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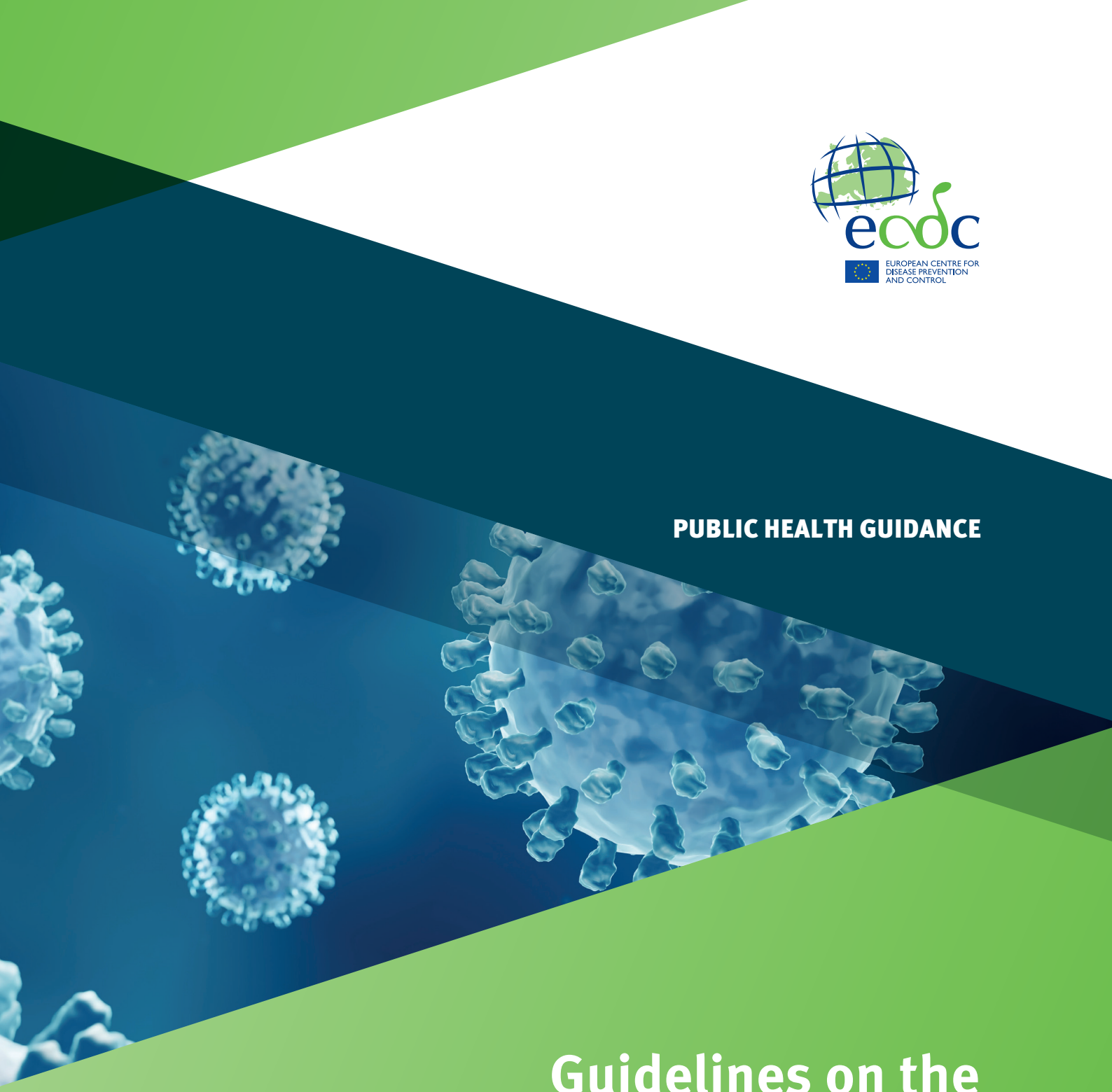

PUBLIC HEALTH GUIDANCE

Guidelines on the prevention of hepatitis B virus transmission through substances of human origin

Technical guidelines supporting the regulation on standards of quality and safety for substances of human origin intended for human application

ECDC PUBLIC HEALTH GUIDANCE

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The content of these guidelines was developed by the European Centre for Disease Prevention and Control (ECDC) with the support of a technical ad hoc scientific panel composed of 17 experts from the EU/EEA countries.

The work of the guidelines development was coordinated by the ECDC SoHO team: Flávia Cunha, Francois-Xavier Lamy and Jenny Mohseni Skoglund.

The following internal ECDC experts contributed to the work that informed the guidelines development:

Francois-Xavier Lamy, Flávia Cunha, Jenny Mohseni Skoglund, Nina Lagerqvist, Erika Duffell, Ndeindo Ndeikoundam Ngangro, and Csaba Kodmon

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Table 1. Chronic hepatitis B virus infection markers by disease phase 12

Abbreviations

ALT	Alanine transaminase
Ag	Antigen
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
COVID-19	Coronavirus disease 2019
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EC	European Commission
EDQM	European Directorate for the Quality of Medicines & Healthcare
EEA	European Economic Area
EIA	Enzyme immunoassay
ESHRE	European Society of Human Reproduction and Embryology
EU	European Union
HBcAg	HBV core antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IU	International units
IVDR	Regulation (EU) 2017/746 on in vitro diagnostic medical devices
LOD	Limit of detection
MAR	Medically Assisted Reproduction
NAT	Nucleic acid test
NCA	National Competent Authorities
OBI	Occult HBV infection
SoHO	Substances of human origin (excluding solid organs)[1]
STI	Sexually transmitted infection

[1] As per the Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC.

Glossary

These guidelines use the definitions of key terms as laid out in Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC [1]. The main key terms used from the Regulation are as follows:

Allogeneic use	The human application of SoHO collected from a person other than the SoHO recipient.
Autologous use	The human application of SoHO collected from a person to the same person.
Blood component	A constituent of blood, such as red blood cells, white blood cells, platelets and plasma, that can be separated from it.
Deceased SoHO donor	A deceased person who has been referred to a SoHO entity with a view to SoHO collection, and from whom consent had been granted in that respect or from whom SoHO collection is permitted, in accordance with national legislation.
Human application	Being inserted, implanted, injected, infused, transfused, transplanted, ingested, transferred, inseminated or otherwise added to the human body in order to create a biological interaction with that body.
Living SoHO donor	A living person who has volunteered to a SoHO entity, or has been presented by a person granting consent on their behalf, in accordance with national legislation, with a view to making a donation of SoHO, for the purpose of use in a person other than themselves, and other than in situations of within-relationship use.
Medically assisted reproduction	Any laboratory or medical intervention, including any preparatory steps, that involves the handling of reproductive SoHO for the purpose of the facilitation of pregnancy or for preservation of fertility.
Offspring from medically assisted reproduction	Children born following medically assisted reproduction.
Reproductive SoHO	Human sperm, oocytes, ovarian and testicular tissue intended to be used for the purpose of medically assisted reproduction or restoring endocrine function; for the purposes of this definition, embryos are considered reproductive SoHO even though they are not collected from the human body. For these guidelines, for clarity, reproductive SoHO are referred to as 'reproductive tissues and cells'.
SoHO donation	A process by which a person voluntarily and altruistically gives SoHO from their own body for persons in need, or authorises the use of such SoHO after their death; it includes the necessary medical formalities, examination and treatments and monitoring of the SoHO donor, irrespective of whether that donation is successful or not; it also includes, where applicable, the consent given by an authorised person in accordance with national legislation. For simplicity, for these guidelines, SoHO donations are referred to as 'donations'.
SoHO donor	A living or deceased SoHO donor.
SoHO entity	An entity legally established in the Union that carries out one or more of the SoHO activities referred to in Article 2(1), point (c) in the Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024. For simplicity, for these guidelines, 'SoHO entity' is referred to as 'entity'.
SoHO recipient	The person to whom SoHO are applied or the human application of SoHO is envisaged, whether by allogeneic, autologous or within-relationship use. 'Recipient' means a SoHO recipient or any person receiving a product manufactured from SoHO, regulated by other Union legislation. For simplicity, for these guidelines, 'SoHO recipients' are referred to as 'recipients'.
Substance of human origin or SoHO	Any substance collected from the human body, whether it contains cells or not and whether those cells are living or not, including SoHO preparations resulting from the processing of such substance.
Third-party donation	A donation of reproductive SoHO to be used for medically assisted reproduction in a SoHO recipient with whom the SoHO donor does not have an intimate physical relationship.
Within-relationship use	The use of reproductive SoHO for medically assisted reproduction between persons with an intimate physical relationship.

Executive summary

These guidelines support adherence to the European Union (EU) Regulation on standards of quality and safety for substances of human origin (SoHO) intended for human application. They provide evidence-based recommendations for assessing SoHO donors within the EU and European Economic Area (EEA) with the aim of preventing transmission of the hepatitis B virus (HBV) to recipients and offspring from medically assisted reproduction (MAR). HBV poses a significant risk to the safety of SoHO due to its potential transmission through blood, tissues, cells, and organs, as well as the lifelong consequences and severity of the disease. The sustained incidence and prevalence of HBV in EU/EEA countries underscores the need for a strategic approach to prevent the transmission of HBV from donors via SoHO.

The guidelines are divided into three main sections corresponding to different types of SoHO: blood and blood components for transfusion, tissues and non-reproductive cells, and reproductive cells.

The guidelines were developed by the European Centre for Disease Prevention and Control (ECDC) with the support of a panel composed of experts from EU/EEA countries. These experts provided specific technical and scientific advice to the ECDC. Expert meetings, supported by evidence synthesis, were conducted to inform guideline recommendations. To develop the statements in these guidelines and to support the expert panel, evidence synthesis based on structured literature searches was provided in a pathogen data sheet. This pathogen data sheet contains microbiological and clinical information on HBV, including virus and disease descriptions, epidemiology, test characteristics and testing approaches, and evidence of transmission through SoHO.

Key requirements

Blood and blood components

For additional information and recommendations, please refer to Requirements and recommendations: Blood and blood components.

If the results of the required donor screening tests are negative, the donation can be released for clinical use.

Testing requirements	Donor screening tests	Outcome of test results		
		Screening test results	Confirmatory test results[a]	Actions
All donors, at each donation • Assessment of recent risks of exposure to HBV is required. • Donors should not be tested in the context of donor evaluation before a period of at least 16 weeks since the last event with a risk of exposure to HBV. In case of the use of a highly sensitive HBV DNA NAT, this period can be reduced to eight weeks.	• **HBsAg + HBV DNA NAT + anti-HBc** Or • **HBsAg + highly sensitive HBV DNA NAT[b]**	HBV DNA NAT reactive and HBsAg reactive or negative	Any	• Do not release donation for clinical use. • Notify donor and refer to relevant clinical care. • Defer until criteria to re-enter donor screening procedures are met[c]. • Initiate look-back procedures.
		HBV DNA NAT negative and HBsAg reactive	Positive and not attributable to recent hepatitis B vaccination	
		HBV DNA NAT negative and HBsAg reactive	Indeterminate	• Do not release donation for clinical use. • Notify donor and refer to relevant clinical care based on risk assessment. • Re-entry in donor screening procedures possible after eight weeks. • Look-back procedures based on risk assessment.
		HBV DNA NAT negative and HBsAg reactive	Negative	• Do not release donation for clinical use. • Re-entry in donor screening procedures possible without a deferral period.
		Anti-HBc reactive and HBV DNA NAT negative and HBsAg negative[d]	Anti-HBc reactivity confirmed	• Test with highly sensitive HBV DNA NAT[b]: if result is reactive, see actions above. • If the highly sensitive HBV DNA NAT is negative, test for anti-HBs: if anti-HBs <100 IU/L, do not release donation for clinical use; if anti-HBs is ≥100 IU/L, the donation can be released for clinical use.

Anti-HBc: Hepatitis B core antibody; DNA: deoxyribonucleic acid; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; IU: international units; LOD: limit of detection; NAT: nucleic acid testing.
[a] Approaches to confirm screening test results should rely on nationally established algorithms.
[b] A 95% LOD of 20 IU/mL or lower could be considered highly sensitive.
[c] Documentation of HBsAg loss and undetectable HBV DNA during clinical follow-up as well as documentation of anti-HBs concentrations demonstrating immunity.
[d] Refer to Requirements and recommendations: Blood and blood components for additional advice and practical considerations on donors with such profiles.

Tissues and non-reproductive cells: living and deceased donors

For additional information and recommendations, please refer to Requirements and recommendations: Tissues and non-reproductive cells.

If all required donor screening test results are negative, the donation can be released for clinical use.

Testing requirements	Donor screening tests	Outcome of test results		
		Screening test results	Confirmatory test results[b]	Actions
All donors, at each donation[a] • Assessment of recent risks of exposure to HBV is required. • Donors should not be tested in the context of donor evaluation before a period of at least 16 weeks since the last event with a risk of exposure to HBV. In case of the use of a highly sensitive HBV DNA NAT, this period can be reduced to eight weeks.	• **HBsAg + HBV DNA NAT + anti-HBc** Or • **HBsAg + highly sensitive HBV DNA NAT[c]**	HBV DNA NAT reactive and HBsAg reactive or negative	Any	• Do not release donation for clinical use. • Initiate look-back procedures if relevant. • Notify donor or the transplant coordination team. • Living donors: refer to relevant clinical care.
		HBV DNA NAT negative and HBsAg reactive	Positive and not attributable to recent hepatitis B vaccination	
		HBV DNA NAT negative and HBsAg reactive	Indeterminate	• Do not release donation for clinical use. • Notify donor or the transplant coordination team based on risk assessment. • Living donors: refer to relevant clinical care based on risk assessment. • Look-back procedures based on risk assessment.
		HBV DNA NAT negative and HBsAg reactive	Negative	• Release the donation for clinical use.
		Anti-HBc reactive and HBV DNA NAT negative and HBsAg negative	Anti-HBc reactivity confirmed	• Test with highly sensitive HBV DNA NAT[c]: if result is reactive, see actions above; if result is negative, the donation can be released for clinical use. • ECDC advises testing for anti-HBs to guide the decision to release the donation for clinical use in immunocompromised recipients.

DNA: deoxyribonucleic acid; HBc(Ag): HBV core (antigen); HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; IU: international units; LOD: limit of detection; NAT: nucleic acid testing.

[a] Should be understood as close as possible to donation and test results should be available before transplantation.

[b] Approaches to confirm screening test results should rely on nationally established algorithms.

[c] A 95% LOD of 20 IU/mL or lower could be considered highly sensitive.

Reproductive cells: third-party donations

For additional information and recommendations, please refer to 'Requirements and recommendations: Reproductive cells'.

If all required donor screening test results are negative, the donation can be released for clinical use.

Testing requirements	Donor screening tests	Outcome of test results		
		Screening test results	Confirmatory test results[b]	Actions
All donors, at each donation[a] • Assessment of recent risks of exposure to HBV is required. • Donors should not be tested in the context of donor evaluation before a period of at least 16 weeks since the last event with a risk of exposure to HBV. In case of the use of a highly sensitive HBV DNA NAT, this period can be reduced to eight weeks.	• **HBsAg + HBV DNA NAT + anti-HBc** Or • **HBsAg + highly sensitive HBV DNA NAT[c]** • In case of donations quarantined for ≥180 days: only tests detecting HBsAg and anti-HBc are required.	HBV DNA NAT reactive and HBsAg reactive or negative	Any	• Do not release the donation for clinical use. • Notify donor and refer to relevant clinical care. • Defer until criteria to re-enter donor screening procedures are met[d]. • Initiate look-back procedures.
		HBV DNA NAT negative and HBsAg reactive	Positive and not attributable to recent hepatitis B vaccination	
		HBV DNA NAT negative and HBsAg reactive	Indeterminate	• Do not release donation for clinical use. • Notify donor and refer to relevant clinical care based on risk assessment. • Re-entry in donor screening procedures possible after eight weeks. • Look-back procedures based on risk assessment.
		HBV DNA NAT negative and HBsAg reactive	Negative	• Do not release donation for clinical use. • Re-entry in donor screening procedures possible without a deferral period.
		Anti-HBc reactive and HBV DNA NAT negative and HBsAg negative	Anti-HBc reactivity confirmed	• Test with highly sensitive HBV DNA NAT[c]: if NAT is reactive, see actions above; if NAT is negative, the donation can be released for clinical use.
		If quarantined: anti-HBc reactive and HBsAg negative	Anti-HBc reactivity confirmed	• Test with highly sensitive HBV DNA NAT[c]: if result is reactive, see actions above; if result is negative, the donation can be released for clinical use.

DNA: deoxyribonucleic acid; HBc(Ag): HBV core (antigen); HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; IU: international units; LOD: limit of detection; NAT: nucleic acid testing.

[a] For oocyte donation, the donation could be considered as the starting date of stimulation, and the testing can hence be performed at the time of stimulation. In the case of serial donations, testing of the donor should be performed at the initial donation and prior to the release of a donation, at least 30 days after the last donation

[b] Approaches to confirm screening test results should rely on nationally established algorithms.

[c] A 95% LOD of 20 IU/mL or lower could be considered highly sensitive.

[d] Documentation of HBsAg loss and undetectable HBV DNA during clinical follow-up as well as documentation of anti-HBs concentrations demonstrating immunity.

Reproductive cells and tissues: within-relationship use

For additional information and for recommendations, please refer to Requirements and recommendations: Reproductive cells.

Testing requirements	Screening tests	Outcome of test results		
		Screening test results	Confirmatory test results	Actions
All partners from whom SoHO are collected • Less than three months before collection. • Maximum of 24 months between tests.	**HBsAg or HBV DNA NAT**	HBsAg or HBV DNA NAT reactive	Positive or indeterminate	• Procedures should be implemented to prevent the risk of infection to the partner and to the offspring. • Refer to ESHRE guidelines on medically assisted reproduction in patients with a viral infection/disease [2].

DNA: deoxyribonucleic acid; ESHRE: European Society of Human Reproduction and Embryology; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; IU: international units; LOD: limit of detection; NAT: nucleic acid testing.

Introduction

These guidelines support adherence to the European Union (EU) Regulation on standards of quality and safety for substances of human origin (SoHO) intended for human application, henceforth referred to as the Regulation [1]. They aim to prevent communicable disease transmission from donors through SoHO in the European Union and European Economic Area (EU/EEA). Following these guidelines should be considered as a means to demonstrate compliance with the standards laid down in the Regulation to ensure a high level of quality and safety. For more information on the legal context, see the Legal background in the Annex.

In this document, SoHO are divided into three categories:

- Blood and blood components (e.g. whole blood, red blood cells, platelets, platelet-rich plasma, and plasma not intended for industrial manufacturing);
- Tissues obtained from deceased or living donors and non-reproductive cells (e.g. corneas, cardiovascular tissues, bones, tendons, skin, amniotic membrane, and hematopoietic progenitor cells), including reproductive tissues when used for allogeneic purposes; and
- Reproductive cells and tissues, including reproductive cells, embryos and reproductive tissues when used for autologous purposes.

These SoHO are used in medical procedures and treatments such as blood transfusions, transplantation therapy and medically assisted reproduction (MAR). They play a pivotal role in enhancing the quality of life and even saving the lives of patients suffering from severe medical conditions or injuries. Despite the life-saving potential of SoHO, the transmission of pathogens through SoHO could lead to infections in recipients and the offspring from MAR, compromising their health and potentially leading to severe complications or even death. Ensuring that these substances are safe and free from avoidable risks, including the transmission of infectious agents, is paramount to protecting the health and wellbeing of patients who receive them.

Objectives and scope

These evidence-based guidelines provide technical requirements and recommendations for evaluating SoHO donors, focusing on the risk of transmitting hepatitis B virus (HBV) to recipients and the offspring from MAR. These guidelines provide the minimum SoHO safety requirements to meet the standards in the Regulation. Countries may, however, apply more stringent measures.

These guidelines aim to provide:

- Requirements and recommendations on laboratory testing methods for screening donors for HBV;
- Requirements and recommendations on testing strategies for HBV; and
- Recommendations on events to consider in donor assessment that may lead to laboratory testing limitations.

The content of these guidelines covers SoHO for allogeneic use (meaning the human application of SoHO collected from a person other than the SoHO recipient), as described in the Regulation.

The SoHO Regulation does not apply to solid organs; therefore, organs are outside the scope of these guidelines. Faecal microbiota and breast milk are not included in this iteration of the guidelines. SoHO for autologous use (meaning the human application of SoHO collected from a person to the same person) except for reproductive tissues for autologous use, is also not included in this iteration of the guidelines. If SoHO intended for autologous use is processed or stored, the individual should be tested for HBV.

SoHO for industrial manufacturing, such as plasma for fractionation, pre-analytical considerations, laboratory quality requirements, storage and detailed tests and algorithms for confirmatory testing, are also out of the scope of this iteration of the guidelines.

The current guidelines will be adapted at a later stage to cover the prevention of HBV transmission from donors through SoHO intended for industrial manufacturing, such as plasma for fractionation. The risk of HBV transmission through faecal microbiota and breast milk will be addressed separately. The plans for these adaptations will be published on ECDC's website[1].

Protection of SoHO recipients and the offspring from MAR other than from transmission of communicable diseases through the application of SoHO, quality requirements for the preparation, use and quality control of blood components, tissues, and cells are not covered in these guidelines. Instead, the European Directorate for the Quality of Medicine and Healthcare (EDQM) 'Guide to the preparation, use and quality assurance of blood components' [3] and 'Guide to the quality and safety of tissues and cells for human application' [4] should be followed.

[1] https://www.ecdc.europa.eu/en/infectious-disease-topics/related-public-health-topics/substances-human-origin/technical-guidelines

Target audience

The target audiences for these guidelines are professionals in the EU/EEA working in SoHO entities, as well as other professionals involved in the selection of blood, tissues, and cells donors. These guidelines may also serve as a reference for National Competent Authorities (NCA) for blood, tissues and cells, and MAR.

Structure of the document

These guidelines are structured into three main sections for the following types of SoHO:

- Blood and blood components;
- Tissues and non-reproductive cells (living and deceased donors); and
- Reproductive cells and tissues (including reproductive cells and embryos, and reproductive tissues for autologous use).

Preceding the SoHO-specific sections, the guidelines outline general requirements and recommendations that apply to all SoHO, which provides a common framework for the subsequent sections. Each SoHO-specific section is subdivided into subsections, addressing requirements and recommendations concerning testing of donors, consequences of test results, risk of exposure to HBV, and other aspects to consider for the described SoHO type. Each set of requirements and recommendations is accompanied by evidence, including expert opinion and justification to support the statements provided. Some of the statements are repeated in the 'Evidence and justification' sections for the sake of clarity; they are consistent with the list of statements in the 'Requirements and recommendations' sections.

Prior to the general and SoHO-specific requirements and recommendations, the guidelines offer an overview of considerations for HBV that is relevant to SoHO safety, as well as a summary of the guideline development process applicable to all SoHO within these guidelines. A summary table outlining key requirements and recommendations is included in the 'Executive summary'.

Further information on the guideline development process, including methods for evidence collection and synthesis and details on the ad hoc scientific expert panel, can be found in the Annex at the end of this document.

The statements in these guidelines are supported by evidence compiled in a pathogen data sheet for HBV (Pathogen data sheet, Annex). Additional details and references are available in the corresponding sections of the pathogen data sheet, as indicated in the guidelines.

In this document, requirements including the term 'should' describe technical requirements to meet the standards set out in the SoHO regulation. Recommendations and practical considerations including the terms 'ECDC advises' or 'is advised' or 'considered' are used to describe additional recommendations or suggestions to consider but that are not required to meet these standards.

Development of the guidelines

Overall development

The development of these technical guidelines on the prevention of HBV transmission from donors through SoHO was coordinated by ECDC with the support of an expert panel convened for this activity. The panel included experts in infectious diseases, donor selection, and donor testing for blood, tissues and reproductive and non-reproductive cells from different EU/EEA countries.

Three expert panel meetings were hosted virtually between May 2024 and September 2024, addressing the topics covered by the present guidelines:

- Laboratory testing methods for screening of donors;
- Testing strategies for screening of donors; and
- Events to consider in donor assessment that may lead to laboratory testing limitations.

Discussions with the expert panel were supported by a pathogen data sheet for HBV developed by ECDC (Pathogen data sheet, Annex). This document served as an evidence base for the expert panel and was intended to support statements agreed with the expert panel.

Conclusions from the expert panel meetings, including discussions on the provided evidence and agreements reached during the meetings, were used to draft these guidelines. The guideline text clarifies when statements rely on the expert opinion expressed during the meetings rather than on the evidence synthesised in the pathogen data sheet. For additional information on the guideline development and the ad hoc expert panel work procedures, see the Annex.

Evidence synthesis

Evidence synthesis supporting the expert panel discussions was provided in the pathogen data sheet for HBV (Pathogen data sheet), containing information on the following topics:

- Description of the virus;
- Description of the disease;
- Epidemiology in the EU/EEA, including risk factors for HBV infection;
- Laboratory testing approaches;
- Current testing requirements in EU/EEA countries;
- Recommendations from other organisations;
- Evidence of transmission through SoHO; and
- Pathogen reduction/inactivation methods.

The evidence for all sections relied on structured but non-systematic literature searches. Quantitative descriptive analysis (range and median values) was performed for laboratory testing approaches and pathogen reduction/inactivation methods; qualitative synthesis was used for all other sections. No assessments for risk of bias were performed. This approach was considered acceptable for HBV as the risk for SoHO is well established, as are the measures to prevent transmission (testing and deferral strategies).

The expert panel had the opportunity to critically review the evidence provided before each meeting and request or offer additional evidence to support discussions and decision-making.

Expert meetings

Prior to each meeting, anonymous surveys were sent to the panel, and results were used as a basis to reach agreements during the meeting. The surveys covered the following topics:

- Which SoHO donors should be tested for HBV?
- When should SoHO donors be tested for HBV?
- Which laboratory screening tests should be used to test SoHO donors for HBV?
- What limit of detection (LOD) should be applied for nucleic acid tests (NAT) detecting HBV deoxyribonucleic acid (DNA)?
- What actions should be performed in case of reactive screening tests, including the deferral of donors?
- Which risks of exposure to HBV are considered relevant for SoHO safety and need to be considered in the SoHO donor assessment?
- What deferral period should be considered for donors with events with a risk of exposure to HBV to ensure reliable test results?

Where survey results indicated a general agreement on a question, ECDC proposed a corresponding statement to the expert panel for formal agreement. Formal agreement was defined as the absence of major disagreement by the participants. In addition to statements proposed by ECDC, agreements reached with the expert panel could rely on expert opinions expressed during the meeting. Summaries of discussions and agreements reached in meetings were detailed in minutes, which were sent for review to the panel after each meeting. All discussions conducted with the panel were inclusive of all SoHO types considered within the scope of these guidelines. Panel members who could not participate in a specific meeting were encouraged to provide written input. If the panel deemed evidence insufficient to reach an agreement on a specific topic, the topic was reconsidered in the subsequent pre-survey and rediscussed in the following meeting, supported by additional evidence in the pathogen data sheet, if available. Approved agreements in the final meeting minutes were a reference for drafting the guidelines.

In cases of major disagreements that could not be resolved, the option to submit the subject to the ECDC SoHO network for consultation was available. However, throughout the panel discussions on the development of the HBV technical guideline, there were no major disagreements. For additional information on the guidance development process and methods, see the Annex.

HBV and the considerations for SoHO safety

Description of HBV infection and disease

HBV infection can present as acute or chronic, with chronic infection resulting from the persistence of the virus. The likelihood of developing chronic infection is primarily determined by the immune status and the age of the individual at the time of acute infection, with a higher risk of chronic HBV infection in younger age groups [5,6].

Acute hepatitis B

Acute HBV infection can have different clinical presentations according to the age at the time of infection. During childhood, acute hepatitis B is usually asymptomatic or subclinical. However, in adults, up to 50% can develop icteric hepatitis [6].

Most acute hepatitis B cases during adult age (~95%) will not progress to chronic hepatitis B, even if the virus can persist lifelong in the form of covalently closed circular DNA (cccDNA) [6-8]. Individuals having recovered from acute HBV infection can reach levels of hepatitis B surface antibodies (anti-HBs) providing lifelong immunity. Fulminant hepatitis can occur following acute hepatitis B, although it is uncommon (0.1 – 0.5%). Fulminant hepatitis can be due to a heightened immune response, causing massive lysis of infected hepatocytes [9].

In the early stage of an acute infection, viremia increases with a doubling time of 2.0 to 2.7 days [10] and is generally detectable in the blood within 15 to 20 days after infection [11]. The hepatitis B surface antigen (HBsAg) increases in parallel and is detectable in the blood after four to 10 weeks [12,13]. Shortly afterwards, hepatitis B core antibodies (anti-HBc) appear, IgM anti-HBc first and IgG anti-HBc later.

Chronic hepatitis B

Chronic HBV infection is implied by the persistence of viral markers such as HBsAg, hepatitis B e antigen (HBeAg), or detectable HBV DNA for a period of more than six months [6,14]. Chronic HBV infection can be divided into five phases, which are not necessarily sequential (Table 1):

HBeAg-positive chronic HBV infection: usually asymptomatic. This phase is characterised by the presence of serum HBsAg, very high levels of HBV DNA and alanine transaminase (ALT) levels in the normal range [12,15]. This phase can last several years in children infected perinatally and is thought to usually cease during the second or third decade of life [16-18].

HBeAg-positive chronic hepatitis B: characterised by high levels of HBV DNA, positive HBeAg, and elevated ALT, with symptoms during this phase ranging from asymptomatic to liver failure with jaundice. During this phase, immune-mediated liver damage will occur and can be identified through necroinflammation on biopsy and varying degrees of fibrosis. Spontaneous flares representing an intensification of the immune response are a typical feature of this phase. Most flares are asymptomatic, but some can be associated with symptoms of acute hepatitis and can result, although rarely, in cirrhosis and death. This phase can last several years [19].

HBeAg-negative chronic HBV infection: usually an asymptomatic phase. During this phase, HBeAg becomes undetectable and antibodies to HBeAg (anti-HBe) appear, with low (<2 000 IU/mL; <10 600 copies/mL, considering a conversion factor of 5.3 copies per IU) or undetectable levels of HBV DNA, as well as normalisation of liver transaminase levels, indicating clinical remission [12,20]. Individuals in this phase have a low risk of progression to cirrhosis, but progression to HBeAg-negative chronic hepatitis B (CHB) is still possible for a minority of patients. During this phase, HBsAg loss can occur spontaneously [15].

HBeAg-negative chronic hepatitis B: symptoms during this phase range from asymptomatic to liver failure with jaundice. This stage is characterised by active liver disease, loss of HBeAg and persistent or fluctuating moderate to high levels of HBV DNA [12,15]. In this phase, liver damage is evidenced by necroinflammation of the liver and fibrosis and is due to the patient's immune response. Patients in this phase have a very low rate of spontaneous disease remission [15].

HBsAg-negative phase or 'occult HBV infection' phase: this phase is characterised by negative HBsAg and usually positive anti-HBc; there can also be detectable anti-HBs in low concentrations. Patients with an occult HBV infection (OBI) usually have low or undetectable serum HBV DNA (DNA levels below the detection limit), but covalently closed circular DNA (cccDNA) can be detected in the liver [15,21]. Anti-HBc is generally detectable in this phase, although rare cases suggest that it may not always be present [22].

Chronic HBV infection can progress to liver cirrhosis in up to 40% of untreated patients [14]. Globally, HBV accounts for 56% of hepatocellular carcinoma cases, but there is considerable regional variation in this proportion [23].

Table 1. Chronic hepatitis B virus infection markers by disease phase

Marker	HBeAg-positive chronic HBV infection	HBeAg-positive chronic hepatitis B	HBeAg-negative chronic HBV infection	HBeAg-negative chronic hepatitis B	HBsAg-negative phase (OBI)
HBsAg	+ (high)	+	+ (low)	+	-
Anti-HBs	-	-	-	-	+/-
HBeAg	+	+	-	-	-
Anti-HBe	-	-/+	+	+	-
Anti-HBc	+	+	+	+	+
HBV DNA	>10^7 IU/mL	10^4-10^7 IU/mL	<2 000 IU/mL	≥2 000 IU/mL	Weak or undetectable

DNA: deoxyribonucleic acid; HBc(Ag): HBV core (antigen); HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; OBI: Occult HBV infection.
Adapted from EASL 2025 [24].

Treatment for hepatitis B

For patients with acute hepatitis B, the goal of treatment is a reduction in the risk of acute or subacute liver failure. Most acutely infected adults will recover spontaneously and do not require treatment. Patients with severe or fulminant acute hepatitis B can benefit from treatment with nucleoside/nucleotide analogues. In case there is no recovery following the treatment, patients with fulminant hepatitis B should be considered for liver transplantation [15].

The management of CHB is complex and aims to improve survival and quality of life by preventing the disease progression to cirrhosis and hepatocellular carcinoma. The two main treatment options for CHB are nucleoside/nucleotide analogues or pegylated interferon alfa. The long-term suppression of HBV DNA is the main endpoint for all treatments, and the normalisation of transaminase levels is considered an additional endpoint. The cure for CHB, i.e. eliminating all replicative forms, including cccDNA and thus reducing the need for lifelong treatment, is not achievable with the currently available treatments.

Infected patients undergoing immunosuppressive therapies are evaluated for the risk of HBV reactivation. The risk depends on the serological status of the patient and the potency of immunosuppressants. Patients at risk for reactivation include those with a resolved infection, those with inactive CHB (detectable HBsAg, undetectable HBV DNA), and those with untreated chronic active hepatitis B (detectable HBsAg and HBV DNA). Prophylaxis is recommended in patients with a higher risk of HBV reactivation due to immunosuppressive therapies. Similarly, prophylaxis is also recommended for women identified as having chronic HBV infection during pregnancy to prevent mother-to-child transmission [15].

The risk of HBV infection through SoHO

Transmission of HBV through SoHO has previously been reported for blood and blood components, cells (except for oocytes), and several tissues [25-35]. The 50% minimum infectious dose, defined as the dose that infects 50% of recipients, is very low and was estimated to be between three to eight HBV DNA copies [36,37]. Considering the low infectious dose and the viral dynamic progression of the infection, transmission of HBV can occur rapidly after exposure. Transmission of HBV can also occur if a donor is in the OBI phase, although the likelihood of transmission from donations during this phase is estimated to be substantially lower than during the initial phase of infection [36]. Recent findings suggest that transmission of HBV from donors, either in the initial phase of the infection or in the OBI phase, is possible with viral load levels below the limits of detection of currently available molecular tests [37,38].

Despite effective donor screening processes in EU/EEA countries and the highly sensitive tests available, there have been reports of 12 HBV transmission events through SoHO in the period from 2017 to 2022 in the serious adverse reactions and events reports published by the European Commission [39,40]. Nine of these events were reported following transfusion of blood and blood components, and three following tissues and cells transplantation. It should be noted that these cases might have occurred prior to the 2017–2022 period when they were reported.

Screening processes for HBV include a combination of thorough donor assessment, considering risks of recent exposure to HBV, and a combination of laboratory testing methods. Laboratory test methods used to screen HBV

in SoHO donors include serological tests detecting the HBsAg and anti-HBc antibodies, as well as HBV DNA NAT. When anti-HBc is used in screening, detection of anti-HBs is used to support the interpretation of these test results for the safe release of donations, as well as for donor management.

During the initial pre-HBsAg window period, i.e. the minimal time from infection to a positive test result, several reports have shown that HBsAg-negative donors can transmit HBV. Transmission events and look-back studies indicate the lack of a clear relationship between infectivity and viral load. The immune status of the recipients largely affects this relationship. Infectivity is also directly related to the amount of plasma transfused [41]. As a result, the viral load may differ only marginally between infectious and non-infectious blood [42]. An HBV DNA NAT with a 95% limit of detection (95% LOD) of 18.6 international units (IU) per mL (98.5 copies per mL, considering a conversion factor of 5.3 copies per IU) is considered to be associated with a window period of 15 days and a 95% LOD of 8.8 IU/mL (46.9 copies/mL) with a window period around 12 days. The initial window period for tests detecting the HBsAg is estimated to be around 30 days on average [28] but could reach up to 60 days [43].

After loss of HBsAg, donors with negative HBsAg and positive anti-HBs, especially in low concentrations (below 100 IU/L), may still transmit HBV, although with a lower infectivity than during the pre-HBsAg phase. Through mathematical modelling, the 50% minimum HBV infectious dose after HBsAg loss was estimated at 316 HBV DNA copies. This model estimated that 3.3% of OBI donations would remain undetected by HBV DNA NAT with a 95% LOD of 43.1 copies/mL and could lead to transmission through transfusion of a blood component containing 20mL of plasma. This proportion was estimated at 14.0% for a volume of 200 mL of plasma [44]. Strong evidence suggests that the infectivity of OBI donations is significantly reduced for donors with high anti-HBs titres [28,38].

The overall notification rate for acute hepatitis B across the EU/EEA declined continuously from 0.7 in 2013 to 0.4 per 100 000 population in 2019. Notification data can be viewed as a proxy for the incidence of infections. The decline mirrored global trends and is considered to reflect the decrease in transmission due to the impact of vaccination. In 2020, the lowest notification rate since 2013 was observed: 0.3 cases per 100 000 individuals, a decrease likely related to the disruption of healthcare and prevention services and behavioural changes due to the COVID-19 pandemic. After 2021, the number of notifications of acute hepatitis B has increased to pre-pandemic levels [45]. This increase might be associated with the end of national and international restrictions due to the pandemic, the reinstitution of regular contact with healthcare, higher migrant inflow in some countries and changes in surveillance and testing, as well as possible increases in transmission. Variations in reporting practices, testing strategies, and underlying local epidemics may explain the differences in notification rates between countries [46]. Overall, an estimated 3.6 million people were living with chronic hepatitis B in the EU/EEA in 2015 [47,48], a large proportion of whom were undiagnosed.

The overall prevalence of HBV and the varying proportion of people with chronic HBV infection who remain undiagnosed in the EU/EEA warrant a strategic approach to prevent transmission of HBV from donors via SoHO. The standards for the prevention of HBV transmission through SoHO in the EU/EEA were established in Directive 2002/98/EC for blood and blood components and Directive 2006/17/EC for tissues and cells [49-51]. Following the application of the SoHO regulation and the repeal of these directives, and considering the potentially life-long consequences of an HBV infection, the severity of the disease, and its sustained incidence in EU/EEA countries, guidelines addressing the prevention of transmission of HBV through SoHO remain relevant for SoHO safety in the EU/EEA.

General requirements and recommendations applicable to all SoHO

These guidelines follow the definitions of SoHO donors described in the Regulation, referred to as 'donors' in these guidelines. For these guidelines, donors are defined as individuals presenting for donation, irrespective of whether that donation was successful or not. Donations should be understood as distinct procurement or collection events. Multiple units of SoHO collected at a single time point are considered a single donation for the purpose of these guidelines.

Donations from donors who do not meet the requirements outlined in these guidelines can be considered for human application, subject to a positive risk-benefit assessment, which should be justified and traceable [1]. Prior to application, specific informed consent should be obtained from the recipient or the recipient's legal representative when necessary. An appropriate follow-up procedure for the recipient should be considered, and adverse outcomes (e.g. transmission) of using such SoHO should be reported through a dedicated national system (e.g. haemovigilance and biovigilance systems) [3,4].

Testing should be performed on the type of specimen required in the manufacturer's instructions for use.

In these guidelines, the term 'screening test' refers to tests used for 'the detection of the presence of, or exposure to, a transmissible agent in blood, blood components, cells, tissues or organs, or in any of their derivatives, in order to assess their suitability for transfusion, transplantation or cell administration' in accordance with the classification of class D devices in the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) [52]. The results from screening tests used to detect HBV in SoHO donors are considered to be reactive or negative. Serological screening tests are typically designed to have a low threshold for detecting possible cases, which carries a risk of false-reactive results. ECDC advises retesting initially reactive samples from serological tests in duplicate using the same sample and the same assays to validate the result and minimise variability during testing. If the sample volume is too low for retesting in duplicate, another sample collected at the same time point as the reactive sample could be considered. If any of the repeat tests are reactive, the sample is considered repeatedly reactive. If both retests are negative, the sample is considered negative for the serological test. These guidelines assume that initially reactive serological screening tests are repeated in duplicates. Hence, the term 'reactive' when used in the context of serological tests can be understood as 'repeatedly reactive' results if these tests are repeated in duplicates. The retesting of samples in duplicates with the same assays only applies to serological tests. For NAT, a reactive result is defined as a reactive result at the individual sample level. This reactive result in an individual sample can be obtained either through the testing of samples individually or through individual testing performed during the resolution of a reactive pool. ECDC advises establishing algorithms at a national level to investigate and consistently resolve reactivity in the screening assay.

Testing of SoHO donors

Requirements and recommendations

Required:

- All tests should comply with class D devices in Regulation (EU) 2017/746 on in vitro diagnostic medical devices.
- All tests should be used according to the manufacturer's specifications and their defined intended purposes.
- Reactive screening test results should lead to further testing to confirm the screening test result.
- Confirmatory testing should be performed as soon as possible following reactive screening test results.
- The confirmatory testing should be performed by an authorised, licensed, or accredited laboratory according to national standards.
- Approaches to confirmation of screening test results should rely on nationally pre-established algorithms. These may differ according to the screening strategy in place.

Advice and practical considerations:

- Concordant reactive HBsAg serological tests and HBV DNA NAT would very likely exclude false reactive results and may be considered equivalent to positive confirmatory testing.
- In the case of a positive confirmatory test result, when possible, ECDC advises obtaining a further sample to reconfirm the test result and to confirm the identity of the donor.

Evidence and justification

Class D in vitro diagnostic medical devices cover general life-threatening conditions and, more specifically, transmissible agents in blood, blood components, cells and tissues intended to be transfused, transplanted or administered to the body [52]. Such transmissible agents can also present a high risk to the wider population.

It is essential to adhere to the manufacturer's specifications when using tests to ensure accuracy, reliability, regulatory compliance, safety, and reproducibility of results, and the use of tests should strictly comply with the conditions of use provided by manufacturers [52]. Deviating from these specifications can compromise the quality of testing and the validity of results and constitute misuse of the device.

In the case of reactive screening test(s), confirmatory testing for the presence of markers of HBV infection in the donor should be performed. For these guidelines, the term 'reactive' is used for screening tests and 'positive' is used for confirmatory test results. Confirmation of the markers of HBV infection is essential to guide appropriate actions to ensure the safety of the SoHO supply as well as the donor's safety and appropriate referral to clinical care, and it should be performed by an authorised, licensed, or accredited laboratory according to national standards.

There are no approved tests to confirm a reactive NAT. However, additional tests (e.g. a different NAT assay) can be performed to verify a NAT that tested reactive in screening. For these tests, approaches to confirmation of screening test results should rely on nationally pre-established algorithms. For these guidelines, the term 'positive confirmatory test result' will include these additional test results verifying NAT reactivity. However, negative results obtained from these additional tests cannot rule out an infection in the donor with a reactive NAT in screening. The initial reactive NAT may reflect a sample with a very low viral load that is not detected by an additional NAT, and serological tests may also yield negative results if seroconversion has not yet occurred (Pathogen data sheet, Section 2). In rare cases, initial NAT reactivity can be due to amplification anomalies or laboratory errors [53]. In these situations, a pre-established algorithm can confirm inaccurate NAT results, and the sample could be considered negative for HBV. However, it is important to consider that the non-repeatability of an initial HBV DNA NAT reactivity may represent OBI, including in low-prevalence settings [54].

Confirmation of HBV infection following HBsAg reactivity is usually done with specific HBsAg neutralisation. At the time of the drafting of these guidelines, there were no approved confirmatory tests for anti-HBc and confirmation of isolated anti-HBc reactivity should instead be based on a pre-established algorithm. Examples of confirmation algorithms include testing for additional HBV markers, repeat testing with one or two different assays in the same sample, or screening for anti-HBc in another sample. No additional testing performed following a reactive anti-HBc result could also be considered as a pre-established confirmation algorithm, where the initial result is accepted. In these guidelines, the term 'confirmed results for reactive anti-HBc' is meant to describe reactive anti-HBc results that have been confirmed using such a pre-established algorithm. In these guidelines, anti-HBc should be understood as 'anti-HBc (total)' and refers to tests detecting IgG and IgM classes of antibodies.

Considering the overall high performance of HBV screening tests and based on expert opinion, concordant reactive results from both the HBsAg serological tests and HBV DNA NAT on the same sample could be considered equivalent to a confirmed positive test result.

Based on good practice principles, in the case of a positive confirmatory test result, if possible, ECDC advises calling back the individual for a second sample and further confirmatory testing to reconfirm the test result and confirm the identity of the donor.

Requirements and recommendations: blood and blood components

Testing of blood donors for HBV

Requirements and recommendations

All blood and blood components

Testing requirements

Required:

- All donors, at each donation, should be tested for HBV.

Screening tests

Required:

- Donors should be tested considering one of the following strategies:
  - An HBV DNA NAT and a serological test detecting HBsAg and a serological test detecting anti-HBc antibodies;

    or
  - A highly sensitive HBV DNA NAT and a serological test detecting HBsAg.

Advice and practical considerations:

- ECDC advises basing the LOD for HBV DNA NAT on a documented risk assessment considering the estimated residual risk. An update of the risk assessment and the residual risk could be performed in case of significant changes to the epidemiology of the disease or a transmission event from donor to recipient.
- A 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT.

Outcome of test results

Required:

- If the results of the required screening tests are negative, the donation can be released for clinical use.
- In case of confirmed results for reactive anti-HBc, with negative HBsAg and negative HBV DNA NAT: the donor should be re-tested with a highly sensitive HBV DNA NAT, unless already performed.
- Donations from donors with a reactive HBsAg serological test and/or HBV DNA NAT should not be released for clinical use.
- In case of reactive HBV DNA NAT or in case of a positive confirmatory test result following reactive HBsAg and which cannot be attributed to a recent vaccination against HBV:
  - The donor should be notified and referred to relevant clinical care.
  - The donor should be deferred until criteria to re-enter donor screening procedures are met.
  - Look-back procedures of previous, potentially infectious donations should be initiated.
- In the case of a negative HBV DNA NAT and where the HBsAg serological test is reactive in screening and followed by an indeterminate confirmatory test result:
  - The decision to notify and refer the donor to clinical care should be based on the likelihood of an infection based on available information, including other available test results obtained during screening and confirmation procedures.
  - The decision to initiate look-back procedures of previous, potentially infectious donations should be based on a risk assessment considering available information, including other available test results obtained during screening and confirmation procedures.
- In case of confirmed results for reactive anti-HBc (including on prior donations), with a negative highly sensitive HBV DNA NAT and negative HBsAg:
  - The donation should be tested with a serological test detecting anti-HBs.
  - If the anti-HBs concentration is below 100 IU/L, the donation should not be released for clinical use. If the anti-HBs concentrations is equal or above 100 IU/L, the donation can be released for clinical use.

Advice and practical considerations:

- In case of confirmed results for reactive anti-HBc, with a negative highly sensitive HBV DNA NAT and negative HBsAg, the following steps could be considered:
  - For first-time donors or for seroconverting repeat donors (i.e. donors anti-HBc negative at previous donation):
    - ECDC advises not releasing the donation for clinical use, even with anti-HBs concentrations above 100 IU/L.
    - ECDC advises deferring the donor for a period of 26 weeks.
    - ECDC advises notifying and referring these donors to clinical care based on available information, including other available test results obtained during screening and confirmation procedures.
    - ECDC advises testing all future donations from these donors for anti-HBs, HBsAg, and with a highly sensitive HBV DNA NAT.
  - For seroconverting repeat donors, ECDC advises initiating look-back procedures for previous, potentially infectious donations following a risk assessment. This assessment could consider available information, including other available test results obtained during screening and confirmation procedures.
- In the case where only the serological test detecting HBsAg is reactive in screening and followed by an indeterminate confirmatory test result, ECDC advises calling back the donor for an additional test on a follow-up sample.

Criteria to re-enter donor screening procedures

Required:

- In case of a negative highly sensitive HBV DNA NAT in screening and a negative confirmatory test result following HBsAg reactivity, the donor can re-enter donor screening procedures without a deferral period.
- In case of a negative highly sensitive HBV DNA NAT in screening and a reactive HBsAg serological test result, which can be attributed to a recent hepatitis B vaccination, the donor can re-enter donor screening procedures without a deferral period if no risks of exposure to HBV have been identified. A period of two weeks from vaccination could be considered to avoid further HBsAg reactivity.
- In case of a negative highly sensitive HBV DNA NAT in screening and an indeterminate confirmatory test result following HBsAg reactivity: the donor can re-enter donor screening procedures, but should not re-enter before a minimum period of eight weeks from the last donor testing, to ensure coverage of the possible HBsAg window period.
- In case of a reactive HBV DNA NAT or in case of a positive confirmatory test result for HBV infection, donors can re-enter screening procedures upon documentation of HBsAg loss and undetectable HBV DNA during clinical follow-up, as well as documentation of anti-HBs concentrations demonstrating immunity.

Advice and practical considerations:

- For donors re-entering screening procedures after a deferral period due to a positive confirmatory test result, reactive HBV DNA NAT, or confirmed test results following reactive anti-HBc, ECDC advises testing all future donations of these donors for anti-HBs, HBsAg, and with a highly sensitive HBV DNA NAT.

Look-back procedure

Required:

- The extent of the look-back procedure should be based on a risk assessment to determine which previous donations are at risk of transmitting HBV. The risk of OBI on previously negative donations should be considered in this assessment. Anti-HBs concentrations can be helpful to assess the risk of OBI in previously negative donations.
- A residual sample of the last negative donation should be retested using a highly sensitive HBV DNA NAT. If the last negative donation cannot be retested, the donation before should be tested. Exemption from retesting previous donations can be considered if the previous donation was tested with a highly sensitive HBV DNA NAT and was negative. If an OBI is likely considering available information, all available donations should be retested for HBV.
- If the last donation is positive in the retest, the retesting of archived samples of previous donations should be performed sequentially until a donation is negative. No further testing of prior archived samples is required if the previous sample is negative in the retest and OBI is unlikely. Recipients who received a donation found positive in the retesting of archived samples should be tested for HBV infection.
- In case of a sample with a positive confirmatory test result, and if no archived samples are available for look-back procedures, the institution that performed the transfusion should test the recipients of the previous potentially infectious donation. The results should be reported to the entity where the donation was performed.

Advice and practical considerations:

- A 95% LOD of 20 IU/mL or lower could be considered for the retesting of the residual sample of the last donation.
- If archived samples test negative upon retesting, additional measures, such as testing recipients who received a potentially infectious donation, could be taken following a risk assessment considering other relevant information.

Evidence and justification

Testing requirements

Considering the documented severity of the disease and the significant consequences on the recipient in case of HBV transmission, members of the expert panel agreed that all donors of blood and blood components should be tested at each donation for HBV infection to reduce the risk of transmission through SoHO (Pathogen data sheet, Section 2).

Screening tests

The expert panel agreed on the benefits of the use of HBV DNA NAT to increase the safety of blood transfusion, in particular as a means to reduce the window period of the acute infection, relative to testing for HBsAg. HBV DNA is also considered a consistent marker for chronic hepatitis B, including after the loss of HBsAg. This opinion is supported by evidence, as the window period in the initial phase of the infection is reduced by a period ranging between 7 to 15 days when using an HBV DNA NAT with a 95% LOD below 20 IU/mL compared to the detection of HBsAg (Pathogen data sheet, Sections 2 and 4). Individuals affected by chronic hepatitis B may also exhibit transient loss of HBsAg but remain infectious (Pathogen data sheet, Sections 2 and 7). While cases of transmission from donors with undetectable HBV DNA, including with highly sensitive HBV DNA NAT, have been reported, these are considered to be rare and less likely after the loss of HBsAg (Pathogen data sheet, Sections 2 and 7).

Similarly, members of the expert panel agreed on the value of using serological tests detecting HBsAg in the HBV screening strategy, due to the possibility of having positive HBsAg with negative HBV DNA NAT, for example, in individuals with HBeAg-negative chronic HBV infection or if the individual is under antiviral treatment (Pathogen data sheet, Section 2).

There was no general agreement among the expert panel members on the use of serological tests detecting anti-HBc in the screening strategy. Due to important variations in the prevalence of anti-HBc in the general and donor populations between EU/EEA countries and regions within the countries (Pathogen data sheet, Section 3), some members of the expert panel expressed concern that the inclusion of these tests in the screening strategy would lead to unsustainable donor loss in EU/EEA countries with high anti-HBc prevalence, even when considering anti-HBs concentrations in the donor eligibility assessment. Conversely, some expert panel members considered that the inclusion of serological tests detecting anti-HBc would increase the safety of blood transfusion, as some transmission cases from donors with undetectable HBsAg and HBV DNA could potentially have been prevented by detecting anti-HBc (Pathogen data sheet, Sections 2 and 7).

In the EU/EEA overall, while the number of notified acute cases is small, reflecting low levels of transmission of infection, there remains a high prevalence of chronic infections across the region, a large proportion of which are undiagnosed (Pathogen data sheet, Section 3). In this context, the risk of HBV transmission through blood transfusion arises not only from failure to detect the HBV infection during the initial infection phase, but also during chronic hepatitis B, in particular in OBI, where HBsAg and HBV DNA may remain undetected in infectious donors (Pathogen data sheet, Sections 2 and 7).

For acute hepatitis B, the risk of not detecting an HBV infection during the initial infection phase is associated with the window period of HBV DNA NAT and HBsAg serology tests. In chronic hepatitis B, in particular during the OBI phase, the risk of not detecting an HBV infection in a donor is associated with loss of HBsAg and transient undetectable HBV DNA. However, some of these cases could be identified by testing for anti-HBc (Pathogen data sheet, Sections 2 and 7), or using highly sensitive HBV DNA NAT. Transfusion-transmitted HBV infection cases from donors with OBI reported in the literature are often associated with undetectable or very low viral loads, supporting an infectious dose that is likely below the LOD of available tests, in certain conditions. A mathematical model relying on look-back studies following transfusion-transmission of HBV suggested that 3.3% of OBI donations would remain undetected by HBV DNA NAT with a 95% LOD of 8.1 IU/mL (43.1 copies/mL) and could lead to transmission through transfusion of a blood component containing 20mL of plasma (Pathogen data sheet, Section 2). In reported cases of transmission from donors with OBI (i.e. anti-HBc positive and HBsAg negative) with detectable HBV DNA, the majority of cases had a viral load above 20 IU/mL (Pathogen data sheet, Section 7).

Considering the use of anti-HBc in screening to identify possible OBI, and as there remains a high prevalence of undiagnosed chronic infections in the region, a minimum LOD threshold for HBV DNA NAT is not required if an anti-HBc test is used in screening. Based on expert opinion, ECDC advises basing the LOD on a risk assessment

considering the estimated residual risk for HBV. The risk assessment could be documented to ensure transparency in case of exchange of blood components to other regions or countries. ECDC advises updating the risk assessment and/or the estimated residual risk in case of significant changes to the HBV epidemiology (as defined by the NCA) or in case of an HBV transmission event from donor to recipient. The risk assessment should be documented to ensure transparency in case of the exchange of blood components to other regions or countries.

Incident HBV infection in repeat donors occurs infrequently in low-prevalence countries (Pathogen data sheet, Section 3; [55,56]). For repeat donors seroconverting for anti-HBc, the time between the donation with a negative result for anti-HBc and the donation with a reactive result remains uncertain and can be below six months in a substantial proportion of seroconverting donors (unpublished data communicated by the expert panel). A period upon which repeat donors should be retested for anti-HBc could not be defined with the panel or based on available evidence: retesting after the identification of new risks is prone to error and may miss risks that occurred at distance from the donation; anti-HBc testing may help identify an infection that was missed at the prior donation due to the window period; acute OBI (primary occult infection without detectable HBsAg) may occur in repeat donors [57]; and retesting after a fixed period of time (e.g. six months after last donation) or testing lapsed donors may pose logistic challenges. Hepatitis B virus DNA levels can fluctuate below detection levels in chronic infections. Therefore, if a highly sensitive HBV DNA NAT is not used for the screening of donors, testing for anti-HBc at each donation was considered a relevant safety measure for the prevention of transfusion-transmission of HBV.

If anti-HBc screening tests are not used, a highly sensitive HBV DNA NAT should be used to mitigate the risk of transmission from donors with OBI. Based on reported transmission cases with detectable HBV DNA, a 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT. It is important to note that although anti-HBc screening can reduce the risk of transmission from donors with OBI, a high 95% LOD for HBV DNA NAT may lead to longer infectious window periods in donors with acute infections. Associated with a long doubling time of the virus (2.0 – 2.7 days; Pathogen data sheet, Section 2), the window period of HBV DNA NAT is estimated at around 15 days for a NAT with a 95% LOD below 20 IU/mL. This window period is expected to increase for tests with lower sensitivity (i.e. higher LOD). Studies evaluating the reduction of the window period of HBV DNA NAT and using 95% LOD of <20 IU/mL compared to HBsAg report a reduction of the window period between 7-24 days (Pathogen data sheet, Section 4).

It is important to note that when it is acceptable in terms of resources and potential donor loss, anti-HBc screening provides an additional layer of safety to highly sensitive HBV DNA NAT in preventing transfusion-transmission of HBV associated with OBI (Pathogen data sheet, Section 7; [22,38]). However, inclusion of anti-HBc may not be acceptable in all settings due to the potential loss of donors with a history of HBV infection, even when considering anti-HBs results, as well as the exclusion of donors with false-reactive anti-HBc results, as there are currently no approved confirmatory tests for anti-HBc. The choice of the screening strategy for HBV could be based on the estimation of the residual risk of HBV transmission in the country.

Outcome of test results

Given the high performance of HBV screening tests, reactive screening tests for HBsAg and/or HBV DNA NAT indicate a high likelihood that the donor has an HBV infection, with a high risk for HBV transmission to the recipient if the donation is used for transfusion (Pathogen data sheet, Section 4). While false-reactive results of serological screening tests are possible, members of the expert panel agreed that donations from donors with reactive screening tests for HBsAg and/or HBV DNA NAT should not be released for clinical use due to the severe impact of an HBV transmission on the recipient.

A positive confirmatory test result following reactive HBsAg or HBV DNA NAT, or an indeterminate confirmatory test result and where an HBV infection is considered likely, considering other available information, should lead to notification and referral of the donor for appropriate clinical care. As acute and, in rare cases, chronic [58], HBV infections may resolve, and the donor may clear HBV DNA and lose HBsAg (Pathogen data sheet, Section 2), re-entry of donors with such a history of resolved HBV infection could be considered under specific circumstances.

In case of isolated positive anti-HBc test results, with a highly sensitive HBV DNA NAT negative result, and with anti-HBs concentrations below 100 IU/L, a deferral period of 26 weeks could be considered to mitigate the risk of transmission. Anti-HBc screening tests can have nontrivial false reactive rates, as high as 20% in low-prevalence populations [59], and reactive anti-HBc in the absence of other reactive markers may be indicative of false reactivity. Twenty-six weeks would cover both HBsAg and anti-HBc seroconversion and facilitate the interpretation of subsequent test results of the donor. Alternatively, this profile could indicate a past infection with waning of anti-HBs or an OBI. There was a general agreement of the expert panel that temporarily deferring these donors would allow SoHO professionals to eventually identify a false reactive result if the donor is anti-HBc negative in screening after re-entry.

For first-time donors and repeat donors previously anti-HBc negative (i.e. donors that have seroconverted), a deferral period of 26 weeks could also be considered, even with anti-HBs concentrations above 100 IU/L. This profile could indicate a past infection; however, among first-time donors, the recency of the past infection is

frequently unknown; for repeat donors previously anti-HBc negative, assessing recency of the infection may be challenging depending on the time that has elapsed since the last donation. According to expert opinion, temporarily deferring such donors could increase safety by confirming this serological profile over a well-defined period. However, these donors could be accepted based on a risk assessment taking into account the incidence of the disease in the donor population. ECDC advises testing donors that had a prior positive anti-HBc with a highly sensitive HBV DNA NAT and anti-HBs for all following donations due to the risk of OBI.

In the case of anti-HBc reactivity in screening, further tests should be performed to confirm the infectious status of the donor, as isolated anti-HBc could indicate a resolved HBV infection. In the case of isolated positive anti-HBc, a highly sensitive HBV DNA NAT should be used, if not used in the initial screening phase, to identify a potential OBI with a low viral load. In addition to the highly sensitive HBV DNA NAT, donors should be tested for anti-HBs. Reported HBV transfusion-transmitted cases from donors with negative HBsAg, negative HBV DNA NAT, and isolated anti-HBc also had low anti-HBs concentrations below 100 IU/L(Pathogen data sheet, Section 7). This HBV marker profile is suggestive of OBI, and, due to the very low infectious dose of HBV, donations from donors with these profiles should not be released for clinical use.

Donors with a resolved HBV infection and repeat donors who were anti-HBc positive at a prior donation, with negative screening test results for HBsAg and highly sensitive HBV DNA NAT, in association with anti-HBs concentrations above 100 IU/L, could be considered eligible to donate. This serological profile corresponds to a past resolved infection and, with anti-HBs concentrations above 100 IU/L, the likelihood of HBV transmission is very low (Pathogen data sheet, Section 7).

Due to the risk of transmission of HBV through blood and blood components (Pathogen data sheet, Section 7), based on good practice and considering expert opinion, in case of a positive confirmatory test result or where both the serological test detecting HBsAg and HBV DNA NAT are reactive in screening in a repeat donor, look-back procedures to identify previous potentially infectious donations should be performed to identify and follow up on recipients who may be at risk of infection. In other cases where HBV infection cannot be definitively ruled out, such as when test results are indeterminate, the decision to initiate look-back procedures should be based on a risk assessment, taking into account other available information, including other test results.

The extent of the look-back procedure should be guided by a risk assessment considering other available test results and relevant information to identify which previous donations may pose a risk of transmitting HBV, and therefore which samples and recipients require testing. The possibility of OBI should be considered in this assessment. If an OBI is considered likely, all available samples from previous donations should be retested for HBV, as it may not be possible to identify when the donor was infected [26]. Although samples from previous donations were found negative in screening, it is possible the donation was made at a moment prior to the appearance of HBsAg, or during its transient disappearance, and with a viral load below the LOD defined for HBV DNA NAT in routine screening. The use of a highly sensitive HBV DNA NAT for look-back procedures would support the identification of low viral loads. In addition, re-testing of negative donations with low viral loads using NAT increases the likelihood of detecting the virus and re-testing previous donations could be considered even if the previous donations were already tested with a highly sensitive HBV DNA NAT.

To ensure the safety of recipients, based on expert opinion, recipients who received a donation found positive in re-testing should be tested for HBV infection. Testing recipients who received a donation negative in retesting could also be considered following a risk assessment and considering other relevant information, since the negative result could reflect a very low viral load. If no archived samples are available for testing in the context of look-back procedures, according to expert opinion, the recipient(s) of the previous potentially infectious donation should be tested for an HBV infection. As a single individual may have previously donated other SoHO, where possible look-back procedures could also consider donations of other SoHO types.

Criteria to re-enter donor screening procedures

A negative confirmatory test following a reactive serological test detecting HBsAg and a negative HBV DNA NAT in screening is very likely indicative of a false-reactive result for the serological test. Therefore, if the confirmatory test following HBsAg reactivity is negative, and if the HBV DNA NAT is negative in screening, the donor is not considered to have an HBV infection, and the donor can be allowed to re-enter donor screening procedures without a deferral period.

Vaccination for HBV can lead to positive results for HBsAg during a short period after vaccination (Pathogen data sheet, Section 4). In the case of a positive HBsAg result and a negative HBV DNA NAT in screening, and where vaccination is the likely cause of the positive result, considering available information, including risks of exposure to HBV and other available test results, the donor can re-enter screening procedures without a deferral period. As vaccination may be considered for individuals at risk of HBV infection, risks of exposure to HBV should be evaluated prior to re-entry.

Further testing is advised in case of an indeterminate confirmatory test result, where only the serological test detecting HBsAg is reactive in screening. For these donors, a minimum period of eight weeks from the last donor testing before re-entry should be followed to cover the possible HBsAg window period (Pathogen data sheet, Section 2).

According to ECDC's opinion, HBV infection is highly likely in donors where both the serological test detecting HBsAg and HBV DNA NAT are reactive, regardless of the confirmatory test result. The concordance of the screening tests significantly reduces the likelihood of a false-reactive screening result. These donors should be treated as donors with a positive confirmatory test result.

Due to the risk of HBV transmission (Pathogen data sheet, Section 2), re-entry following a positive confirmatory test result or where an infection cannot be ruled out taking into account all information available (including where both HBV DNA NAT and the serological screening test detecting HBsAg are reactive), should only be considered for donors with a documented loss of HBsAg and clearance of HBV DNA in clinical follow-up, as well as concentration levels of anti-HBs demonstrating immunity. An anti-HBs concentration of 10 IU/L or above is generally considered to provide immunity to HBV after vaccination [60]. However, to ensure the safety of future donations and avoid unnecessary discarding of donations, a concentration of 100 IU/L or above could be considered for the re-entry of these donors. After re-entry, ECDC advises testing all future donations of these donors for anti-HBs, HBsAg, and with a highly sensitive HBV DNA NAT.

Limitations

The evidence is based on studies evaluating individual test performance and not the comparison of a combination of test methods. The infectious dose for HBV DNA is uncertain but likely very low. The factors (e.g. anti-HBs concentrations) influencing the likelihood of transmission of HBV from a donor with a very low or undetectable viral load remain unclear. The expected safety benefit of including anti-HBc as mandatory testing in EU/EEA countries relative to the impact of such a requirement on supply cannot be established at the EU/EEA level. The relative effectiveness for the safety of blood supply of both proposed testing alternatives (i.e. including anti-HBc or not) is uncertain. However, available evidence supports the use of anti-HBc as an additional layer of safety to highly sensitive HBV DNA NAT in preventing transfusion-transmission of HBV associated with OBI.

Risk of exposure to HBV and testing limitations in case of recent exposure to be considered for each donation

Requirements and recommendations

Donors should receive accurate and understandable information about HBV transmission and risks of exposure to HBV, as well as the risks to recipients posed by communicable disease transmission through transfusion, as detailed in the latest version of the EDQM guide to the preparation, use, and quality assurance of blood components [3]. Donors should be given the opportunity to self-defer. ECDC advises recommending the donor seeks clinical advice and testing in case of recent risk of exposure to HBV before considering donation.

Injection of non-prescription drugs refers to the use of any drug or other substance not prescribed by a registered healthcare professional and self-administered via injection. This includes both illicit drugs (e.g. heroin, methamphetamine) and performance enhancing drugs (e.g. testosterone) when injected and which can be associated with needle sharing.

The events listed below aim to represent evidence-based risks of exposure to HBV and are not intended to be the exact questions asked or the physical examination performed during the donor assessment. They are provided to support entities in developing their donor eligibility assessment strategies.

All blood and blood components

Risk of exposure to HBV

Required:

- All donors, at each donation, should be assessed for recent risks of exposure to HBV when assessed for donor eligibility.

Advice and practical considerations:

The following events are considered risks of exposure to HBV. ECDC advises considering the occurrence of these events in the previous 16 weeks when assessing donor eligibility:

- Active sexually transmitted infection (STI);
- Close household contact with an individual with acute or chronic HBV infection;
- Recent healthcare encounter: receipt of a SoHO (including human organs), haemodialysis, intravenous fluid/contrast infusion;
- Needle sharing and/or injection of non-prescription drugs;
- Non-occupational needlestick injuries, tattoos/piercing in unhygienic conditions;
- Exposure to known or suspected HBV infection following an occupational incident (e.g. needlestick injury);
- Condomless* sex with an individual with an ongoing acute or chronic HBV infection;

- Condomless* sex with a partner who injects non-prescription drugs;
- Condomless* sex in exchange for money, drugs, or other payments.

* *Condomless should be understood as a situation where no condom was used or where one was used incorrectly (e.g. breaking, leaking, or slipping off during sexual activity).*

Testing limitations in case of recent risk of exposure to HBV

Required:

- Donors should not be tested in the context of donor evaluation before a period of at least 16 weeks since the last event with a risk of exposure to HBV listed above. In case of the use of a highly sensitive HBV DNA NAT, this period can be reduced to eight weeks since the last event.

Advice and practical considerations:

- Due to the possibility of HBsAg reactivity following an HBV vaccination, it advised to not test donors during a period of two weeks following a recent HBV vaccination.

Evidence and justification

Risk of exposure to HBV

Based on evidence and expert opinion, the above-described events include the major risks of exposure to HBV, relevant for SoHO safety, in EU/EEA countries (Pathogen data sheet, Section 3).

Some healthcare encounters may carry an increased risk of HBV infection according to literature reports [61,62] (Pathogen data sheet, Section 3); however, there was no overall agreement in the expert panel on the relevance of this event as an exposure risk for HBV in the EU/EEA. Inadequate infection prevention and control measures in the healthcare setting may increase the risk of exposure to HBV, although such situations are not considered frequent in the EU/EEA by the members of the expert panel. Specifically for blood donations, patients undergoing haemodialysis or who have received a SoHO transfusion or transplantation are unlikely to become donors in a time frame consistent with the risk of a window period transmission.

Similarly, the risk of exposure to HBV following new tattoos or piercings was considered very low by the expert panel since authorised facilities in the EU/EEA are considered to follow appropriate hygienic measures. However, the risk of infection may be increased for events performed in situations with suboptimal hygienic conditions. Considering the high likelihood of transmission of HBV and the long-term consequences of the disease, based on expert opinion, ECDC advises considering all the above risk events when assessing donor eligibility. These events may be unlikely for prospective blood donors or can be challenging to assess through interviews, particularly in the context of risk events concerning the partner, as well as the correct usage of condoms, as condom failure is not always recognised [63].

It is important to note that the most documented transmission mode of acute HBV infection in the EU/EEA is sexual (Pathogen data sheet, Section 3). In this perspective, condomless sex with new or multiple partners with an unknown HBV infection status in areas with high prevalence of HBV could be considered a risk of exposure to HBV. This risk is not listed in the advice and practical considerations above, as it could lead to unnecessary donor loss in low-prevalence countries; however, it could be considered in areas with high prevalence of HBV infection. Active STI are associated with an increased likelihood of carrying HBV (Pathogen data sheet, Section 3). In the available evidence, the specific infections associated with HBV are not comprehensively described, but include infections with *T. pallidum*, *Chlamydia trachomatis*, or *Neisseria gonorrhoeae*.

There was no general agreement in the expert panel on taking into account the vaccination status of the donor against HBV when assessing the recent risks of exposure. While complete HBV vaccination is effective and provides lifelong immunity, breakthrough infections can occur [64,65]. An accurate assessment of a complete HBV vaccination schedule (three doses) was also considered challenging and could lead to misclassification of partial vaccination profiles as fully vaccinated. In addition, the expert panel identified vaccination against HBV in individuals at risk of HBV infection as a potential safety concern. Hence, the inclusion of vaccination status in the criteria for the assessment of recent risks of exposure was not supported.

Testing limitations in case of risk of exposure to HBV

Given the high performance of the HBV screening tests required in these guidelines, the risk for transmission through SoHO is related to newly infected potential donors tested during the window period (Pathogen data sheet, Section 2). To avoid window period transmissions from newly infected donors, donors who have been at risk of exposure to HBV should not be tested until the test results are considered reliable.

Based on expert opinion, a multiplication factor of the window period should be considered to ensure reliability of the test results and reduce the risk of a window period transmission after a recent infection. The risks of exposure identified in this section do not address the risk of a transmission in the OBI phase.

A period of 16 weeks after an event with a risk of exposure to HBV covers more than three times the maximum window period of HBV DNA NAT and HBsAg, including when considering testing in pools (Pathogen data sheet, Sections 2 and 4). If a highly sensitive HBV DNA NAT is used, as HBsAg is unlikely to be present if the viraemia is undetectable (Pathogen data sheet, Sections 2 and 4), this period can be reduced to eight weeks.

Limitations

The events with increased risk of exposure to HBV are based on targeted literature searches and not on systematic literature reviews and so may not be comprehensive. The interval between potential exposure to HBV and testing to be considered to ensure reliability of the test results is based on an arbitrary multiplication factor of the window period; however, it is consistent with the deferral periods currently used in several EU/EEA countries in which transmissions of HBV are rare events (Pathogen data sheet, Section 7). It may not be possible to transpose these events/wording directly to the donor eligibility assessment and adaptations may be needed in particular to account for the acceptability and feasibility of including these questions and limit potential donor loss.

Donor eligibility is assessed for all pathogens at once, and the list of events in the current guidelines only covers HBV. The list of events with an increased risk of exposure to other pathogens will be completed by ECDC as new guidelines are published. Pending these updates, the EDQM guide to the preparation, use, and quality assurance of blood components can be used as a resource for additional events to consider in the assessment of donor eligibility [3].

Affected individuals and other situations to consider

Requirements and recommendations

All blood and blood components

Required:

- Individuals with a medical diagnosis of chronic HBV infection or receiving treatment for chronic HBV infection should not be considered eligible for donation and should be deferred until HBsAg loss, undetectable HBV DNA, and anti-HBs concentrations demonstrating immunity are documented in the context of clinical follow-up.

Advice and practical considerations:

- In case of uncertainty of a medical diagnosis of chronic HBV infection, the prospective donor could be tested for anti-HBc, as described in the section Testing of blood donors for HBV.
- Regarding individuals meeting the criteria to re-enter screening procedures after a medical diagnosis of chronic HBV infection, ECDC advises testing all future donations of these donors for anti-HBs, HBsAg, and with a highly sensitive HBV DNA NAT.

Evidence and justification

Individuals with a medical diagnosis of chronic HBV infection have a low likelihood of spontaneous remission. Chronic HBV infections may progress to an HBsAg-negative phase or 'occult HBV infection', where very low levels of viraemia of HBV DNA may not be identified through testing, but could nonetheless lead to transmission through transfusion (Pathogen data sheet, Sections 2 and 4). Treatment for chronic HBV infection is not curative, and while it could lead to undetectable levels of HBV DNA, a risk of transmission cannot be excluded. Based on evidence and expert opinion, considering the severity and long-term consequences of the disease, the route of transmission, and the low infectious dose of HBV, all individuals with a known diagnosis of chronic HBV infection or treatment for chronic HBV infection should be deferred to reduce the risk of transmission. Eligibility for donation should only be considered for individuals with a documented loss of HBsAg and clearance of HBV DNA in clinical follow-up, as well as concentration levels of anti-HBs demonstrating immunity. An anti-HBs concentration of 10 IU/L or above is generally considered to provide immunity to HBV after vaccination [60]. However, to ensure the safety of future donations and avoid unnecessary discarding of donations, a concentration of 100 IU/L or above could be considered for individuals re-entering screening procedures after a medical diagnosis of chronic HBV infection.

Limitations

The risk of transmission of HBV through transfusion from individuals with remission from chronic HBV infection is uncertain, and the eligibility of these individuals may be reconsidered when further evidence is available.

Requirements and recommendations: tissues and non-reproductive cells

Testing of tissues and non-reproductive cells donors for HBV

Reproductive tissues used for allogeneic purposes should be considered as tissues.

In the context of the evaluation of deceased children, the birth mother should be understood as the person who carried and gave birth to the child. The person breastfeeding the child should be understood as the person who feeds the child with their own breast milk, either directly or by milk expression.

For these guidelines, 'at donation' should be understood as close as possible to donation, and test results should be available before transplantation and the conditioning regimen of hematopoietic progenitor cells recipients. Sample collection and time of sampling should be in accordance with the latest EDQM guide to the quality and safety of tissues and cells for human application [4].

The potential impact of haemodilution and haemolysis on screening test results should be considered, according to the recommendations of the EDQM guide to the quality and safety of tissues and cells for human application [4].

Requirements and recommendations

Living donors

Testing requirements

Required:

- All donors, at each donation, should be tested for HBV.

Screening tests

Required:

- Donors should be tested considering one of the following strategies:
  - An HBV DNA NAT and a serological test detecting HBsAg and a serological test detecting anti-HBc antibodies; *or*
  - A highly sensitive HBV DNA NAT and a serological test detecting HBsAg.

Advice and practical considerations:

- ECDC advises basing the LOD for HBV DNA NAT on a documented risk assessment considering the endemicity of the disease. An update of the risk assessment could be performed in case of significant changes to the epidemiology of the disease or a transmission event from donor to recipient.
- A 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT.
- ECDC advises testing donors with a serological test detecting anti-HBc antibodies independently of the LOD for HBV DNA NAT for donations used in immunocompromised recipients.

Outcome of test results

Required:

- If the results of the required screening tests are negative, the donation can be released for clinical use.
- Donations from donors with a reactive HBsAg and/or HBV DNA NAT, in the absence of a negative confirmatory test result, should not be released for clinical use.
- In the case of a negative confirmatory test result, and provided a negative HBV DNA NAT in screening, the donation can be released for clinical use.
- In case of reactive HBV DNA NAT or in case of a positive confirmatory test result following reactive HBsAg, and which cannot be attributed to a recent vaccination against HBV:
  - The donor should be notified through any means available and referred to relevant clinical care.
  - The donor should be deferred until criteria for further donations are met.
  - A risk assessment should be performed to determine if any previous donations are at risk of transmitting HBV and if look-back procedures should be initiated.
- In the case of a negative HBV DNA NAT and where the HBsAg serological test is reactive in screening and followed by an indeterminate confirmatory test result:
  - The decision to notify and refer the donor to clinical care should be based on the likelihood of an infection based on available information, including other available test results obtained during screening and confirmation procedures.

  - A risk assessment should be performed to determine if any previous donations are at risk of transmitting HBV and if look-back procedures should be initiated.
- In case of confirmed results for reactive anti-HBc, with negative HBsAg and negative HBV DNA NAT:
  - The donor should be re-tested with a highly sensitive HBV DNA NAT, unless already performed.
  - If the highly sensitive HBV DNA NAT is negative, the donation can be released for clinical use.

Advice and practical considerations:

- A 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT.
- In case of confirmed results for reactive anti-HBc, with negative HBsAg and a negative highly sensitive HBV DNA NAT, ECDC advises considering additional test results (e.g. anti-HBs) to guide the decision to release the donation for clinical use in immunocompromised recipients.
- In the case where only the serological test detecting HBsAg is reactive in screening and followed by an indeterminate confirmatory test result, ECDC advises calling back the donor for an additional test on a follow-up sample.

Criteria for further donations

Required:

- In case of a negative highly sensitive HBV DNA NAT in screening and an indeterminate confirmatory test result following HBsAg reactivity: the donor can re-enter donor screening procedures but should not re-enter before a minimum period of eight weeks from the last donor testing, to ensure coverage of the possible HBsAg window period.
- In case of a reactive HBV DNA NAT or in case of a positive confirmatory test result for HBV infection, donors can re-enter screening procedures upon documentation of HBsAg loss and undetectable HBV DNA during clinical follow-up, as well as documentation of anti-HBs concentrations demonstrating immunity. All future donations should be tested for HBsAg and with a highly sensitive HBV DNA NAT.

Deceased donors

Testing requirements

Required:

- All donors, at donation, should be tested for HBV.

Screening tests

Required:

- Donors should be tested considering one of the following strategies:
  - An HBV DNA NAT and serological tests detecting the HBsAg and anti-HBc antibody; *or*
  - A highly sensitive HBV DNA NAT and a serological test detecting HBsAg.
- If the sample needs to be diluted prior to testing, the dilution factor should be documented.

Advice and practical considerations:

- ECDC advises basing the LOD for HBV DNA NAT on a documented risk assessment considering the endemicity of the disease.
- A 95% LOD of 20 IU/mL or lower could be considered as highly sensitive HBV DNA NAT.

Outcome of test results

Required:

- If the results of the required screening tests are negative, the donation can be released for clinical use.
- Donations from donors with a reactive HBsAg serological test and/or HBV DNA NAT, in the absence of a negative confirmatory test result, should not be released for clinical use.
- In the case of a negative confirmatory test result and provided a negative HBV DNA NAT in screening, the donation can be released for clinical use.
- In case of confirmed results for reactive anti-HBc, with negative HBsAg and negative HBV DNA NAT:
  - The donor should be re-tested with a highly sensitive HBV DNA NAT, unless already performed.
  - If the highly sensitive HBV DNA NAT is negative, the donation can be released for clinical use.
- In the absence of a negative confirmatory test result following reactive HBsAg or in case of reactive HBV DNA NAT screening test results: the transplant coordination team(s) should be informed. A risk assessment should be performed based on available information, including other available test results, to determine if other or previous donations are at risk of transmitting HBV and if look-back procedures should be initiated. Recipients who received potentially infectious donations should be tested for HBV.

Advice and practical considerations:

- A 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT.

Paediatric donors: specific requirements for neonates and young children

Children 12 months of age or less

Testing requirements and recommendations

Required:

- The birth mother should be tested with a highly sensitive HBV DNA NAT and a serological test detecting HBsAg.
- If it is not possible to test the mother, but an HBV infection can be ruled out through other means in the birth mother, the child aged two months or above can be tested with an HBV DNA NAT and with a serological test detecting HBsAg. Recent vaccination (within the past two weeks) against HBV should be considered when interpreting the HBsAg test results.
- If the birth mother has a known HBV infection and the child received appropriate immunoprophylaxis according to national recommendations and is more than nine months of age, the child should be tested with an HBV DNA NAT, a serological test detecting HBsAg, and a serological test detecting anti-HBs.
- Children of 12 months of age or less whose birth mother has a known HBV infection and who have not received appropriate immunoprophylaxis according to national recommendations should not be considered for donation.

Advice and practical considerations:

- In the context of donor assessment, serological testing for anti-HBc for children up to 12 months old is not advised.

Outcome of test results

- If the results of the required screening tests are negative, the donation can be released for clinical use.
- Donations from children with a reactive HBsAg serological test and/or HBV DNA NAT, in the absence of a negative confirmatory test result, should not be released for clinical use.
- In the case of a negative confirmatory test result and provided a negative HBV DNA NAT in screening for the child or the birth mother, the donation can be released for clinical use.
- If the birth mother has a known HBV infection and the child has received appropriate immunoprophylaxis for HBV according to national recommendations, donations should not be released for clinical use if the anti-HBs concentration for the child is lower than 10 IU/L.

Children who have been breastfed by a person with an HBV infection

Testing requirements and recommendations

Required:

- For children who have been breastfed by a person with a known HBV infection, the testing strategy should be performed considering the requirements that apply according to their age (i.e. below 12 months of age, or following the general requirements applicable to tissues and non-reproductive cells donors) but should not be tested before a period of 16 weeks since the last occurrence, or eight weeks if a highly sensitive HBV DNA NAT is used.

Advice and practical considerations:

- If the person breastfeeding has a known HBV infection and the child is fully vaccinated for HBV according to national recommendations, ECDC advises not releasing donations from children with anti-HBs concentrations below 10 IU/L.

Evidence and justification

Testing of donors

All tissue donors and donors of non-reproductive cells

Considering the documented severity of the disease and the significant consequences for the recipient in case of HBV transmission, members of the expert panel agreed that all donors of tissues and non-reproductive cells should be tested at each donation for HBV infection to reduce the risk of transmission through SoHO (Pathogen data sheet, Section 2).

Screening tests

All tissue donors and donors of non-reproductive cells

Members of the expert panel agreed on the benefits of the use of HBV DNA NAT to increase the safety of transplantation of tissues and non-reproductive cells, in particular as a means to reduce the window period of the acute infection, relative to testing for HBsAg. HBV DNA is also considered a consistent marker for chronic hepatitis B, including after the loss of HBsAg. This opinion is supported by evidence as the window period in the initial phase of the infection is reduced by a period ranging between seven and 15 days when using an HBV DNA NAT with a 95% LOD below 20 IU/mL compared to the detection of HBsAg (Pathogen data sheet, Sections 2 and 4). Individuals affected by chronic hepatitis B may also exhibit transient loss of HBsAg but remain infectious (Pathogen data sheet, Sections 2 and 7). While cases of transmission from donors with undetectable HBV DNA, including with highly sensitive HBV DNA NAT, have been reported, these are considered to be rare and less likely after the loss of HBsAg (Pathogen data sheet, Sections 2 and 7).

Similarly, members of the expert panel agreed on the use of serological tests detecting HBsAg in the HBV screening strategy, due to the possibility of having positive HBsAg with negative HBV DNA NAT, for example, in individuals with HBeAg-negative chronic HBV infection or if the individual is under antiviral treatment (Pathogen data sheet, Section 2).

There was no general agreement among the expert panel members on the use of serological tests detecting anti-HBc in the screening strategy. Due to important variations in the prevalence of anti-HBc in the general and donor populations between EU/EEA countries and regions within the countries (Pathogen data sheet, Section 3), some members of the expert panel expressed concern that the inclusion of these tests in the screening strategy would lead to unsustainable donor loss in EU/EEA countries with high anti-HBc prevalence. This loss was viewed as unjustified for tissues and non-reproductive cells in the context of HBV DNA NAT testing. Conversely, some members of the expert panel considered that the inclusion of serological tests detecting anti-HBc could increase the overall safety of tissue and non-reproductive cells transplantation. This consideration was based on the low infectious dose of the virus and the known transmission cases occurring through blood transfusion from donors with undetectable HBsAg and HBV DNA (Pathogen data sheet, Sections 2 and 7) as well as through liver transplantation [66]. It was noted by several experts that, contrary to the field of blood transfusion, the association of high anti-HBs concentrations and increased safety of donations is not established for tissues and non-reproductive cells transplantation [67]. Due to the high risk of HBV transmission to immunocompromised recipients, ECDC advises always testing for anti-HBc for donations intended for such recipients, including if highly sensitive HBV DNA NAT is used, to identify a possible OBI in the donor.

In the EU/EEA, overall, while the number of notified acute cases is small, reflecting low levels of transmission of infection, there remains a high prevalence of chronic infections across the region, a large proportion of which are undiagnosed (Pathogen data sheet, Section 3); in this context, the risk of HBV transmission through transplantation arises not only from failure to detect the HBV infection during the initial infection phase but also during chronic hepatitis B, in particular in OBI, where HBsAg and HBV DNA may remain undetected in infectious donors (Pathogen data sheet, Sections 2 and 7).

In acute hepatitis B, the risk of not detecting an HBV infection during the initial infection phase is associated with the window period of HBV DNA NAT and HBsAg serology tests. In chronic hepatitis B, in particular during the OBI phase, the risk of not detecting an HBV infection in a donor is associated with loss of HBsAg and transient undetectable HBV DNA. However, some of these cases could be identified by testing for anti-HBc (Pathogen data sheet, Sections 2 and 7), or using highly sensitive HBV DNA NAT. Most of the available evidence on transmission of HBV through SoHO concerns blood transfusion. Transfusion-transmitted HBV infection cases from donors with OBI reported in the literature are often associated with undetectable or very low viral loads, supporting an infectious dose that is likely below the LOD of available tests, in certain conditions. A mathematical model relying on look-back studies following transfusion-transmission of HBV suggested that 3.3% of OBI donations would remain undetected by HBV DNA NAT with a 95% LOD of 8.1 IU/mL (43.1 copies/mL) and could lead to transmission through transfusion of a blood component containing 20mL of plasma (Pathogen data sheet, Section 2). In reported cases of transmission through blood transfusion from donors with OBI, where most of the evidence is available, the majority of donors with detectable HBV DNA had a viral load above 20 IU/mL (Pathogen data sheet, Section 7).

Considering the use of anti-HBc in screening to identify possible OBI, and as there remains a high prevalence of undiagnosed chronic infections in the region, a minimum LOD threshold for HBV DNA NAT is not required if an anti-HBc test is used in screening. Based on expert opinion, ECDC advises basing the LOD on a risk assessment considering the local epidemiological situation and the endemicity of the disease. The risk assessment could be documented to ensure transparency in case of exchange of tissues or non-reproductive cells to other regions or countries. ECDC advises updating the risk assessment in case of significant changes to the HBV epidemiology (as defined by the NCA) or in case of an HBV transmission event from donor to recipient. The risk assessment should be documented to ensure transparency in case of exchange of tissues or cells to other regions or countries.

If anti-HBc screening tests are not used, a highly sensitive HBV DNA NAT should be used to mitigate the risk of transmission from donors with OBI. Based on reported transmission cases from the field of blood transfusion, a 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT. It is important to note that although anti-HBc screening can reduce the risk of transmission from donors with OBI, an HBV DNA NAT with a high 95% LOD, which can be used if anti-HBc screening is used, may lead to longer infectious window periods in donors with acute infections. Due to the presence of inhibitory substances in post-mortem samples following death by circulatory criteria, some HBV DNA NAT may require a dilution of the sample to ensure valid test results [68,69]. In these situations, the dilution factor should be documented to facilitate the interpretation of the results.

Associated with a long doubling time of the virus (2.0 – 2.7 days; Pathogen data sheet, Section 2), the window period of HBV DNA NAT is estimated at around 15 days for a NAT with a 95% LOD below 20 IU/mL. This window period is expected to increase for tests with lower sensitivity (i.e. higher LOD). Studies evaluating the reduction of the window period of HBV DNA NAT and using 95% LOD of <20 IU/mL compared to HBsAg report a reduction of the window period between 7-24 days (Pathogen data sheet, Section 4). Due to the presence of inhibitory substances in post-mortem samples following death by circulatory criteria, some NAT may require a dilution of the sample to ensure valid test results [68,69]. In these situations, the dilution factor should be documented to support the interpretation of the results. However, it should be noted that the exact impact of such a dilution on the analytical sensitivity of the test is currently unknown.

Specific requirements for paediatric donors: neonates and young children

Mother-to-child transmission of HBV through delivery is very likely and, without intervention, is reported to occur in 90% of cases where the mother is HBsAg-positive and HBeAg-positive, and up to 20% of cases where the mother is HBsAg-positive and HBeAg-negative [70]. The use of immunoprophylaxis in the child, comprised of hepatitis B vaccination and administration of hepatitis B immunoglobulin, can reduce the transmission rate to approximately 1% of infants [70-72]. Ruling out perinatal transmission of HBV following immunoprophylaxis can be done by testing for HBsAg and HBV DNA after nine months of age, and one to two months after the last vaccination dose. Adequate protection after vaccination is considered for anti-HBs concentrations above 10 IU/L in the child [71,72], further reducing the likelihood of an infection. As the evidence on the accuracy of screening tests for HBV in newborns has mainly been established after the first month of age, if it is not possible to test the birth mother, children aged below two months should not be screened for HBV DNA or HBsAg, and donation from these children should not be considered. Anti-HBc testing is not recommended before at least 12 months old due to the possibility of passive transfer from the mother to the child [71].

HBV is considered unlikely to be transmitted through breast milk [71,73]; however it can be transmitted through cracked or bleeding nipples. Breastfeeding by persons with a known HBV infection could be considered as a close household contact, and testing the child for HBV infection should not be performed for a period of 16 weeks since the last event to reduce the risk of an infection remaining undetected due to the window period of the tests used (see Risk of exposure to HBV). With appropriate HBV vaccination for the child prior to being breastfed, the risk of HBV transmission through breastfeeding is virtually null [71] and ECDC advises ascertaining the vaccination status and anti-HBs concentration of a child who has been breastfed by a person with a known HBV infection to guide the decision to release the donation. Children with anti-HBs concentrations above 10 IU/L are considered sufficiently protected by vaccination.

Outcome of test results

All tissue donors and donors of non-reproductive cells

Given the high performance of HBV screening tests, reactive screening tests for HBsAg and/or HBV DNA NAT indicate a high likelihood that the donor has an HBV infection, with a high risk for HBV transmission to the recipient if the donation is used (Pathogen data sheet, Section 4). Members of the expert panel agreed that donations from donors with reactive screening tests for HBsAg and/or HBV DNA NAT should not be released for clinical use.

Due to the nature of screening tests and based on expert opinion, a reactive screening result for HBsAg followed by a negative confirmatory test result and provided a negative HBV DNA NAT in screening, suggests a false-reactive result in screening. In this situation the donation can be released for clinical use [74]. Due to the risk of transmission of HBV from a donor with OBI to an immunocompromised recipient, in the case of confirmed results for anti-HBc with negative HBsAg and HBV DNA NAT, additional tests such as anti-HBs could be considered to guide the decision to release the donation for clinical use.

Living donors

Due to the risk of transmission of HBV through tissues and cells (Pathogen data sheet, Section 7), based on good practice principles and considering expert opinion, in case of a positive confirmatory test result or where both the serological test detecting HBsAg and HBV DNA NAT are reactive in screening, look-back procedures to identify previous potentially infectious donations should be performed to identify and follow up on recipients who may be at risk of infection. As samples from previous donations were found negative in screening, it is possible the donation was made at a moment prior to HBsAg appearance, or following loss of HBsAg, and with a viral load below the LOD defined for HBV DNA NAT in routine screening. The use of a highly sensitive HBV DNA NAT for look-back procedures would support the identification of low viral loads. In addition, re-testing of negative donations with low viral loads using NAT increases the likelihood of detecting the virus and re-testing previous donations could be considered even if the previous donations were already tested with a highly sensitive HBV DNA NAT. According to expert opinion, to ensure the safety of recipients, recipients who received a potentially infectious donation should be tested for HBV infection. As a single individual may have previously donated other SoHO, where it is possible look-back procedures could also consider donations of other SoHO types.

ECDC advises calling back the living donor for further testing on a follow-up sample, in case of discordant results between a reactive HBV DNA NAT in screening and the results of additional tests performed to confirm the initial HBV DNA NAT results, where a higher viral titre may be detected. ECDC also advises further testing in the case of an indeterminate confirmatory test result, where only the serological test detecting HBsAg is reactive in screening. For these donors, a minimum period of eight weeks from the last donor testing before re-entry should be followed to cover the possible HBsAg window period (Pathogen data sheet, Section 2).

There was an agreement among the expert panel members that due to the risk of HBV transmission (Pathogen data sheet, Section 2), living donors with a positive confirmatory test result for HBV, or with reactive HBV DNA NAT in screening, or where an infection cannot be ruled out, taking into account all information available, should be notified and referred to relevant clinical care. As acute and, in rare cases, chronic [58], HBV infections may resolve, and the donor may clear HBV DNA and lose HBsAg (Pathogen data sheet, Section 2), donors with such a history of resolved HBV infection may be considered for further donations. Due to the high risk of transmission of HBV, donors with a confirmed HBV infection should only be considered for further donations after a documented loss of HBsAg and clearance of HBV DNA in clinical follow-up, as well as concentration levels of anti-HBs demonstrating immunity. An anti-HBs concentration of 10 IU/L or above is generally considered to provide immunity to HBV after vaccination [60]. However, to ensure the safety of the future donations for all recipients, a concentration of 100 IU/L or above could be considered for the re-entry of these donors.

According to ECDC's opinion, HBV infection is highly likely in donors where both the serological test detecting HBsAg and HBV DNA NAT are reactive, regardless of the confirmatory test result. The concordance of the screening tests significantly reduces the likelihood of a false-reactive screening result. These donors should be deferred and treated as donors with a positive confirmatory test result.

Deceased donors

Based on good practice principles and expert opinion, in the case of a positive confirmatory test result or indeterminate confirmatory test result for HBV, or with reactive HBV DNA NAT in screening, or where an HBV infection is considered likely, taking into account other available information, the transplant coordination team should be notified and inform all entities which received SoHO of the donor. Due to the risk of transmission of HBV through tissues and cells (Pathogen data sheet, Section 7), based on good practice and considering expert opinion, in case of a reactive HBV DNA NAT or a reactive HBsAg serological test in the absence of negative confirmatory test in a multi-tissues and organs donor, a risk assessment should be performed, including other available test results to determine if there are other potentially infectious donations and if look-back procedures should be initiated. Recipients who may be at risk of infection should be identified and tested for HBV. As a single individual may have previously donated other SoHO, where it is possible look-back procedures could also consider donations of other SoHO types

Specific requirements for paediatric donors: neonates and young children

Due to the high risk of mother-to-child HBV transmission in the absence of intervention [71,72], children born from mothers with a known HBV infection that did not receive appropriate immunoprophylaxis should not be considered for donation. Similarly, children who received appropriate immunoprophylaxis but have not achieved adequate levels of anti-HBs (above 10 IU/L) remain at risk of vertical and horizontal transmission of HBV, and donations from these children should not be released for clinical use.

While HBsAg can be found in breast milk, transmission of HBV through breast milk is considered very unlikely and is virtually null if the child has received a full vaccination course in accordance with national recommendations prior to being breastfed [71,73]. Children who have achieved adequate levels of anti-HBs (above 10 IU/L) are unlikely to be infected by HBV through breastfeeding.

Limitations

The evidence is based on studies evaluating individual test performance and not the comparison of a combination of test methods. The infectious dose for HBV DNA is uncertain but likely very low. The factors (e.g. anti-HBs concentrations) influencing the likelihood of transmission of HBV from a donor with a very low or undetectable viral load remain unclear. Evidence supporting the low risk of transmission from tissue donations with isolated anti-HBc irrespective of anti-HBs concentrations is very limited, and the recommendations for anti-HBs concentrations for tissues and non-reproductive cells transplantation may be modified when additional evidence becomes available.

Risk of exposure to HBV and testing limitations in case of recent exposure to be considered for each donation

Requirements and recommendations

Standards, good practice guidelines, and recommendations for the evaluation of donors of tissues and non-reproductive cells are detailed in the latest version of the EDQM guide to the quality and safety of tissues and cells for human application [4].

Injection of non-prescription drugs refers to the use of any drug or other substance not prescribed by a registered healthcare professional and self-administered via injection. This includes both illicit drugs (e.g. heroin, methamphetamine) and performance enhancing drugs (e.g. testosterone) when injected and which can be associated with needle sharing.

The events listed below aim to represent evidence-based risks of exposure to HBV and are not intended to be the exact questions asked or the physical examination performed during the donor assessment. They are provided to support entities in developing their donor eligibility assessment strategies. For deceased donors, the availability of information on the infectious disease risks related to the donor should be considered as part of the risk assessment.

Risk of exposure to HBV

Required:

- All donors, at each donation, should be assessed for recent risks of exposure to HBV when considered for donor eligibility.

Advice and practical considerations:

The following events are considered risks of exposure to HBV. ECDC advises considering the occurrence of these events in the previous 16 weeks when assessing donor eligibility:

- Active sexually transmitted infection (STI);
- Close household contact with an individual with acute or chronic HBV infection;
- Recent healthcare encounter: receipt of a SoHO (including human organs), haemodialysis, intravenous fluid/contrast infusion;
- Needle sharing and/or injection of non-prescription drugs;
- Non-occupational needlestick injuries, tattoos, piercing in unhygienic conditions;
- Exposure to known or suspected HBV infection following an occupational incident (e.g. needlestick injury);
- Condomless* sex with an individual with an ongoing acute or chronic HBV infection;
- Condomless* sex with a partner who injects non-prescription drugs;
- Condomless* sex in exchange for money, drugs, or other payments.

* *'Condomless' should be understood as a situation where no condom was used or where one was used incorrectly (e.g. breaking, leaking, or slipping off during sexual activity).*

Testing limitations in case of recent risk of exposure to HBV

Required:

- Donors should not be tested in the context of donor evaluation before a period of at least 16 weeks since the last event with a risk of exposure to HBV. In case of the use of a highly sensitive HBV DNA NAT, this period can be reduced to eight weeks since the last event.

Advice and practical considerations:

- Due to the possibility of HBsAg reactivity following an HBV vaccination, ECDC advises not testing donors during a period of two weeks following a recent HBV vaccination.

Evidence and justification

Risk of exposure to HBV

All tissue donors and donors of non-reproductive cells

Based on available evidence and expert opinion, the above-described events include the major risks of exposure to HBV, relevant for SoHO safety, in EU/EEA countries (Pathogen data sheet, Section 3).

Some healthcare encounters may carry an increased risk of HBV infection, according to literature reports [61,62] (Pathogen data sheet, Section 3); however, there was no overall agreement in the expert panel on the relevance of this event as an exposure risk for HBV in the EU/EEA. Inadequate infection prevention and control measures in the healthcare setting may increase the risk of exposure to HBV, although such situations are not considered frequent in the EU/EEA by the members of the expert panel. Specifically for donors of non-reproductive cells, patients undergoing haemodialysis or who have received a SoHO transfusion or transplantation are unlikely to become donors in a time frame consistent with the risk of a window period transmission. Similarly, the risk of exposure to HBV following new tattoos or piercings was considered very low by the expert panel since authorised facilities in the EU/EEA are considered to follow appropriate hygienic measures. However, the risk of infection may be increased for events performed in situations with suboptimal hygienic conditions. Considering the high likelihood of transmission of HBV and the long-term consequences of the disease, based on expert opinion ECDC advises considering all the above risk events when assessing donor eligibility. These events may be unlikely for some donors of tissue and non-reproductive cells or can be challenging to assess through interviews, particularly in the context of risk events concerning the partner, as well as the correct usage of condoms, as condom failure is not always recognised [63].

It is important to note that the most documented transmission mode of acute HBV infection in EU/EEA is sexual (Pathogen data sheet, Section 3). In this perspective, condomless sex with new or multiple partners with an unknown HBV infection status in areas with high prevalence of HBV could be considered as a risk of exposure to HBV. This risk is not listed in advice and practical considerations above as it could lead to unnecessary donor loss in low-prevalence countries, however it could be considered in areas with high prevalence of HBV infection. Active STI are associated with an increased likelihood of carrying HBV (Pathogen data sheet, Section 3). In the available evidence, the specific infections associated with HBV are not comprehensively described, but include infections with *T. pallidum*, *Chlamydia trachomatis*, or *Neisseria gonorrhoeae*.

There was no general agreement in the expert panel on taking into account the vaccination status of the donor against HBV when assessing the recent risks of exposure. While complete HBV vaccination is effective and provides lifelong immunity, breakthrough infections can occur [64,65]. An accurate assessment of a complete HBV vaccination schedule (three doses) was also considered challenging and could lead to misclassification of partial vaccination profiles as fully vaccinated. In addition, vaccination against HBV for individuals at risk of HBV infection was also identified as a potential safety concern for the expert panel which did not support taking into account vaccination status when assessing recent risks of exposure. As a result of these discussions, no specific considerations for HBV vaccination were included in the assessment of recent risks of exposure.

Testing limitations in case of risk of exposure to HBV

All tissue donors and donors of non-reproductive cells

Given the high performance of the HBV screening tests required in these guidelines, the risk for transmission through SoHO is related to newly infected potential donors tested during the window period (Pathogen data sheet, Section 2). To avoid window period transmissions from newly infected donors, donors who have been at risk of exposure to HBV should not be tested until the test results are considered reliable. As this does not apply to deceased donors, deceased donors with recent risks of exposure should be excluded.

Based on expert opinion, a multiplication factor of the window period should be considered to ensure reliability of the test results and reduce the risk of a window period transmission after a recent infection. The risks of exposure identified in this section do not address the risk of transmission in the OBI phase.

A period of 16 weeks after an event with a risk of exposure to HBV covers more than three times the maximum window period of HBV DNA NAT and HBsAg, including when considering testing in pools (Pathogen data sheet, Sections 2 and 4). If a highly sensitive HBV DNA NAT is used, as HBsAg is unlikely to be present if the viraemia is undetectable (Pathogen data sheet, Sections 2 and 4), this period can be reduced to eight weeks.

For deceased donors, assessing the precise time since the most recent event with a risk of exposure to HBV may be challenging, due to limited information; in such cases, the likelihood of a recent risk should be estimated based on the existing data.

Limitations

The events with increased risk of exposure to HBV are based on targeted literature searches and not on systematic literature reviews and may not be comprehensive. The interval between potential exposure to HBV and testing to be considered to ensure reliability of the test results is based on an arbitrary multiplication factor of the window period; however, it is consistent with the deferral periods currently used in several EU/EEA countries where transmissions of HBV are rare events (Pathogen data sheet, Section 7). It may not be possible to transpose these events/wording directly to the donor eligibility assessment and adaptations may be needed in particular to account for the acceptability and feasibility of including these questions and limit potential donor loss.

Donor eligibility is assessed for all pathogens at once, and the list of events in the current guidelines only covers HBV. The list of events with an increased risk of exposure to other pathogens will be completed by ECDC as new guidelines are published. Pending these updates, the EDQM guide to the quality and safety of tissues and cells for human application can be used as a resource for additional events to consider in the assessment of donor eligibility [4].

Affected individuals and other situations to consider

Requirements and recommendations

Required:

- Individuals with a medical diagnosis of chronic HBV infection or receiving treatment for chronic HBV infection should not be considered eligible for donation and should be deferred until HBsAg loss, undetectable HBV DNA, and anti-HBs concentrations demonstrating immunity are documented in the context of clinical follow-up.

Advice and practical considerations:

- In case of uncertainty of a medical diagnosis of chronic HBV infection, the prospective donor could be tested for anti-HBc, as described in the section Testing of tissues and non-reproductive cells donors for HBV.
- For individuals meeting criteria to re-enter screening procedures after a medical diagnosis of chronic HBV infection, ECDC advises testing these donors for anti-HBs, HBsAg and with a highly sensitive HBV DNA NAT.

Evidence and justification

All tissue donors and donors of non-reproductive cells

Individuals with a medical diagnosis of chronic HBV infection have a low likelihood of spontaneous remission. Chronic HBV infections may progress to an HBsAg-negative phase or 'occult HBV infection', where very low levels of viraemia of HBV DNA may not be identified through testing, but could nonetheless lead to transmission through the use of SoHO, as evidenced through liver transplantation [66] and blood transfusion (Pathogen data sheet, Sections 2 and 4). Treatment for chronic hepatitis B is not curative, and while it could lead to undetectable levels of HBV DNA, a risk of transmission cannot be excluded. Based on evidence and expert opinion, considering the severity and long-term consequences of the disease, the route of transmission, and the low infectious dose of HBV, all individuals with a known diagnosis of chronic HBV or treatment for chronic HBV should be deferred to reduce the risk of transmission. Eligibility for donation should only be considered for individuals with a documented loss of HBsAg and clearance of HBV DNA in clinical follow-up, as well as concentration levels of anti-HBs demonstrating immunity. An anti-HBs concentration of 10 IU/L or above is generally considered to provide immunity to HBV after vaccination [60]. However, to ensure the safety of future donations for all recipients, a concentration of 100 IU/L or above could be considered for individuals re-entering screening procedures after a medical diagnosis of chronic HBV infection.

Limitations

The risk of transmission of HBV through transplantation from individuals with remission from chronic HBV infection is uncertain, and the eligibility of these individuals may be reconsidered when further evidence is available.

Requirements and recommendations: reproductive cells and tissues

Testing of partners within-relationship use and third-party donors

For these guidelines, embryo donors who contributed their reproductive cells to the embryo should be considered and tested as sperm or oocyte donors.

For embryo donation, partners within-relationship use who contributed to the embryo with their reproductive cells should be tested as sperm or oocyte donors at the time of embryo donation or at the time of the procurement of partner gametes.

Reproductive tissues used for autologous purposes should be considered as within-relationship use.

For these guidelines, 'at donation' should be understood as close as possible to donation, and test results should be available before treatment. The timing of sampling should be in accordance with the latest EDQM guide to the quality and safety of tissues and cells for human application [4].

For oocyte donation, the donation could be considered as the start of stimulation, and the testing can hence be performed at the time of stimulation.

For these guidelines, serial sperm donations are considered a process where a sperm donor donates sperm on multiple occasions in a frequent and repetitive manner during a limited time period. If two donations are separated by a period of 90 days or more, these should not be considered serial donations.

Requirements and recommendations

Reproductive cells: third-party donation

Testing requirements

Required:

- All oocyte donors, at each donation, should be tested for HBV.
- All sperm donors should be tested for HBV at each donation, or in the case of serial donations, at the initial donation and at least 30 days after the last donation in the series. The second test should be done before the release of any of the donations from the series of donations.

Screening tests

Required:

- Donors should be tested considering one of the following strategies:
  - An HBV DNA NAT and a serological test detecting HBsAg and a serological test detecting anti-HBc antibodies; *or*
  - A highly sensitive HBV DNA NAT and a serological test detecting HBsAg.
- In case of donations quarantined for 180 days or more, and if the donor is retested after the quarantine period, the donor does not need to be tested with HBV DNA NAT at donation and after the quarantine period, and only serological tests detecting HBsAg and anti-HBc are required.

Advice and practical considerations:

- ECDC advises basing the LOD for HBV DNA NAT on a documented risk assessment considering the endemicity of the disease.
- A 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT.

Outcome of test results

Required:

- If the results of the required screening tests are negative, the donation can be released for clinical use.
- Donations from donors with reactive HBsAg serological test and/or HBV DNA NAT should not be released for clinical use.
- In case of reactive HBV DNA NAT or in case of a positive confirmatory test result following reactive HBsAg, and which cannot be attributed to a recent vaccination against HBV:
  - The donor should be notified and referred to relevant clinical care.
  - The donor should be deferred until the criteria to re-enter donor screening procedures are met.
  - Look-back procedures of previous, potentially infectious donations should be initiated.

- In the case of a negative HBV DNA NAT and where the HBsAg serological test is reactive in screening and followed by an indeterminate confirmatory test result:
  - The decision to notify and refer the donor to clinical care should be based on the likelihood of an infection based on available information, including other available test results obtained during screening and confirmation procedures.
  - The decision to initiate look-back procedures of previous, potentially infectious donations should be based on a risk assessment considering available information, including other available test results obtained during screening and confirmation procedures.
- In case of confirmed results for reactive anti-HBc, with negative HBsAg and negative HBV DNA NAT (or with only negative HBsAg if the donation is quarantined):
  - The donor should be re-tested with a highly sensitive HBV DNA NAT, unless already performed.
  - If the highly sensitive HBV DNA NAT is negative, the donation can be released for clinical use.

Advice and practical considerations:

- A 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT.
- In the case where only the serological test detecting HBsAg is reactive in screening and followed by an indeterminate confirmatory test result, ECDC advises calling back the donor for an additional test on a follow-up sample.
- If the donor was negative for anti-HBc prior to quarantine and has confirmed results for reactive anti-HBc after the quarantine period, ECDC advises not releasing the donation for clinical use.

Criteria to re-enter donor screening procedures

Required:

- In case of a negative highly sensitive HBV DNA NAT in screening and a negative confirmatory test result following HBsAg reactivity, the donor can re-enter donor screening procedures without a deferral period.
- In case of a negative highly sensitive HBV DNA NAT in screening and a reactive HBsAg serological test result, which can be attributed to a recent vaccination, the donor can re-enter donor screening procedures without a deferral period if no risks of exposure to HBV have been identified. A period of two weeks from vaccination could be considered to avoid further HBsAg reactivity.
- In case of a negative highly sensitive HBV DNA NAT in screening and an indeterminate confirmatory test result following HBsAg reactivity: the donor can re-enter donor screening procedures but should not re-enter before a minimum period of eight weeks from the last donor testing, to ensure coverage of the possible HBsAg window period.
- In case of a reactive HBV DNA NAT or in case of a positive confirmatory test result for HBV infection, donors can re-enter screening procedures upon documentation of HBsAg loss and undetectable HBV DNA during clinical follow-up, as well as documentation of anti-HBs concentrations demonstrating immunity. All future donations should be tested for HBsAg and with a highly sensitive HBV DNA NAT.

Look-back procedure

Required:

- The extent of the look-back procedure should be based on a risk assessment to determine which previous donations are at risk of transmitting HBV. The risk of OBI on previously negative donations should be considered in this assessment. Anti-HBs concentrations can be helpful to assess the risk of OBI in previously negative donations.
  - A residual sample of the last negative donation should be retested using a highly sensitive HBV DNA NAT. In case of a serial donation, the initial sample of the serial donation should be retested. If the last negative donation cannot be retested, the donation before should be tested. Exemption from retesting previous donations can be considered if the previous donation was tested with a highly sensitive HBV DNA NAT and was negative. If an OBI is likely, considering available information, all available donations should be retested for HBV.
  - If the residual sample is positive in the retest, the retesting of archived samples of previous donations should be performed sequentially until a donation is negative. In case of serial donation, testing of the initial and final sample of the previous series of donations should be performed sequentially until both samples are negative. No further testing of prior archived samples is required if the previous sample is negative in the re-test and OBI is unlikely. Recipients who received a donation positive in the retesting of archived samples should be tested for HBV infection.
  - In case of a sample with a positive confirmatory test result, and if no archived samples are available for look-back procedures, the centre that performed the treatment should test the recipients of the previous potentially infectious donation. The results should be reported to the entity where the donation was performed.

Advice and practical considerations:

- A 95% LOD of 20 IU/mL or lower could be considered for the retesting of the residual sample of the last donation.
- If archived samples test negative upon retesting, additional measures, such as testing recipients who received a potentially infectious donation, could be taken following a risk assessment considering other relevant information.

Reproductive cells and tissues: within-relationship use

Testing requirements

Required:

- Partners within the relationship should be tested for HBV not more than three months before collection. For additional collection, testing should be repeated no later than 24 months after the first or previous testing, or when a new risk is identified and according to national legislation.

Screening tests

Required:

- The partners should be tested with a serological test detecting HBsAg *or* with an HBV DNA NAT.

Outcome of reactive tests

Required:

- In the case of a positive confirmatory test result or of an indeterminate confirmatory test result that cannot be resolved:
  - Proceeding with the within-relationship use is to be discussed with the partners and the clinical team, including a specialist in HBV care; please refer to European Society of Human Reproduction and Embryology (ESHRE) guidelines [2].
  - Procedures should be implemented to prevent the risk of infection to the partner and to the offspring; please refer to ESHRE guidelines [2].

Advice and practical considerations:

- In the case of a positive confirmatory test result, ECDC advises obtaining a further sample to reconfirm the test result.

Evidence and justification

Testing requirements

Third-party donations

Considering the documented severity of the disease and the significant consequences on the recipient in case of HBV transmission, members of the expert panel agreed that all donors of reproductive cells should be tested at each donation for HBV infection to reduce the risk of transmission through SoHO (Pathogen data sheet, Section 2).

For oocyte donation, testing can be performed at the time of stimulation to avoid unnecessary stimulation of the donor in case of reactive screening test(s).

Semen can be collected in a repetitive manner with short intervals between the donations during a limited period, so-called serial donations. In the case of serial donations for sperm, the donor should be tested for HBV at the initial donation and at least 30 days after the last donation in the series. The second test should be performed before the release of any of the donations from the series of donations. Thirty days corresponds to the upper limit of the HBV DNA NAT window period for a 95% LOD of 20 IU/mL (Pathogen data sheet, Sections 2 and 4). ECDC recommends that the maximum period for serial donations should be clearly defined at a national level, not exceeding a period of 90 days. ECDC has previously assessed a maximum period of 90 days between testing in the context of serial donations as a safe alternative to testing at each donation. This assessment was conducted with an external expert panel, considering the estimated residual risk of HBV transmission through semen donation. The risk model used for this assessment took into account the incidence, prevalence, and the window period for HBV infection [75]. This is provided the donations are stored in a manner that mitigates cross-contamination risks and that the test results are negative before the release of any of the donations between the two periodically repeated screening tests. If the period of serial donations extends beyond 90 days, ECDC recommends to retest every 90 days for as long as the serial donations are ongoing. In addition, if two donations are separated by 90 days or more, then these should not be considered serial donations [75].

Within-relationship use

Due to the risk of vertical transmission to the offspring and to protect the receiving partner within the relationship, all partners from whom SoHO are collected for within-relationship use should be tested for HBV. It has been demonstrated that for MAR, within-relationship use, testing the partners at entry and at fixed time intervals up to a maximum of 24 months would not diminish the level of safety of the cells concerned, compared to more frequent testing, as long as appropriate safety and quality systems are in place [76]. These requirements are based on the assumption that storage is performed in a manner that mitigates cross-contamination risks during cryopreservation, both to the material used within the relationship and to any other donations [2].

Screening tests

Third-party donations

Members of the expert panel agreed on the benefits of the use of HBV DNA NAT to increase the overall safety of third-party donations of reproductive cells, in particular as a means to reduce the window period of the acute infection, relative to testing for HBsAg. HBV DNA is also considered a consistent marker for chronic hepatitis B, including after the loss of HBsAg. This opinion is supported by evidence as the window period in the initial phase of the infection is reduced by a period ranging between 7 to 15 days when using an HBV DNA NAT with a 95% LOD below 20 IU/mL compared to the detection of HBsAg (Pathogen data sheet, Sections 2 and 4). Individuals affected by chronic hepatitis B may also exhibit transient loss of HBsAg but remain infectious (Pathogen data sheet, Sections 2 and 7). While cases of transmission from donors with undetectable HBV DNA, including with a highly sensitive HBV DNA NAT, have been reported, these are considered to be rare and less likely after the loss of HBsAg (Pathogen data sheet, Sections 2 and 7).

Similarly, members of the expert panel agreed on the use of serological tests detecting HBsAg in the HBV screening strategy, due to the possibility of having positive HBsAg with negative HBV DNA NAT, for example, in individuals with HBeAg-negative chronic HBV infection or if the individual is under antiviral treatment (Pathogen data sheet, Section 2).

There was no general agreement among the expert panel members on the use of serological tests detecting anti-HBc in the screening strategy. Due to important variations in the prevalence of anti-HBc in the general and donor populations between EU/EEA countries and regions within the countries (Pathogen data sheet, Section 3), some members of the expert panel expressed concern that the inclusion of these tests in the screening strategy would lead to unneeded donor loss in EU/EEA countries with high anti-HBc prevalence. Conversely, some members of the expert panel considered that the inclusion of serological tests detecting anti-HBc could increase the overall safety of third-party donation. This consideration was based on the low infectious dose of the virus and the known transmission cases occurring through blood transfusion from donors with undetectable HBsAg and HBV DNA (Pathogen data sheet, Sections 2 and 7) as well as through liver transplantation [66].

In the EU/EEA, while the number of notified acute cases is small, reflecting low levels of transmission of infection, there remains a high prevalence of chronic infections across the region, a large proportion of which are undiagnosed (Pathogen data sheet, Section 3); in this context, the risk of HBV transmission through SoHO arises not only from failure to detect the HBV infection during the initial infection phase but also during chronic hepatitis B, in particular in OBI, where HBsAg and HBV DNA may remain undetected in infectious donors (Pathogen data sheet, Sections 2 and 7).

In acute hepatitis B, the risk of not detecting an HBV infection during the initial infection phase is associated with the window period of HBV DNA NAT and HBsAg serology tests. In chronic hepatitis B, in particular during the OBI phase, the risk of not detecting an HBV infection in a donor is associated with loss of HBsAg and transient undetectable HBV DNA. However, some of these cases could be identified by testing for anti-HBc (Pathogen data sheet, Sections 2 and 7), or using highly sensitive HBV DNA NAT. In reported cases of transmission through blood transfusion from donors with OBI, where most of the evidence is available, the majority of donors with detectable HBV DNA had a viral load above 20 IU/mL (Pathogen data sheet, Section 7).

Considering the use of anti-HBc in screening to identify possible OBI, and as there remains a high prevalence of undiagnosed chronic infections in the region, a minimum LOD threshold for HBV DNA NAT is not required if an anti-HBc test is used in screening. Based on expert opinion, ECDC advises basing the LOD on a risk assessment considering the local epidemiological situation and the endemicity of the disease. ECDC advises updating the risk assessment in case of significant changes to the HBV epidemiology (as defined by the NCA) or in case of an HBV transmission event from donor to recipient. The risk assessment should be documented to ensure transparency in case of exchange of reproductive cells or embryos to other regions or countries.

If anti-HBc screening tests are not used, a highly sensitive HBV DNA NAT should be used to mitigate the risk of transmission from donors with OBI. Based on reported transmission cases from blood transfusions, a 95% LOD of 20 IU/mL or lower could be considered as a highly sensitive HBV DNA NAT (Pathogen data sheet, Section 7). It is important to note that although anti-HBc screening can reduce the risk of transmission from donors with OBI, a high 95% LOD for HBV DNA NAT may lead to longer infectious window periods in donors with acute infections.

It should also be noted that no transmission of HBV through reproductive cell donation has been reported in the EU/EEA between 2017 and 2022 (Pathogen data sheet, Section 7), including in countries that rely solely on

screening for HBsAg after a quarantine period of 180 days (Pathogen data sheet, Sections 5 and 7). The requirements described above are based on the precautionary principle and include the possibility of testing with HBV DNA NAT for early detection of donors in case no quarantine period is considered. These requirements are meant to harmonise the safety of third-party donation of reproductive cells in the EU/EEA. It is also important to note that there is no evidence of the value of high anti-HBs concentrations as a marker of safe donations for reproductive cells.

Donors whose donations are quarantined for 180 days or more, tested at each donation, and retested with serological tests detecting HBsAg and anti-HBc after the quarantine period, do not need to be tested with HBV DNA NAT at donation and after the quarantine period. The quarantine period allows sufficient time for seroconversion for HBV, but also for HIV and HCV, reducing the need for additional HBV DNA NAT.

Within-relationship use

As the need for tests with short window periods to detect recent infections is less critical within-relationship use, the use of either HBV DNA NAT or serological tests detecting HBsAg can be considered to detect HBV infections among partners within-relationship use.

Outcome of test results

Third-party donations

Given the high performance of HBV screening tests, reactive screening tests for HBsAg and/or HBV DNA indicate a high likelihood that the donor has an HBV infection, with a possible risk for HBV transmission if the donation is used (Pathogen data sheet, Section 4). While false-reactive results of serological screening tests are possible, members of the expert panel agreed that donations from donors with reactive screening tests for HBsAg and/or HBV DNA NAT should not be released for clinical use due to the severe impact of an HBV transmission on the recipient.

In the case of serial donations, the requirement to not release donations from donors with reactive screening tests applies to both the initial and final donation in the series. For the initial donation, if the HBV DNA NAT or test detecting HBsAg is reactive, the donation should not be released for clinical use, and the serial donations should not proceed. If HBV DNA NAT or test detecting HBsAg is reactive in the final sample, none of the donations within that series, going back to the last (initial) negative donation, should be released. In the case of a positive confirmatory test result, or if both the HBV DNA NAT and the test detecting HBsAg are reactive in screening, as well as in other situations where HBV infection cannot be ruled out and is considered likely, taking into account other available information (including other test results), the donor should be informed and referred to relevant clinical care. In the case of donations quarantined for 180 days or more, a seroconversion for anti-HBc (i.e. negative prior to quarantine and reactive after quarantine) could indicate an incident HBV infection during the quarantine period. However, it may also indicate an acute HBV infection which was not identified with the tests used at the time of donation. In this situation, ECDC advises not releasing the donation for clinical use, as it carries a risk for transmission of HBV.

A positive confirmatory test result following a reactive HBsAg serological test or HBV DNA NAT, or an indeterminate confirmatory test result and where an HBV infection is considered likely, considering other available information, should lead to notification and referral of the donor for appropriate clinical care. As acute and, in rare cases, chronic [58], HBV infections may resolve, and the donor may clear HBV DNA and lose HBsAg (Pathogen data sheet, Section 2), re-entry of donors with such a history of resolved HBV infection could be considered under specific circumstances.

The extent of the look-back procedure should be guided by a risk assessment considering other available test results and relevant information to identify which previous donations may pose a risk of transmitting HBV, and therefore which samples and recipients require testing. The possibility of OBI should be considered in this assessment. Although samples from previous donations were found negative in screening, it is possible the donation was made at a moment prior to HBsAg appearance, or following the loss of HBsAg, and with a viral load below the LOD defined for HBV DNA NAT in routine screening. Using a highly sensitive HBV DNA NAT for look-back procedures would support the identification of low viral loads and should be used for the residual sample of the previous donation, or in case of serial donations, the initial sample in the series. In case of a reactive test result, the initial and final samples in the previous series of donations should be tested. If either of the tests is reactive in the previous series of donations, all donations in the series are to be considered potentially infectious. Re-testing of negative donations with low viral loads using NAT increases the likelihood of detecting the virus, and re-testing previous donations could be considered even if the previous donations were already tested with a highly sensitive HBV DNA NAT. According to expert opinion, to ensure the safety of recipients, recipients who received a donation found positive in re-testing should be tested for HBV infection. Testing recipients who received a donation negative in retesting could also be considered following a risk assessment and considering other relevant information, since the negative result could reflect a very low viral load. If no archived samples are available for testing in the context of look-back procedures, according to expert opinion, the recipient(s) of the previous potentially infectious donation should be tested for an HBV infection. As a single individual may have previously donated other SoHO, where it is possible look-back procedures could also consider donations of other SoHO types.

Within-relationship use

Confirmed markers of HBV infection status, or in other cases where an HBV infection cannot be ruled out, means a risk for HBV transmission to the partner or to the offspring. Based on good practice principles, in case of a positive confirmatory test result, ECDC advises calling back the individual for a second sample and further confirmatory testing to reconfirm the test result and confirm the identity of the individual. Members of the expert panel agreed that, in case of confirmed markers of HBV infection within the relationship or in cases where HBV infection cannot be ruled out, assisted reproduction is to be discussed with the partners and the clinical team, including a specialist in HBV care. Procedures should be implemented to prevent the risk of infection to the partner and to the offspring following ESHRE's guidelines [2].

Criteria to re-enter donor screening procedures

Third-party donations

A negative confirmatory test following a reactive serological test and a negative HBV DNA NAT in screening is very likely indicative of a false-reactive result for the serological test. Therefore, if the confirmatory test following HBsAg reactivity is negative, and if the HBV DNA NAT is negative in screening, the donor is not considered to have an HBV infection, and the donor can be allowed to re-enter donor screening procedures without a deferral period.

Vaccination for HBV can lead to positive results for HBsAg during a short period after vaccination (Pathogen data sheet, Section 4). Donors with a positive HBsAg result and a negative HBV DNA NAT in screening, and where vaccination is the likely cause of the positive result, considering available information, including risks of exposure and other available test results, the donor can re-enter screening procedures without a deferral period. As vaccination may be considered for individuals at risk of HBV infection, risks of exposure to HBV should be evaluated prior to re-entry.

Further testing is advised in case of an indeterminate confirmatory test result, where only the serological test detecting HBsAg is reactive in screening. For these donors, a minimum period of eight weeks from the last donor testing before re-entry should be followed to cover the possible HBsAg window period (Pathogen data sheet, Section 2).

According to ECDC's opinion, HBV infection is highly likely in donors where both the serological test detecting HBsAg and HBV DNA NAT are reactive, regardless of the confirmatory test result. The concordance of the screening tests significantly reduces the likelihood of a false-reactive screening result. These donors should be treated as donors with a positive confirmatory test result.

Due to the risk of HBV transmission (Pathogen data sheet, Section 2), re-entry following a positive confirmatory test result or where an infection cannot be ruled out taking into account all information available (including where both HBV DNA NAT and the serological screening test detecting HBsAg are reactive), should only be considered for donors with a documented loss of HBsAg and clearance of HBV DNA in clinical follow-up, as well as concentration levels of anti-HBs demonstrating immunity. An anti-HBs concentration of 10 IU/L or above is generally considered to provide immunity to HBV after vaccination [60]. However, to ensure the safety of future donations and avoid unnecessary discarding of donations, a concentration of 100 IU/L or above could be considered for the re-entry of these donors.

Limitations

The evidence is based on studies evaluating individual test performance and not the comparison of a combination of test methods. The infectious dose for HBV DNA is uncertain but likely very low. The factors (e.g. anti-HBs concentrations) influencing the likelihood of transmission of HBV from a donor with a very low or undetectable viral load remain unclear. The likelihood of transmission of HBV through third-party donation of reproductive cells is uncertain but is likely to be low in the EU/EEA, and further evidence supporting a low likelihood of transmission may lead to a modification of the requirements provided in these guidelines.

Risk of exposure to HBV and testing limitations in case of recent exposure to be considered for each donation

Requirements and recommendations

Reproductive cells: third-party donations

Injection of non-prescription drugs refers to the use of any drug or other substance not prescribed by a registered healthcare professional and self-administered via injection. This includes both illicit drugs (e.g. heroin, methamphetamine) and performance enhancing drugs (e.g. testosterone) when injected and which can be associated with needle sharing.

ECDC advises recommending the donor seeks clinical advice and testing in case of recent risk of exposure to HBV before considering donation.

The events listed below aim to represent evidence-based risks of exposure to HBV and are not intended to be the exact questions asked or the physical examination performed during the donor assessment. They are provided to support entities in developing their donor eligibility assessment strategies.

Risk of exposure to HBV

Required:

- All donors, at each donation, should be assessed for recent risks of exposure to HBV when considered for donor eligibility.

Advice and practical considerations:

The following events are considered risks of exposure to HBV. ECDC advises considering the occurrence of these events in the past 16 weeks when assessing donor eligibility:

- Active sexually transmitted infection (STI);
- Close household contact with an individual with acute or chronic HBV infection;
- Recent healthcare encounter: receipt of a SoHO (including human organs), haemodialysis, intravenous fluid/contrast infusion;
- Needle sharing and/or injection of non-prescription drugs;
- Non-occupational needlestick injuries, tattoos, piercing in unhygienic conditions;
- Exposure to known or suspected HBV infection following an occupational incident (e.g. needlestick injury);
- Condomless* sex with an individual with an ongoing acute or chronic HBV infection;
- Condomless* sex with a partner who injects non-prescription drugs;
- Condomless* sex in exchange for money, drugs, or other payments.

* '*Condomless' should be understood as a situation where no condom was used or where one was used incorrectly (e.g. breaking, leaking, or slipping off during sexual activity).*

Testing limitations in case of recent risk of exposure to HBV

Required:

- Donors should not be tested in the context of donor evaluation before a period of at least 16 weeks since the last event with a risk of exposure to HBV. In case of the use of a highly sensitive HBV DNA NAT, this period can be reduced to eight weeks since the last event.

Advice and practical considerations:

- Due to the possibility of HBsAg reactivity following an HBV vaccination, ECDC advises not testing donors during a period of two weeks following a recent HBV vaccination.

Reproductive cells and tissues: within-relationship use

Risk of exposure to HBV

Advice and practical considerations:

- ECDC advises considering the risk of exposure to HBV for partners within-relationship use.

Considerations for testing due to risk of exposure to HBV

Advice and practical considerations:

- ECDC advises considering test results as not reliable before a period of at least 16 weeks (eight weeks if a highly sensitive HBV DNA NAT is used) since the last event with a risk of exposure to HBV.
- If an event with a risk of exposure to HBV occurred within the above-described period, ECDC advises testing the individuals within the relationship after the corresponding period since the last event has passed.

Evidence and justification

Risk of exposure to HBV

Reproductive cells: third-party donations

Based on available evidence and expert opinion, the above-described events include the major risks of exposure to HBV, relevant to the safety of reproductive cells, in EU/EEA countries (Pathogen data sheet, Section 3).

Some healthcare encounters may carry an increased risk of HBV infection, according to literature reports [61,62] (Pathogen data sheet, Section 3); however, there was no overall agreement in the expert panel on the relevance of this event as an exposure risk for HBV in the EU/EEA. Inadequate infection prevention and control measures in the healthcare setting may increase the risk of exposure to HBV, although such situations are not considered frequent in the EU/EEA by the members of the expert panel. Specifically for donors of reproductive cells, patients undergoing haemodialysis or who have received a SoHO transfusion or transplantation are unlikely to donate in a time frame consistent with the risk of a window period transmission. Similarly, the risk of exposure to HBV following new tattoos or piercings was considered very low by the expert panel since authorised facilities in the EU/EEA are considered to follow appropriate hygienic measures. However, the risk of infection may be increased for events performed in situations with suboptimal hygienic conditions. Considering the high likelihood of transmission of HBV and the long-term consequences of the disease, based on expert opinion, ECDC advises considering all the above risk events when assessing donor eligibility. These events may be unlikely for some donors of reproductive cells or can be challenging to assess through interviews, particularly in the context of risk events concerning the partner, as well as the correct usage of condoms, as condom failure is not always recognised [63].

It is important to note that the most documented transmission mode of acute HBV infection in the EU/EEA is sexual (Pathogen data sheet, Section 3). In this perspective, condomless sex with new or multiple partners with an unknown HBV infection status in areas with high prevalence of HBV could be considered as a risk of exposure to HBV. This risk is not listed in the advice and practical considerations above, as it could lead to unnecessary donor loss in low-prevalence countries; however, it could be considered in areas with high prevalence of HBV infection. Active STI are associated with an increased likelihood of carrying HBV (Pathogen data sheet, Section 3). In the available evidence, the specific infections associated with HBV are not comprehensively described, but include infections with *T. pallidum*, *Chlamydia trachomatis*, or *Neisseria gonorrhoeae*.

There was no general agreement in the expert panel on taking into account the vaccination status of the donor against HBV when assessing the recent risks of exposure. While complete HBV vaccination is effective and provides lifelong immunity, breakthrough infections can occur [64,65]. An accurate assessment of a complete HBV vaccination schedule (three doses) was also considered challenging and could lead to misclassification of partial vaccination profiles as fully vaccinated. In addition, vaccination against HBV for individuals at risk of HBV infection was also identified as a potential safety concern for the expert panel which did not support taking into account vaccination status when assessing recent risks of exposure. As a result of these discussions, no specific considerations for HBV vaccination were included in the assessment of recent risks of exposure.

Testing limitations in case of risk of exposure to HBV

Reproductive cells: third-party donations

Given the high performance of the HBV screening tests required in these guidelines, the risk for transmission through SoHO is related to newly infected potential donors tested during the window period (Pathogen data sheet, Section 2). To avoid window period transmissions from newly infected donors, donors who have been at risk of exposure to HBV should not be tested until the test results are considered reliable.

Based on expert opinion, a multiplication factor of the window period should be considered to ensure reliability of the test results and reduce the risk of a window period transmission after a recent infection. The risks of exposure identified in this section do not address the risk of transmission in the OBI phase.

A period of 16 weeks after an event with a risk of exposure to HBV covers more than three times the maximum window period of HBV DNA NAT and HBsAg, including when considering testing in pools (Pathogen data sheet, Sections 2 and 4). If a highly sensitive HBV DNA NAT is used, as HBsAg is unlikely to be present if the viraemia is undetectable (Pathogen data sheet, Sections 2 and 4), this period can be reduced to eight weeks.

Risk of exposure to HBV and considerations for testing

Within-relationship use

Similarly to what is described for donors, test results cannot be considered fully reliable before a minimum period of 16 weeks after an event with a risk of exposure to HBV. If one of the partners within the relationship had an event with an increased risk of exposure to HBV less than 16 weeks prior to testing for HBV, ECDC advises re-testing the individual for HBV after a minimum period of 16 weeks from the event to ensure the reliability of the test results.

Limitations

The events with increased risk of exposure to HBV are based on targeted literature searches and not on systematic literature reviews and may not be comprehensive. The interval between potential exposure to HBV and testing to be considered to ensure reliability of the test results is based on an arbitrary multiplication factor of the window period; however, it is consistent with the deferral periods currently used in several EU/EEA countries where transmissions of HBV are rare events (Pathogen data sheet, Section 7). It may not be possible to transpose these events/wording directly to the donor eligibility assessment, and adaptations may be needed in particular to account for the acceptability and feasibility of including these questions and limit potential donor loss.

Donor eligibility is assessed for all pathogens at once, and the list of events in the current guidelines only covers HBV. The list of events with an increased risk of exposure to other pathogens will be completed by ECDC as new guidelines are published. Pending these updates, the EDQM guide to the quality and safety of tissues and cells for human application can be used as a resource for additional events to consider in the assessment of donor eligibility [4].

Affected individuals and other situations to consider

Requirements and recommendations

Reproductive cells: third-party donations

Required:

- Individuals with a medical diagnosis of chronic HBV infection or receiving treatment for chronic HBV infection should not be considered eligible for donation and should be deferred until HBsAg loss, undetectable HBV DNA, and anti-HBs concentrations demonstrating immunity is documented in the context of clinical follow-up.

Advice and practical considerations:

- In case of uncertainty of a prior medical diagnosis of HBV infection, the prospective donor could be tested for anti-HBc as described in the section Testing of partners within-relationship use and third-party donors.
- Regarding individuals meeting the criteria to re-enter screening procedures after a medical diagnosis of chronic HBV infection, ECDC advises testing all future donations of these donors for anti-HBs, HBsAg, and with a highly sensitive HBV DNA NAT, including for donations that are quarantined.

Reproductive cells and tissues: within-relationship use

Advice and practical considerations:

- Individuals with an acute or chronic HBV infection:
  - Proceeding with the within-relationship use is to be discussed with the couple and the clinical team, including a specialist in HBV care; please refer to ESHRE guidelines [2].
  - Procedures should be implemented to prevent infection of the partner and the offspring; please refer to ESHRE guidelines [2].

Evidence and justification

Third-party donations

Individuals with a medical diagnosis of chronic HBV have a low likelihood of spontaneous remission. Chronic HBV infections may progress to an HBsAg-negative phase or 'occult HBV infection', where very low levels of viraemia of HBV DNA may not be identified through testing, but could nonetheless lead to transmission through the use of SoHO, as evidenced through liver transplantation [66] and blood transfusion (Pathogen data sheet, Sections 2 and 4). Treatment for chronic HBV is not curative, and while it could lead to undetectable levels of HBV DNA, a risk of transmission cannot be excluded. Based on evidence and expert opinion, considering the severity and long-term consequences of the disease, the route of transmission, and the low infectious dose of HBV, all individuals with a known diagnosis of chronic HBV or treatment for chronic HBV should be deferred to reduce the risk of transmission. Eligibility for donation should only be considered for individuals with a documented loss of

HBsAg and clearance of HBV DNA in clinical follow-up, as well as concentration levels of anti-HBs demonstrating immunity. An anti-HBs concentration of 10 IU/L or above is generally considered to provide immunity to HBV after vaccination [60]. However, to ensure the safety of future donations for all recipients, a concentration of 100 IU/L or above could be considered for individuals re-entering screening procedures after a medical diagnosis of chronic HBV infection.

There is no evidence of transmission through oocyte MAR treatment (Pathogen data sheet, Section 7). However, as precautionary measures and to contribute to the safety of healthcare workers, the requirements also apply for third-party oocyte donors.

Within-relationship use

HBV can be transmitted from mother-to-child. There is also a risk for horizontal transmission for partners and individuals in the household (Pathogen data sheet, Section 2). To protect the partner and the offspring, it is recommended to implement procedures to prevent the risk of infection and follow ESHRE's guidelines for medically assisted reproduction in patients with a viral infection/disease [2].

Next steps

ECDC will update these guidelines when significant new evidence becomes available or if the scope of the guidelines should be expanded to cover the needs of SoHO entities, such as considerations on pre-analytical requirements, or to cover additional SoHO currently not in scope of these guidelines.

An important step towards the harmonisation of SoHO safety in the EU/EEA could be accomplished by defining a common threshold for the residual risk of transmission of HBV through SoHO application, particularly for blood and blood components. A common threshold for residual risk would ensure a similar level of safety in each country, considering the endemicity of the disease in the country, and the local organisation of donor screening. As such, a maximum required threshold for a residual risk of transmission would impact the content of the present guidelines and lead to an update of this document. Defining such a threshold would require an agreement across EU/EEA countries.

ECDC will also follow significant developments in the epidemiology (e.g. changes in associated risks), in the prevention, in the available laboratory screening test methods for SoHO donors, and in the treatment of HBV that may significantly change the assessments in the current guidelines. During meetings of the ECDC SoHO network, on the basis of such developments, ECDC will evaluate, with the support of the SoHO network, the need for an update of these guidelines.

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Annex

Legal background

On 14 July 2024, the new regulation on standards of quality and safety for substances of human origin intended for human application was published. After entry into force, in August 2027 for most provisions, the regulation will repeal the Blood Directive (2002/98/EC) and the Tissues and cells Directive (2004/23/EC).

Standards for the quality and safety of SoHO, including the prevention of communicable diseases transmission, are currently defined in the directives. In the SoHO regulation, guidelines for the implementation of standards for the prevention of communicable diseases transmission through SoHO are no longer listed. The regulation establishes the ECDC as an expert body for developing and updating technical guidelines on the safety and quality of SoHO from a communicable disease threat perspective. With regards to standards concerning donor, recipient and offspring protection, the regulation stipulates in the absence of Union legislation describing particular procedures to be applied and followed to meet the standards set out in this regulation, following the guidelines of the ECDC regarding communicable disease transmission through SoHO donation and EDQM for issues of quality and safety beyond the risks of communicable disease transmission should be considered as a means to demonstrate compliance with the standards laid down in the regulation to ensure high level of quality, safety and efficacy. SoHO entities should be permitted to follow other guidelines, provided that it has been demonstrated that those other guidelines achieve the same level of quality, safety and efficacy.

These guidelines support the regulation in the prevention of communicable disease transmission from donors through SoHO in the EU/EEA.

Detailed guideline development process and methods

Ad hoc expert panel

Selection of the panel

The ad hoc scientific expert panel members for this guideline were identified through the ECDC Expert Directory, suggestions from the ECDC Advisory Forum, ECDC experts and ECDC Coordinating Competent Bodies. A call for interest was sent through ECDC relevant networks (SoHO, Emerging and vector-borne diseases, HIV/AIDS, sexually transmitted infections and hepatitis B/C, and the Microbiology network) as well as SoHO professional associations (European Association of Tissue and Cell Banks (EATCB), European Blood Alliance (EBA), European Society of Human Reproduction and Embryology (ESHRE), International Society of Blood Transfusion (ISBT), International Plasma and Fractionation Association (IPFA), European eye bank association (EEBA), European Hematology Association (EHA), European Group for Blood & Marrow Transplantation (EBMT), International Council for Commonality in Blood Banking Automation (ICCBBA), International Haemovigilance Network (IHN), Nordic cryobank group, World Marrow Donor Association (WMDA)). The call for interest was also sent to the EU/EEA national competent authorities for SoHO.

The expert panel members were selected by ECDC based on their expertise in the technical field of the guidelines and their professional skills. Panel members were expected to have experience in evidence-based decision-making. The selected experts primarily come from the clinical field and public health institutes. While selecting experts, ECDC has ensured sufficient representation for the different types of SoHO as well as geographical representativeness. The principles of diversity, equity, and inclusion, and the absence of conflict of interests have been applied.

Following a selection based on the criteria described above, all panel members signed a declaration of interest, which has been reviewed by the ECDC expert responsible for the panel with the help of ECDC compliance office. One expert (Ana Avellón) received research funding from Diasorin, and one expert (Silvia Sauleda) received funding from Grifols. The following mitigation measures were proposed for these two experts: no participation in final advice related to the choice of test methods and careful monitoring of participation by ECDC.

The EDQM, also cited as an expert body establishing guidelines in the regulation, was represented by two observers selected by EDQM in the scientific expert panel.

The ECDC Advisory Forum was consulted regarding their opinion on the suitability of the proposed members of the panel, prior to formal appointment by the ECDC Director. The ECDC Advisory Forum had no objections to the proposed panel.

Terms of reference

The terms of reference of the ad hoc scientific panel, including a description of the requirements for the expert panel, are found in 'Supporting documents'.

Work procedures

ECDC prepared guideline statements on screening strategies, test methods for donors, and circumstances for deferring donors. These statements were discussed with the scientific expert panel during four virtual meetings between May 2024 and September 2024. Pre-meeting surveys were sent prior to each meeting including questions on the following topics:

- Which SoHO donors should be tested for HBV?
- When should SoHO donors be tested for HBV?
- Which laboratory screening tests should be used for the testing of SoHO donors with regards to HBV?
- What limit of detection that should be applied for HBV DNA NAT?
- What actions should be performed in case of reactive screening tests, including the deferral of donors?
- Which risks of exposure to HBV are considered relevant for SoHO safety and need to be considered in the SoHO donor assessment?
- What deferral period should be considered for donors with events leading to a risk of exposure to HBV?

All meetings also covered and discussed similar questions for hepatitis C virus as both pathogens were addressed at the same time.

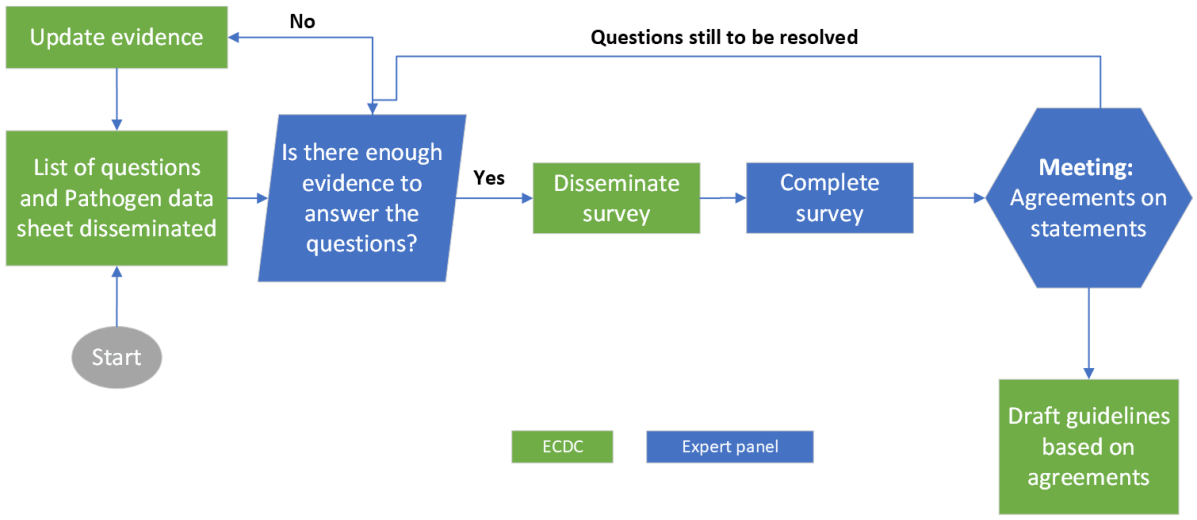
Supporting the development of these guidelines and discussions with the scientific expert panel, a 'pathogen data sheet' on HBV containing relevant epidemiological, microbiological, and clinical information was developed by ECDC based on methods detailed in the pathogen data sheet document (see Pathogen data sheet). For each question posed by ECDC, the panel assessed if there is sufficient evidence to support a reply to the question with the possibility of requiring additional evidence from ECDC prior to each meeting. Where no or limited evidence was available, this was communicated during meetings to the expert panel.

Pre-meeting survey results were presented and discussed during meetings, if results indicated a general consensus on a topic, corresponding statements were proposed by ECDC for agreement. Due to the limited number of experts per SoHO type, agreements were not reached through consensus voting but through the confirmation of the absence of major disagreements for a statement. All meetings with the scientific expert panel were virtual and audio-recorded for the purpose of drafting meeting minutes; recordings were deleted after the minutes were finalised. All experts have accepted the terms of reference, including an agreement to the audio recording of the panel meetings. Draft meeting minutes were circulated to the entire expert panel for comments and all members (including those who could not participate in the meeting) were invited to comment on the minutes and in particular on the agreed statements. The final minutes were then made available to the expert panel and to the observers.

The final meeting minutes served as reference documents for the drafting of the guidelines. In the case of serious disagreements that could not be resolved, the agreed process was to submit the subject to SoHO-Net for consultation. However, no unresolved disagreements were encountered during the development of the HBV statements.

Figure 1 describes the overall working procedures for agreements on statements with the expert panel.

Figure 1. Overall working procedures for agreements on statements



Responsibilities of ECDC during guidelines development

During the development of these guidelines, the responsibility of ECDC was to:

- Select and establish an independent ad hoc scientific expert panel to support ECDC in the development of the guidelines.
- Provide a 'pathogen data sheet' for HBV.
- Provide additional evidence upon request of the scientific expert panel.
- Provide technical and secretarial support to the meetings.
- Draft and finalise meeting minutes concluding on agreements reached by the scientific expert panel on donor testing and deferral strategies (including deferral periods) and testing methods for the group of pathogens covered by the terms of reference.
- Draft technical guidelines on the prevention of HBV transmission through SoHO and coordinate the review of the draft guidelines with the ECDC SoHO network.
- Coordinate the consultation of external stakeholders on the final draft of the technical guidelines on the prevention of HBV transmission through SoHO.
- Preparation of the final guideline document.

Responsibilities of scientific expert panel members during guidelines development

- Attend the meetings, according to availability, or provide their contribution by other means.
- Critically review evidence provided by ECDC in the pathogen data sheets.
- Provide additional evidence relevant to the discussion.
- Define the need for additional evidence, if required, to support panel discussions on specific pathogens.
- Provide expertise on donor selection, screening testing, and testing methods.
- Provide feedback as requested via surveys sent out by the ECDC SoHO team.
- Provide ECDC with expert advice on testing strategies and methods and deferrals (including deferral periods) for the pathogens under discussion.
- Help to develop and give opinions on proposals on testing and deferral strategies.
- Review draft meeting minutes covering discussions and agreements reached by the panel, to ensure alignment with the best practical in the field.
- Provide and keep up to date, at least on a yearly basis, declarations of interest throughout the guideline development process, including informing in a timely fashion ECDC of any potential conflict of interest that might affect their decisions and/or actions.

The expert panel also supported ECDC in the resolution of comments from external stakeholders.

Responsibilities of the observers during guidelines development

- Attend the meetings, according to availability, or provide their contribution by other means.
- Liaise with the EDQM guide working groups to avoid gaps and inconsistencies between the ECDC technical guidelines and the EDQM blood and tissues and cells guides.
- Treat in strictest confidence and not make use of or divulge to third party any information or documents which are linked to the tasks of the scientific expert panel. To continue to be bound by this undertaking after completion of the tasks unless it becomes public.
- Provide and keep up to date, at least on a yearly basis, declarations of interest throughout the guideline development process, including informing in a timely fashion ECDC of any potential conflict of interest that might affect their decisions and/or actions.

Review and stakeholder consultation

The draft guidelines were sent for review to the ECDC SoHO network and the comments from the ECDC SoHO network have been addressed. These guidelines were also sent to the list of stakeholders included in the list of stakeholder organisations interested in participating in ad hoc meetings with representatives of members of the Competent Authorities on Substances of Human Origin Expert Group. The guidelines were sent at the same time for consultation to third parties, e.g. EDQM, EMA, and WHO. The comments from the stakeholders and third parties have been addressed.

The final guidelines were sent for consultation regarding the scientific excellence and independence of activities and opinions to the ECDC Advisory Forum.

Methods of evidence collection and synthesis

The pathogen data sheet supporting the statements in the present technical guidelines consists of eight separate sections:

- Description of the pathogen;
- Disease description;
- Epidemiology of the disease;
- Laboratory testing approaches;
- Current testing requirements in EU/EEA countries;
- Recommendations from other organisations;
- Transmission through SoHO; and
- Pathogen reduction.

Below is a description of how the evidence was systematically searched for and selected, including the different information sources used and criteria for inclusion or exclusion of the evidence found for the first seven sections in the pathogen data sheet.

The searches were conducted for both HBV and HCV using a single search strategy comprising both viruses. The evidence was analysed in two separate data sheets.

Section 1: Description of the pathogen

Search questions and objectives:

- The objectives of this section are to describe the biological characteristics of HBV and to describe the pathogenesis of HBV.

General search strategy:

- As this section aimed to provide a general overview of the pathogen, the search strategy was limited to a targeted review with pre-selected sources.

Section 2: Disease description

Search questions and objectives:

- The objectives of this section are to describe the disease, including severity, long-term outcomes, diagnostic possibilities, duration of infectivity and infectious dose, and treatment options.

General search strategy:

- As this section aimed to provide a general overview of the disease, the search strategy was limited to a targeted review with pre-selected sources.

Section 3: Epidemiology

Search questions and objectives:

- The objectives of this section are to describe the prevalence and incidence of HBV infections in EU/EEA countries in the general population and the SoHO donor population; to describe known risk factors for HBV; to describe any other relevant issue related to SoHO safety and HBV (e.g. vaccination).

General search strategy:

- As this section aimed to provide a general overview of the incidence and prevalence of HBV in EU/EEA countries and provide an overview of the risk factors of the infection and did not intend to answer a specific question, the search strategy was limited to a targeted review with pre-selected sources.

Section 4: Laboratory testing approaches

Search questions and objectives:

- To describe the characteristics and test accuracy properties of laboratory tests that are approved or used for the screening of HBV or HCV in SoHO donors (living and deceased donors).
- To describe the test accuracy properties of the NAT tests approved or used for the screening of HBV or HCV in blood donations according to pooled or individual donation use.
  - Population: SoHO donors.
  - Intervention: HBV or HCV tests used for the screening of SoHO donors.
  - Comparators = reported reference standards.
  - Outcome: HBV and HCV test accuracy metrics: sensitivity (clinical and analytical) and specificity, genotype and variants detection capability, window period.

Search and eligibility:

- Searches were restricted from January 2001 to the present and will cover MEDLINE only.
- Customised searches of grey literature using generic web search engines (e.g. Google) combined with searches in targeted websites were also conducted. Only publications available in English were considered, and letters and commentaries, conference abstracts, case reports, and case series were excluded.

Index tests considered for inclusion were:

- Enzyme immunoassays (EIA);
- Enzyme-linked immunosorbent assay (ELISA);
- Indirect Fluorescent Antibody assay (IFA);
- Chemiluminescent immunoassay (CMIA or ChLIA/CLIA);
- Nucleic acid amplification test (NAT).

No reference standard was prespecified.

Additional eligibility criteria:

- In-house (i.e. not commercial) tests were in scope if they were used in EU/EEA countries. In-house tests used outside EU/EEA were excluded as not considered relevant for the EU/EEA context.
- Studies reporting accuracy metrics in the context of proficiency testing were excluded.

Main outcomes:

- Type (e.g. ELISA, EIA, NAT);
- Target (e.g. HBsAg);
- Manufacturer;
- Sensitivity: clinical and analytical;
- Genotype and mutant capacity detection;
- Specificity;
- Window period;
- Performance (specificity in plasma/serum collected postmortem).

Data extraction:

- Studies were assessed for relevance, first by title/abstract and then by full text, excluding at each step studies which did not satisfy the inclusion criteria. The studies were assessed by a single reviewer. Data was extracted by a single reviewer using a standardised data extraction form, but the extracted data were reviewed by a second reviewer.

Strategy for data synthesis:

- The extracted data were described in a tabular format; no meta-analysis was conducted. The outcome data were presented by the type and target of the test. Test accuracy metrics that were not reported but could be calculated from the reported information were calculated.

No risk of bias assessment was performed.

Analysis of subgroups or subsets:

- Donor type (living, deceased) sample

Keywords for the search (PubMed):

Concept	No.	Query	Results
HBV or HCV	1	"hepatitis b virus"[MeSH Terms] OR "Hepatitis B/diagnosis"[Mesh] OR "Hepacivirus"[MeSH Terms] OR "Hepatitis C/diagnosis"[Mesh] OR "hepatitis b"[text word] OR "Hepatitis C"[text word] OR Hepacivirus*[tw] OR "hepatitis c-like virus*"[tw] OR HBV[tw] OR HCV[tw]	200,796
SoHO	2	"Tissue Donors"[Mesh] OR "Tissue Transplantation"[Mesh] OR "Blood Transfusion"[Mesh] OR "donor*"[Title/Abstract] OR "donat*"[Title/Abstract] OR "transfus*"[Title/Abstract] OR "transplant*"[Title/Abstract] OR "tissue graft*"[Title/Abstract]	1,080,936
Test methods	3	"polymerase chain reaction/methods"[MeSH Terms] OR "enzyme linked immunosorbent assay/methods"[MeSH Terms] OR "immunoassay/methods"[MeSH Terms] OR (("screen*"[Title/Abstract] OR "test*"[Title/Abstract] OR "assay*"[Title/Abstract] OR "detect*"[Title/Abstract] OR "diagnos*"[Title/Abstract] OR "immunoassay*"[Title/Abstract]) AND ("antigen*"[Title/Abstract] OR "antibod*"[Title/Abstract] OR "serolog*"[Title/Abstract] OR "sero log*"[Title/Abstract] OR "PCR"[Title/Abstract] OR "nucleic acid amplification*"[Title/Abstract] OR "polymerase chain reaction*"[Title/Abstract] OR "seroconvert*"[Title/Abstract] OR "EIA"[Title/Abstract] OR "ELISA"[Title/Abstract] OR "IFA"[Title/Abstract] OR "CMIA"[Title/Abstract] OR "CLIA"[Title/Abstract] OR "ChLIA"[Title/Abstract] OR "NAT"[Title/Abstract] OR "NATs"[Title/Abstract] OR "multiplex"[Title/Abstract]))	1,343,665
Accuracy metrics	4	"Sensitivity and Specificity"[MeSH Terms:noexp] OR "Predictive Value of Tests"[MeSH Terms] OR "sensitivity"[Title/Abstract] OR "specificity"[Title/Abstract] OR "negative predictive value*"[Title/Abstract] OR "positive predictive value*"[Title/Abstract] OR "characteristic*"[Title/Abstract]	3,351,914
All	5	#1 AND #2 AND #3 AND #4	1,683
All, >2001	**6**	**#5 AND 2001:3000 [dp]**	**995**

Section 5: Current testing requirements in EU/EEA countries

Search questions and objectives:

- The objectives of this section are to describe the laboratory testing procedures for blood donors and for tissue and cell donors in use in EU/EEA countries.

General search strategy:

- Data published by the European Directorate for the Quality of Medicines & HealthCare on the collection, testing and use of blood and blood components in Europe.
- Data published by the European Commission on the Mapping of More Stringent Blood Donor Testing Requirements (Mapping Exercise 2015).
- Input from the scientific expert panel.

Section 6: Recommendations from other organisations

Search questions and objectives:

- The objectives of this section are to describe the recommendations for the prevention of transmission of HBV through the application of SoHO from relevant organisations.

General search strategy:

- As this section aimed to describe recommendations published by recognised organisations and authorities in the field of SoHO, the search strategy was limited to a targeted review with pre-selected sources. The following organisations were considered:
  - European Commission (EC);
  - European Directorate for the Quality of Medicines & HealthCare (EDQM);
  - US Food and Drug Administration (FDA);
  - Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (JPAC); and
  - World Health Organization (WHO).

Section 7: Transmission through SoHO

Search questions and objectives:

- The objectives of this section are to provide evidence of demonstrated transmission of HBV through SoHO and to describe the number of HBV transmissions through SoHO in the EU/EEA.

General search strategy

- As this section aimed to describe the possibility of transmission through SoHO rather than provide a comprehensive overview of all transmission events, the search strategy was limited to a targeted review, including a search on the Notify library (www.notifylibrary.org).
- The number of HBV transmissions through SoHO in EU/EEA was based on Serious Adverse Reactions and Events (SARE) data provided by the European Commission for the period from 2017 to 2022.

Section 8: Processing and pathogen inactivation approaches

Search questions and objectives:

- To describe the effectiveness properties, specific to HBV and HCV, of pathogen inactivation or reduction methods that are approved or used in each type of relevant SoHO: blood (including all blood products), tissues, or cells (including reproductive cells).
  - Population: SoHO.
  - Intervention: pathogen inactivation or reduction methods.
  - Comparator: not applicable.
  - Outcomes: pathogen reduction values reported for HBV and HCV.
- To describe the impact of specific SoHO processing steps on HBV or HCV levels in each type of relevant SoHO: blood (including all blood products), tissues, or cells (including reproductive cells).
  - Population: SoHO.
  - Intervention: processing steps:
  - Sperm wash;
  - Density gradient centrifugation;
  - Filtration;
  - Freezing;
  - Lyophilisation;
  - Glycerolization;
  - Vitrification.
  - Comparator: not applicable.
  - Outcomes: pathogen reduction values reported for HBV or HCV.

Searches and eligibility:

- Searches were restricted from January 2001 to the present and covered MEDLINE only. Customised searches of grey literature using generic web search engines (e.g. Google) combined with searches in targeted websites were also conducted.
- Only publications available in English were considered, and letters and commentaries, conference abstracts, case reports, and case series were excluded.

Main outcomes:

- Reduction/inactivation methods:
  - Qualitative assessment of effectiveness (e.g. pathogen no longer detectable);
  - Quantitative assessment of effectiveness (e.g. log reduction).
- Processing methods:
  - Qualitative assessment of impact on pathogen reduction;
  - Quantitative assessment of impact on pathogen reduction (e.g. log reduction).
- Data extraction:
  - Studies were assessed for relevance, first by title/abstract and then by full text, excluding at each step studies which do not satisfy the inclusion criteria. The studies were assessed by a single reviewer. Data were extracted by a single reviewer using a standardised data extraction form.
- Strategy for data synthesis:
  - The extracted data were described in a tabular format and no meta-analysis was conducted. The data corresponding to the protocol outcomes were presented by the type of reduction/inactivation or processing method.

No risk of bias assessment was performed.

Analysis of subgroups or subsets:

- SoHO type, processing method

Keywords for the search (PubMed):

- Search strategy – reduction or inactivation

Concept	No.	Query	Results
Donors and SoHO	1	"Tissue Donors"[Mesh] OR "Tissue Transplantation"[Mesh] OR "Blood Transfusion"[Mesh] OR "donor*"[Title/Abstract] OR "donat*"[Title/Abstract] OR "transfus*"[Title/Abstract] OR "transplant*"[Title/Abstract] OR "graft*"[Title/Abstract] OR "soho*"[Title/Abstract] OR "mpho*"[Title/Abstract] OR "blood"[Title/Abstract] OR "cell*"[Title/Abstract] OR "tissue*"[Title/Abstract] OR "plasma"[Title/Abstract] OR "cornea*"[Title/Abstract] OR "bone*"[Title/Abstract] OR "tendon*"[Title/Abstract] OR "skin"[Title/Abstract] OR "islet*"[Title/Abstract] OR "valve*"[Title/Abstract] OR "rbc"[Title/Abstract] OR "platelets"[Title/Abstract] OR "sperm*"[Title/Abstract] OR "oocyte*"[Title/Abstract]	11,271,417
Pathogen reduction or inactivation	2	"pathogen"[Title/Abstract] AND ("reduction"[Title/Abstract] OR "inactivation"[Title/Abstract] OR "solvent-detergent"[Title/Abstract] OR "methylene blue"[Title/Abstract] OR "ultraviolet"[Title/Abstract] OR "amotosalen"[Title/Abstract] OR "alkylating agents"[Title/Abstract] OR "washing"[Title/Abstract] OR "riboflavin"[Title/Abstract] OR "uv light"[Title/Abstract]) AND (effect*[Text Word] OR effic*[Text Word] OR impact[Text Word])	9,158
Virus, HBV and HCV	3	"hepatitis b virus"[MeSH Terms] OR "Hepatitis B/diagnosis"[Mesh] OR "Hepacivirus"[MeSH Terms] OR "Hepatitis C/diagnosis"[Mesh] OR "hepatitis b"[text word] OR "Hepatitis C"[text word] OR Hepacivirus*[tw] OR "hepatitis c-like virus*"[tw] OR HBV[tw] OR HCV[tw]	203,367
All	4	#1 AND #2 AND #3	80
All, >2001	**5**	**#4 AND 2001:3000 [dp]**	**77**

Search strategy – processing methods

Concept	No.	Query	Results
Donors and SoHO	1	"Tissue Donors"[Mesh] OR "Tissue Transplantation"[Mesh] OR "Blood Transfusion"[Mesh] OR "donor*"[Title/Abstract] OR "donat*"[Title/Abstract] OR "transfus*"[Title/Abstract] OR "transplant*"[Title/Abstract] OR "graft*"[Title/Abstract] OR "soho*"[Title/Abstract] OR "mpho*"[Title/Abstract] OR "blood"[Title/Abstract] OR "cell*"[Title/Abstract] OR "tissue*"[Title/Abstract] OR "plasma"[Title/Abstract] OR "cornea*"[Title/Abstract] OR "bone*"[Title/Abstract] OR "tendon*"[Title/Abstract] OR "skin"[Title/Abstract] OR "islet*"[Title/Abstract] OR "valve*"[Title/Abstract] OR "rbc"[Title/Abstract] OR "platelets"[Title/Abstract] OR "sperm*"[Title/Abstract] OR "oocyte*"[Title/Abstract]	11,271,417
Processing methods	2	(("pathogen"[Title/Abstract] OR "safety"[Title/Abstract] OR "microb*"[Title/Abstract]) AND ("processing"[Title/Abstract] OR "wash*"[Title/Abstract] OR "density gradient centrifugation"[Title/Abstract] OR "filtration"[Title/Abstract] OR "freezing"[Title/Abstract] OR "lyophilis*"[Title/Abstract] OR "glyceroli"[Title/Abstract] OR "Vitrification"[Title/Abstract]))	46,206
Virus, HBV and HCV	3	"hepatitis b virus"[MeSH Terms] OR "Hepatitis B/diagnosis"[Mesh] OR "Hepacivirus"[MeSH Terms] OR "Hepatitis C/diagnosis"[Mesh] OR "hepatitis b"[text word] OR "Hepatitis C"[text word] OR Hepacivirus*[tw] OR "hepatitis c-like virus*"[tw] OR HBV[tw] OR HCV[tw]	203,367
All	4	#1 AND #2 AND #3	236
All, >2001	**5**	**#4 AND 2001:3000 [dp]**	**223**

Supporting documents

Terms of reference: expert panel

See supporting document 'Terms of reference for the scientific expert panel convened for the development of the ECDC technical guidelines on the prevention of donor-derived transmission of communicable diseases through Substances of Human Origin'.

Conclusions from the ad hoc expert panel meeting

Abridged versions of the meeting minutes containing only decisions reached during the meetings can be provided by ECDC upon request.

Pathogen data sheet

See the supporting document 'Data sheet to support the development of the ECDC technical guidelines on the prevention of donor-derived transmission of Hepatitis B Virus (HBV) through Substances of Human Origin (6 September 2024)'.

European Centre for Disease Prevention and Control (ECDC)

Gustav III:s Boulevard 40
16973 Solna, Sweden

Tel. +46 858 60 10 00
ECDC.info@ecdc.europa.eu

www.ecdc.europa.eu

Publications Office
of the European Union