms (CRFs) Lyophilized No unitage	HIV-1 CRFs 11GJ, 02AG, 01AE, 01AGJU,BG24; subtypes J, G, C; group O	NIBSC
	2nd International Reference Panel HIV-1 subtypes Lyophilized No unitage	HIV-1 subtypes A, B, C, D, AE, F, G, AG–GH; groups N and O	NIBSC
HIV-2 RNA	1st International Standard Lyophilized 1000 IU/vial	–	NIBSC
HBsAg	3rd International Standard Lyophilized 50 IU/mL	–	NIBSC
	Dilutional panel 8.25; 2.06; 0.52; 0.13 IU/vial	–	NIBSC
	1st International Reference Panel HBV genotypes Lyophilized No unitage	HBV genotypes A–F, H	PEI

Table A4A1.1 *continued*

Marker	Preparation	Details	Custodian
HBV DNA	4th International Standard Lyophilized 955 000 IU/mL	–	NIBSC
	1st International Reference Panel HBV genotypes Lyophilized No unitage	HBV genotypes A–G	PEI
anti-HBc	1st International Standard Lyophilized 50 IU/vial	–	NIBSC
HCV core	1st International Standard Lyophilized 3200 IU/mL	–	PEI
HCV RNA	5th International Standard Lyophilized 100 000 IU/mL	–	NIBSC

Appendix 2

Examples for estimation of residual risks

Example 1: HCV screening by anti-HCV EIA

Centre A; observation period 01.06.2011–31.05.2012

49 660 *repeat donors*; 100 313 *donations*; 45 *anti-HCV pos (EIA)*

11 452 *first-time donors*; 11 452 *donations*; 89 *anti-HCV pos (EIA)*

Table A4.1 (see section 6.2 main text) – anti-HCV EIA: vDWP = 60 days = 0.164 years

Table A4.2 (see section 7 main text) – anti-HCV EIA: maximal virus concentration: 10^8 IU HCV RNA/mL plasma of vDWP donation

A. Residual risk (RR) per blood donation from repeat donors

$$\text{Incidence} = \frac{\text{number of repeat donors tested positive during one year}}{\text{total number of repeat donors in the year}} \times 100\,000$$

$$= \frac{45}{49\,660} \times 100\,000$$

$$= 90.61 \text{ HCV infections per } 100\,000 \text{ donor years}$$

RR per blood donation = vDWP × incidence

$$= 0.164 \times 0.000\,906\,1 = 0.000\,148\,600$$

$$= 148.60 \text{ per million donations}$$

Number (N) of vDWP blood donations from repeat donors

$$N = 100\,313 \times \frac{148.60}{1\,000\,000} = 14.90$$

B. Residual risk (RR) per blood donation from first-time donors

Positive screening test results for first-time donors represent mainly old (prevalent) infections. The rate of recent infections can be determined by specific investigations (for example, recency assays or NAT-only positive results).

In the absence of incidence data, the worst-case assumption is a three-fold incidence in first-time donors compared to the corresponding repeat donors.

RR = 0.000 148 61 × 3 = 0.000 445 = 445 per million donations

Number (N) of vDWP blood donations from first-time donors:

$$N = 11\,452 \times \frac{445}{1\,000\,000} = 5.10$$

C. Expected number (N) and risk of window-phase donations for repeat and first-time donors combined (Centre A; observation period of 1 year)

$$N = 14.90 + 5.10 = 20.00$$

$$RR = \frac{20}{100\,313 + 11\,452} = 0.000\,179 = 179 \text{ per million donations}$$

Example 2: HBV screening by HBsAg RDT; HBV adjustment factor

Centre A; observation period 01.06.2011–31.05.2012
49 660 *repeat donors*; 100 313 *donations*; 184 *HBsAg RDT pos*
11 452 *first-time donors*; 11 452 *donations*; 291 *HBsAg RDT pos*

Table A4.1 (section 6.2 main text) – HBsAg RDT: vDWP = 55 days = 0.15 years
Table A4.3 (section 10.2.1 main text) – HBsAg RDTs: HBV marker detection period = 44 days

Average number of donations per repeat donor: 100 313/49 660 = 2.02

Interdonation interval (IDI)

$$IDI = \frac{365 \text{ days}}{\text{average number of donations per repeat donor}} = 180.69 \text{ days}$$

A. Residual risk (RR) per blood donation from repeat donors (without adjustment for transient HBsAg)

$$\text{Incidence} = \frac{\text{number of repeat donors tested positive during one year}}{\text{total number of repeat donors in the year}} \times 100\,000$$

$$= \frac{184}{49\,660} \times 100\,000$$

$$= 370.52 \text{ HBV infections per 100 000 donor years}$$

vDWP = 55 days = 0.15 years

RR = vDWP × incidence

    = 0.15 × 0.003 705 2 = 0.000 555 78

    = 555.78 per million donations

B. HBV incidence adjustment factor
Probability (*P*) for HBsAg detection

$$P = 70\% \times \frac{\text{HBV marker detection period}}{\text{IDI}} + 5\%$$

$$= 70\% \times \frac{44 \text{ days}}{180.69 \text{ days}} + 5\% = 70\% \times 0.24 + 5\% = 21.8\%$$

HBV incidence adjustment factor =

$$\frac{100\%}{P} = \frac{100\%}{21.8\%} = 4.58$$

C. Residual risk (RR) per blood donation from repeat donors (with adjustment for transient HBsAg)

Adjusted RR = 4.58 × 0.000 555 78 = 0.002 545 = 2545 per million donations.