World Health Organization

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WHO GUIDELINE ON ESTIMATION OF RESIDUAL RISK OF HIV, HBV OR HCV INFECTIONS VIA CELLULAR BLOOD COMPONENTS AND PLASMA

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed *WHO Guideline on estimation of residual risk of HIV, HBV or HCV infections via cellular blood components and plasma*, to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. **Written comments proposing modifications to this text MUST be received by 16th September 2016 in the Comment Form available separately** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Essential Medicines and Health Products (EMP). Comments may also be submitted electronically to the Responsible Officer: **Dr C Micha Nübling** at email: **nueblingc@who.int.**

The outcome of the deliberations of the Expert Committee on Biological Standardization will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide" (WHO/IMD/PUB/04.1).

Recommendations and guidelines published by 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 Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Guidelines be made only on condition that modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments or examples for additional guidance intended for manufacturers and NRAs, which may benefit from those details.

SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT

WHO Guideline on Estimation of Residual Risk of HIV, HBV or HCV Infections via Cellular Blood Components and Plasma

Endorsement of the "residual risk guideline" project by the WHO Expert Committee on Biological Standardization (ECBS), based on requests from low- and middle-income countries aiming to use recovered plasma for manufacture of plasma derived medicinal products	15.-19.10.2012
Discussions on outline and necessary elements of the guidance document at the "WHO Workshop on Blood Testing and Risk Assessment as part of GMP in blood establishments", Jakarta, Indonesia	09.-12.06.2014
Working group of experts in the fields of epidemiology and blood safety testing; meeting at WHO HQ, Geneva, Switzerland	17.-18.06.2015
Circulation of draft guideline among working group members and international experts	Aug / Sep 2015
Presentation and discussion of draft guideline at the WHO AFRO "Regional workshop on the development of regional strategy for blood safety and the establishment of national regulatory system for blood and blood products", Cotonou, Benin	23.-25.09.2015
Presentation and discussion of draft guideline at the WHO Expert Committee on Biological Standardization (ECBS)	12.-16.10.2015
Presentation and discussion of draft guideline at the Blood Regulator Network (BRN)	15.10.2016
Presentation and discussion of draft guideline at the "12th Arab Transfusion Medicine Forum (ATMF), Cairo, Egypt	20.-23.11.2015
Presentation and discussion of draft guideline at the WHO EMRO "Regional Meeting of Directors of National Blood Transfusion Services", Tunis, Tunisia	17.-19.05.2016
Presentation and discussion of draft guideline at the "IPFA / PEI 23rd International Workshop on Surveillance and Screening of Blood Borne Pathogens", Lisboa, Portugal	25.-26.05.2016
Circulation of draft guideline among working group members and international experts	Apr – Jun 2016
Circulation of final draft guideline version for public consultation	Jun – Sep 2016
Consolidation of comments received and review of feedback	Oct 2016
Presentation to the WHO Expert Committee on Biological Standardization for adoption	17.-21.10.2016
Any other follow-up action as required	

Executive Summary

This guideline advises on estimation of the residual risk of HIV, HBV or HCV being present in cellular blood components and plasma. This estimation has implications for the safety of non- (or incompletely) inactivated blood or plasma products. There are large differences in the prevalence and incidence of viral infections in blood donors around the world. The impact of these epidemiological differences on blood safety needs to be assessed together with the sensitivity of the testing strategy applied. These estimations may be used for strategic decisions on the choice of assays to interdict virus-positive blood and plasma units and as a basis for cost benefit analysis of different testing scenarios most suitable in the region. The factors influencing the risk of virus transmissions by blood components are described as well as simple mathematical formulas to calculate its probability. Similarly, the probability and potential level of viral contamination of plasma pools used for manufacture of plasma derived medicinal products can be calculated and subsequently the infectivity risk of plasma products can 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 the potential virus risks and quality concerns. It is hoped that this document can help in rationalising decision making on the use of plasma units for fractionation on the basis of residual risk estimations.

Since the performance of assays is a key element in minimizing residual risk of blood components and guaranteeing safety of plasma products, an annex to this guideline gives advice on assessment of *in vitro* diagnostics in studies using specimen panels from the region. This limited performance evaluation of new assays may be performed prior to acceptance of a new blood screening assay in the country.

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Glossary

Analytical sensitivity: the smallest amount of the target marker that can be precisely detected by an 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".

Blood product: any therapeutic substance derived from human blood, including whole blood, blood components and plasma-derived medicinal products.

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

Diagnostic window period: the time interval from infection to the time point when a blood sample from that infected person first yields a positive result in a diagnostic or screening assay for that agent (e.g. specific antibodies). 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 followed by the ramp up phase where the virus concentration increases exponentially in the blood (viraemic phase). Blood components prepared from a blood donation during the viraemic phase of the diagnostic window (the potentially infectious window period) can transmit infection to the transfusion recipient, or respective plasma may contaminate the plasma pool used for manufacturing of plasma derived medicinal products (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: (large-scale) process by which plasma is separated into individual protein fractions that are further purified for medicinal use (variously referred to as "plasma derivatives", "fractionated plasma products" or "plasma-derived medicinal products"). The term "fractionation" is usually used to describe a sequence of processes, including: plasma protein separation steps (typically precipitation and/or chromatography), purification steps (typically ion-exchange or affinity chromatography) and one or more steps for the inactivation or removal of blood-borne 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 immune deficiency syndrome.

Incidence: the rate of newly acquired infection identified over a specified time period in a defined population.

Nucleic acid amplification technique: a testing method to detect the presence of a targeted area of a defined nucleic acid (e.g. viral genome) using amplification techniques such as polymerase chain reaction or transcription mediated amplification.

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: recovered or apheresis plasma used for the production of plasma-derived medicinal products.

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 pathogens.

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

Plasmapheresis: see "Apheresis"

Prevalence: the rate of identified infection, including both past and present infections, at a specified point in time in a defined population.

Recovered plasma: plasma recovered from a whole blood donation and used for transfusion or for fractionation into plasma-derived medicinal products.

Sensitivity: see "analytical sensitivity" or "diagnostic sensitivity"

Source plasma: plasma obtained by apheresis (see apheresis plasma) for further fractionation into plasma-derived medicinal products.

Viraemic phase of diagnostic window period: part of the diagnostic window period during which viruses are present in 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 (doubling time).

Window period: see "diagnostic window period"

Abbreviations

antiHBc	anti HBV core protein
antiHBs	anti HBV surface antigen
CE	conform with European requirements
CLIA	chemiluminescence assay
EIA	enzyme immune assay
FDA	Food and Drug Administration
HBsAg	HBV surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	human immunodeficiency virus
ID NAT	individual donation nucleic acid amplification based technique
IDI	interdonation interval
IU	International Unit
IVD	in vitro diagnostic
MP NAT	mini pool nucleic acid amplification based technique
NAT	nucleic acid amplification based technique
NIBSC	National Institute for Standardization and Control
OBI	occult HBV infection
P	probability
PCR	polymerase chain reaction
PDMP	plasma derived medicinal products
PEI	Paul-Ehrlich-Institut
RDT	rapid diagnostic test
RR	residual risk
TGA	Therapeutic Good Administration
TMA	transcription mediated amplification
US	United States
vDWP	viraemic phase of diagnostic window period
WHO	World Health Organization

Introduction

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

(1) Course of HIV, HBV and HCV infections

The course of infection in humans differs for HBV, HCV and HIV depending on the biological features of the virus and on the individual immunological response to the infection. In principal, chronically persistent virus infections can be distinguished from infection courses leading to clearance of the virus. Both infection 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.

Acute infection

The acute viraemic phase of infection is followed by the humoral and cellular immune response, resulting in seroconversion and potential clearance of the virus. For some infections the immunity also protects against re-infection. The acute viraemic phase of virus infection in blood donors may be detected by antigen assays or, more sensitively, by nucleic acid amplification techniques (NAT). Antibody assays are less useful for detection of acute infections, but have been long used for detection of persistent infection (HIV, HCV). Usually there is an overlap of immunoglobulin (Ig) detection, e.g. of class M (IgM), and the declining phase of viraemia.

For HBV both acute resolving and chronic persistent infection courses occur. The frequencies are dependent on different factors, e.g. the age of the individual getting infected. It has been

estimated that in 70% of HBV-infected donors the Hepatitis B surface antigen (HBsAg) may be detected transiently in blood, 5% develop chronic HBV infection with continuous antigenaemia, and 25% does not show detectable antigenaemia. In principal the marker HBV DNA follows the same transient pattern as HBsAg but the median length of viraemia is longer. The transient nature of these HBV blood screening markers requires introducing an adjustment factor when calculating rates of new infections (incidence) *(1)*.

Chronic persistent infection

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

The persistent viraemic infections are usually detectable by both serology and NAT. An exception is HBV where low level HBV-DNA positive carriers (HBsAg negative, antiHBcore (antiHBc) positive) have been described as so-called occult Hepatitis B infections (OBI) *(3, 4)*. In some low prevalence countries the potential OBI transmission risk has been eliminated by introduction of testing for HBV core antibodies (antiHBc). In large parts of the world where HBV is endemic screening for this marker would lead to loss of an unacceptable proportion of donors. Blood components from donors with OBI have transmitted HBV at a low frequency (approximately 3%) while presence of detectable levels of antibody levels against HBsAg (antiHBs) were found to protect against infection, with few exceptions *(5-9)*. The OBI-associated input of HBV into plasma pools used for manufacture of PDMPs appears negligible when compared to diagnostic window period donations.

(2) Residual risk origins

The residual risk of HIV, HBV or HCV infections in blood or plasma donations is defined as the probability of a viraemic donation from a donor infected with one of these blood borne viruses not being detected by the routine screening assay(s).

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

a) Assay failures

Assay failures in blood screening can happen due to viral variants escaping detection (for example by oligonucleotide mismatches in NAT methods or monoclonal antibodies not detecting antigen of mutant virus) *(10-12)*. Another potential root cause of assay failures is the assay performance in the screening laboratory, for example deficiencies of instrument or software. Such assay performance failures can be recognized when an adequate quality management system with external quality control procedures is in place. 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 this guideline. Nevertheless it is important to continuously survey quality features of screening assays and to identify potential causes of false test results. Post market surveillance of assay safety, quality and performance is a mechanism to detect, investigate and act on issues identified and defines the need for continuous improvement of assays *(13)*.

b) Diagnostic window periods

Historically the phase elapsing between the time point of infection and 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 on 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. This non-viraemic phase is followed by the viraemic ramp-up phase during which the virus concentration increases in a log-linear fashion in the plasma. For each of the three blood-borne viruses (HIV, HBV and HCV) a specific constant replication rate is apparent until a peak or a 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 is when one virus can be 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 when 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)*. The viral replication characteristics in the early phase of infection are rather consistent among recently infected individuals. This phenomenon results in doubling times for the virus amount in plasma characteristic for HIV, HBV and HCV. By knowing the viral replication kinetics of HIV, HCV or HBV in the early infection phase along with the diagnostic sensitivity of the screening assay, the length of the viraemic phase can be extrapolated for a certain screening assay.

HIV

HIV replicates with an average doubling time of 20 hours (0.85 days) to reach the peak level of viraemia of up to 10^7 IU HIV-RNA/ml *(15)*. This virus concentration decreases in parallel to development of specific antibodies detectable by antiHIV assays. The currently most sensitive antigen assays can detect HIV p24 antigen at 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: the corresponding HIV-RNA concentration for detection by state of the art combo assays is around 10^5 IU/ml *(15,16)*.

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 antiHCV-negative plateau phase of several weeks characterized by high-level viraemia with up to 10^8 IU HCV-RNA/ml *(17, 18)*. HCV core antigen appears to be detectable by core antigen assays during the major part of this antiHCV-negative phase, namely the entire plateau phase and the last part of the ramp up phase. Similar to HIV, the antigen detection efficiency by current HCV combo assays is less than that of the antigen assays. Combo assays have an overall detection rate of approximately 40% of the antiHCV negative window period specimens, and they preferentially detect those with virus concentrations above 10^6 IU/ml *(19)*.

HBV

The replication rate of HBV in early infection phase as determined by the increase in viraemia is significantly lower when compared to HIV or HCV; the HBV mean doubling time is 2.6 days *(20, 21)*. HBV viraemia in early infection phase is detected earlier by NAT than HBsAg assays. In the absence of NAT, sensitive HBsAg assays are key for detection of early infection.

(3) Screening assay categories and diagnostic window periods

In this document screening assays are discussed by category. While antibody assays are designed to detect both recent and chronic persistent infections, the additional benefit of antigen or viral genome detection is mainly to further reduce the diagnostic window. The length of the diagnostic window period varies greatly by assay category.

(a) Nucleic acid amplification technique (NAT) based assays detect viral nucleic acids after in vitro amplification of a target region of the viral genome; NAT assays are performed on individual donations (ID NAT) or in small pools of donations (mini pools; MP NAT). A true infection may not be detectable by NAT if the concentration of viral genomes is below the

detection limit of the assay. Without virus enrichment steps (e.g. ultracentrifugation) in pooled specimens the length of the window period increases with the mini pool size and is minimal with ID NAT. At low virus concentrations in the early ramp up phase of the window period the detection probability by NAT follows a Poisson distribution. The concentration range between a 5% to 95% probability of detection may be 100 fold, and this complicates the estimation of window period reduction that can be achieved by NAT. In this guideline the threefold concentration of the 95% detection probability has been taken as worst case assumption for reliable NAT detection (for estimate of virus concentration in a potentially contaminated plasma pool). However, NAT window periods may be significantly shorter at lower bond of uncertainty range. The Poisson distribution property of the analytes detected by NAT is often considered for more accurate estimate of virus transmission risk by non-inactivated blood components *(22, 23)*.

(b) Antigen assays have been optimized for the detection of viral proteins (antigens) which are part of the virus particle, such as viral capsids (e.g. HIV p24 or HCV core) or virus envelopes, or are subviral particles (e.g. HBsAg). For recently infected individuals non-reactive test results of antigen assays are due to either absence of viral proteins or presence of antigens with concentration below the detection limit of the assay.

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

(d) Antibody assays report an infection by the detection of specific antibodies against the pathogen; for recently infected individuals non-reactive test results of antibody assays can be caused by absence of specific antibodies, antibody concentration insufficient to obtain 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.

(e) Rapid diagnostic tests (RDT) are diagnostic devices of simple design, often based on immunochromatographic (lateral flow) or immunofiltration (flow through) technologies, without need for complex equipment, and giving a test result within short time frame (15 – 30 minutes). Though often not claimed by the manufacturer for use in blood screening, these devices are sometimes used for blood safety testing in resource-limited settings or in emergency situations. The RDT technology is associated with a lower sensitivity when compared to more sophisticated immunoassays developed specifically for blood screening *(24, 25)*.

NAT assays are generally able to detect a recent infection prior to antigen assays, followed by combination assays and antibody assays. These differential capacities in 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 more sensitive methods of another category. For example, currently the most sensitive HIV1/2 antibody asssay provides a shorter diagnostic window period than the least sensitive CE-marked HIV1/2 combo assay. This is true both for assays prequalified by WHO and for CE-marked assays. Furthermore, assays may have differing sensitivity for 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 represent mainly viral genotypes and subtypes prevalent in the US and Europe, which are HIV subtype B, HCV genotype 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 *(26)*.

Mean estimates of the viraemic diagnostic window periods of assays representing the so-called "state of the art" are presented by assay categories in Table 1. These estimates should be used for risk calculation unless there is more detailed information available for 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 in Table 1, the more accurate data for this assay should be taken for the residual risk estimation.

Table 1

	Length of the viraemic phase of the diagnostic window period (vDWP) for assay categories (in days)							
	ID NAT	**MP (16) NAT**	**antigen EIA / CLIA**	**combo EIA / CLIA**	**antibody EIA / CLIA**	**antigen RDT**	**combo RDT**	**antibody RDT**
HIV	**8**	**11**	**14**	**16**	**21**	**---**	**20**	**28**
HBV	**27**	**37**	**42**	**---**	**---**	**55**	**---**	**---**
HCV	**5**	**7**	**9**	**38**	**60**	**---**	**---**	**80**

<u>Explanations to Table 1</u>

NAT assays: Only a limited number of NAT assays claiming blood screening as intended use has been CE-marked or FDA-approved so far; for a worst case scenario, diagnostic window periods of less sensitive NAT assay versions have been taken as examples in Table 1. It was further assumed that the consistent analytical NAT sensitivity ("100%") corresponds to the threefold 95% cut-off concentration, analogous to the assumption for determination of the whole system failure rate in the Common Technical Specifications of the EU IVD

Directive *(27)*. For more accurate estimate of transmission risk with ID and MP-NAT options one also can take into account the probability of detection in the early ramp up phase of viremia which significantly reduces the infectious window periods *(22,23)*.

EIA/CLIA: In this categories (antibodies, antigen, combo) FDA-approved, CE-marked and/or WHO prequalified assays of medium sensitivity have been chosen as examples *(17, 19, 24, 25, 28, 29)*.

RDT: For rapid diagnostic tests (RDTs) there is a wide range of sensitivity among different assays; values of medium sensitive RDTs have been taken for Table 1 *(24, 25)*.

Viraemic phase of the diagnostic window period (vDWP): this phase has been defined as the period with a virus concentration of ≥1 virus particle in a red blood cell unit containing 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.

(4) Virus concentrations during diagnostic window period

For risk modelling of plasma pool contamination the maximum 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. Maximal viral loads of window period donations are listed in Table 2 as worst case for the different assay categories, corresponding to Table 1.

Table 2

	Maximal concentration of viral genomes in the viraemic phase of the diagnostic window period (vDWP) (in International Units per millilitre (IU/ml))							
	ID NAT	**MP (16) 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

(5) Confirmation of reactive screening results

The residual risk estimations rely on reactive screening assay results representing true infection events. Initially reactive test results should be repeated in duplicate in the same assay. If reactivity is repeatedly obtained in the routine screening assay, the test result should still be checked by a confirmation strategy *(30)*.

Confirmation strategies may include more specific assays (e.g. HIV Western blot or immunoblot, HCV immunoblot, HBsAg neutralisation assay) or another screening or diagnostic assay of different design. NAT results should be checked by testing an independent aliquot of the donation to exclude contamination and/or by testing of 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 risks.

(6) 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 are targeting for an established population of repeat donors undergoing constant selection for absence of infectious markers.

First time donors

Positive screening test results in first time donors may be an indication of infections which 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 are the major contribution to the residual risk of window period infections. The distinction between prevalent and incident infections requires more detailed investigations: 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). The 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 this subpopulation *(14, 31)*. If results from these investigations are not available for a specific

first time donor population, the incidence rate of these donors can be derived from the rate of repeat donors by applying an adjustment factor. Scientific investigations for HIV, HBV and HCV in different donor populations investigated incidence in the first time and repeat donors. Some studies showed a two- to threefold higher rate of recent infections in the first time compared to the corresponding repeat donors; however, results of other studies are not consistent *(32-37)*. In the absence of incidence data specific to the first time donor population one has the option to assume a threefold higher incidence of virus infections as the worst case for this subpopulation when compared to the corresponding repeat donor subpopulation of the same blood establishment. This factor will be referred to as "first time donor incidence adjustment factor" in section 7.

Repeat donors

For repeat donors any confirmed positive screening test result indicates a new infection having likely occurred during the interdonation interval, the time period between the most recent donation (tested positive) and the previous donation (tested negative). However it is also possible that the previous donation (tested negative) was drawn just in the diagnostic window period of the screening assay. The relative frequency of this possibility depends on the length of the interdonation interval, with smaller interdonation intervals (IDIs) increasing the probability of a viraemic window period (vDWP) donation tested negative in the screening assay. Hence the risk of a screening assay to miss a viraemic window period donation is defined as the length of the vDWP divided by the average IDI.

(7) Estimation of incidence and window period modelling of risks

Incidence

The incidence rate of new infections in repeat donors is defined as the number of NAT conversions or seroconversions divided by the total number of person years of observation of all donors during the study period *(38-40)*. Person years of observation requires a computer systems that record 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 this guideline, both the estimation of incidence and the estimation of the residual risk per blood donation are derived from data of 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 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.

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

Formula 1: Incidence (per 100.000 person years)

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

Residual risk per blood donation

For calculating the probability of the residual risk that a blood donation has been collected during the viraemic phase of the diagnostic window period, different factors play a role:

- The frequency of new infections (incidence) in the repeat donor population.
- The donation frequency of repeat donors or the average length of the interdonation intervals (IDIs).
- The length of the viraemic phase of the diagnostic window period (vDWP) for the assay used (Table 1).

The donation frequency of repeat donors (average number of donations per repeat donor) determines the average size of the interdonation interval (IDI). The interdonation interval (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. The smaller the IDI, the higher is the probability that a donor (unaware of the infection) donates during the viraemic diagnostic window period of the screening assay.

The residual risk (RR) for a blood donation from a repeat donor to have been collected during the viraemic phase of the diagnostic window period (vDWP) of the screening assay used can be calculated by the formula 2.

Formula 2: Residual Risk per donation (RR)

$$\text{RR} = \frac{\text{vDWP}}{\text{IDI}} \text{ x } \frac{\text{number of seroconverters among repeat donors}}{\text{number of donations from repeat donors}}$$

RR is usually expressed as per million donations (for which one has to multiply the calculated RR figure above with 1 000 000).

Formula 2 can be directly used to calculate the residual risk per donation (RR) for HIV and HCV infections in repeat donors, for HBV infections R calculated by this formula has to be multiplied by an HBV incidence adjustment factor.

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 resolving after the acute phase. A transient infection course is seen in adults for the majority of HBV infections (95%) wheras 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 interdonation intervals (IDIs) and the assay sensitivity. For each assay category a mean detection period for the transient HBV marker (HBsAg, 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) *(1)*. Scientific literature provides different estimates for the length of transient antigenaemia *(1, 21, 41)*. The differences 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 3.

Table 3

HBV DNA and HBsAg detection period (days) for assay categories			
NAT ID	**NAT MP (16)**	**HBsAg EIA / CLIA**	**HBsAg RDT**
90	70	60	44

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

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

The probability P (in %) of detection by NAT testing (Table 3) may be calculated as

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

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

To determine the HBV infection residual risk per donation, RR obtained for HBV (Formula 2) is multiplied by the adjustment factor for the specific assay category used.

First time donor incidence adjustment factor

In the absence of specific incidence data for first time donors, a threefold higher residual risk may be assumed for blood donations from first time donors when compared to the repeat donors of the same donor population (see section 6).

Accordingly, the residual risk (RR) for a blood donation from a first time donor to have been collected during the viraemic phase of the diagnostic window period of the screening assay may be assumed to be threefold higher than the risk calculated for a blood donation obtained from the corresponding repeat donors of the same blood establishment.

Adjustment for interdonation intervals

The incidence / window period modelling of residual risk, as described above, assumes that the donation behaviour with regard to timing and frequency of donations is the same for infected versus non-infected donors. In scientific literature evidence can be found that seroconverting donors sometimes delay their return to blood donation, and therefore have larger average interdonation intervals (IDI) when compared to non-infected donors, resulting in a lower residual risk *(42)*. There are mathematical models available to reflect this difference in donor behaviour *(43)*. For high incidence settings the mean IDI (in days) of the seroconverting repeat donors (this is the period between the last negative and the first positive donation after infection) may be compared with the overall IDI of non-infected repeat donors. The residual risk calculation may then be adjusted by the relative IDI difference. If, however, only a few acute infections are found it is adviced to take the average IDI of all repeat donors.

(8) Residual risks

Infection of recipients of non-inactivated blood components

The actual infection risk in recipients of non-inactivated blood products is dependent on factors like the amount of intact viruses transmitted, the presence of potentially neutralising antibodies in the donation or recipient, virus properties and recipient immunological factors *(29)*. Using worst case scenarios, the frequency of viraemic donations escaping screening can be estimated using formula 2. For whole blood donations different blood components (erythrocytes, thrombocytes, plasma) may be manufactured 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 nondetection by the screening assay(s) and the infectivity of the virus after storage of the blood component are important factors influencing the infection risk but are beyond the scope of this guideline *(23, 29)*.

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 e.g. immunoglobulins, albumin or clotting factors, manufactured from plasma pools. These may be contaminated with HIV, HBV or HCV by inclusion of plasma units originating from window period donations not detected by the screening assays. The extent of potential plasma pool contamination depends on different factors:

- The expected frequency for donations from the viraemic phase of the diagnostic window period (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 frequency of viraemic plasma units is estimated by the residual risk (RR) 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 maximum viral load of window period donations (the information in Table 2 for the different assay categories) should be taken as worst case scenario, even though only a minority of window period plasma units will reach the maximum viral load.

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Annex 1

Targeted evaluation of new blood screening assays

Dependant 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 assays. It is recommended that previous assessments of quality features of the assay performed by experienced regulatory authorities (e.g. US FDA approval, European CE certification, Australian TGA or Health Canada marketing authorisations) or by the WHO Prequalification of in In Vitro Diagnostic programme should be taken into account. Previous assessments by such stringent regulatory bodies include the review of analytical and clinical performance data submitted by the manufacturer, the manufacturer`s quality management system, the batch to batch consistency and in the case of WHO Prequalification, an independent performance evaluation.

Therefore in a country the assessment of manufacturer`s documentation with focusing on the 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 prior to implementation (e.g. by a national reference laboratory) it is recommended that the evaluation be focused on essential features through selective testing.

Assessment of documents

Documents provided by the IVD manufacturer may be assessed, with special focus on regional peculiarities. This focus may include the questions whether stability studies cover the regional environmental conditions, e.g. in regard to temperature and humidity, or whether instructions for use are appropriate for the target users.

Performance evaluation studies documented by the IVD manufacturer may be reviewed for the representation of specimens reflecting the regional situation, e.g. with regard to viral genotypes or variants or for potential interference of other regionally more prevalent infections with the test result.

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 on regionally important quality aspects. This refers e.g. to 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 data base obtained with a number of assays may then be the scientific basis for definition of criteria for acceptance of new assays and for identification of less suitable assays.

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

Furthermore, WHO offers through its IVD standardization program different kinds of biological reference preparations which may be useful for the confirmation of basic assay features. WHO International Standards, expressed in International Units, are available for confirmation of analytical sensitivity, and WHO International Reference Panels representing the major viral genotypes could be used to check genotype detection efficiency.

These WHO reference preparations are usually lyophilized in order to facilitate worldwide shipping. They are listed on the WHO homepage (http://www.who.int/bloodproducts/catalogue/en/). They can be obtained from the WHO Collaborating Centers acting in this field as WHO custodians, the National Institute for Biological Standardization and Control (NIBSC), UK, or the Paul-Ehrlich-Institut (PEI), Germany.

Table 4 summarizes the currently available most important WHO reference preparations in the field of blood screening.

Table 4: WHO reference preparations (blood screening)

Marker	Preparation	Details	Custodian
antiHIV-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 1,000 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
	1st International Reference Panel HBV Genotypes Lyophilized No unitage	HBV genotypes A-F, H	PEI
	Dilutional panel 8.25; 2.06; 0.52; 0.13 IU/vial		NIBSC
HBV DNA	3rd International Standard Lyophilized 850,000 IU/vial		NIBSC
	1st International Reference Panel HBV Genotypes Lyophilized No unitage	HBV genotypes A-G	PEI
antiHBc	1st International Standard Lyophilized 50 IU/vial		NIBSC
HCV core	1st International Standard Lyophilized 3,200 IU / ml		PEI
HCV RNA	5th International Standard Lyophilized 100,000 IU/ml		NIBSC

Annex 2

Examples for estimation of residual risks

Example 1

HCV screening by antiHCV EIA

Center A, observation period 01.06.2011-31.05.2012

49 660 ***repeat donors****, 100 313 donations, 45 antiHCV pos (EIA)*

11 452 ***first time donors****, 11 452 donations, 89 antiHCV pos (EIA)*

a) Residual risk (RR) per blood donation from repeat donors

Interdonation interval (IDI)

$$\text{Donations per donor} = \frac{100\ 313}{49\ 660} = 2.01$$

$$\text{IDI} = \frac{\text{observation period}}{\text{donations per donor}} = \frac{365 \text{ days}}{2.01} = 181.59 \text{ days}$$

Residual risk (RR) per blood donation from repeat donors

vDWP = 60 days (according to Table 1: antiHCV EIA)

$$\text{RR} = \frac{\text{vDWP}}{\text{IDI}} \text{ x } \frac{\text{number of seroconverters among repeat donors}}{\text{number of donations from repeat donors}}$$

$$\text{RR} = \frac{60 \text{ days}}{181.59 \text{ days}} \text{ x } \frac{45}{100\ 313} = 0.000\ 148 = 148\ per\ million$$

Number of vDWP blood donations from repeat donors (Center A, observation period)

$$\text{No.} = 100\ 313 \text{ x } \frac{148}{1\ 000\ 000} = 14.84$$

b) Residual risk (RR) per blood donation from first time donors

Positive screening test results represent mainly old (prevalent) infections. The rate of recent infections can be determined by specific investigations, e.g. recency assays, NAT only`s.

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

$$\text{R} = 0.000\ 148\ x\ 3 = 0.000\ 444 = 444\ per\ million$$

Number of vDWP blood donations from first time donors (Center A, observation period)

$$\text{No.} = 11\ 452 \text{ x } \frac{444}{1\ 000\ 000} = 5.08$$

c) Expected number and risk of window phase donations for repeat and first time donors combined (Center A, observation period)

$$\text{No.} = 14.84 + 5.08 = 19.92$$

$$\text{R} = \frac{19.92}{100\ 313 + 11\ 452} = 0.000\ 178 = 178\ per\ million$$

Example 2

HBV screening by HBsAg rapid diagnostic assay (RDT), HBV adjustment factor

Center 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*

a) Residual risk (RR) per blood donation from repeat donors

Interdonation interval (IDI), as calculated for Example 1

$$\text{IDI} = 181.59 \text{ days}$$

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

vDWP = 55 days (according to Table 1: HBsAg RDT)

$$\text{RR} = \frac{\text{vDWP}}{\text{IDI}} \text{ x } \frac{\text{number of seroconverters among repeat donors}}{\text{number of donations from repeat donors}}$$

$$\text{R} = \frac{55 \text{ days}}{181.59 \text{ days}} \text{ x } \frac{184}{100\,313} = 0.000\,555 = 555 \; per \; million$$

HBV incidence adjustment factor

Probability (P) for HBsAg detection

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

$$= 70\% \text{ x } \frac{44 \text{ days}}{181.59 \text{ days}} + 5\% = 70\% \text{ x } 0.24 + 5\% = 21.8\%$$

HBV incidence adjustment factor = $\frac{100\,\%}{\text{P}} = \frac{100\,\%}{21.8\,\%} = 4.58$

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

Adjusted residual risk = $4.58 \text{ x } 0.000\,555 = 0.002\,541 = 2\,541$ per million

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