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EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

PUBLIC HEALTH GUIDANCE

# Guidelines on the prevention of hepatitis C 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

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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.

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# Abbreviations

| | |
|---|---|
| Ag | Antigen |
| DAA | Direct-acting antiviral agent |
| 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 |
| EUDA | European Union Drugs Agency |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| 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 |
| RNA | Ribonucleic acid |
| SoHO | Substances of human origin (excluding solid organs)[1] |
| SVR | Sustained virological response |

[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 C virus (HCV) to recipients and offspring from medically assisted reproduction (MAR). HCV 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 HCV in EU/EEA countries underscores the need for a strategic approach to prevent the transmission of HCV 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 HCV, 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**<br>• Assessment of recent risks of exposure to HCV is required.<br>• Donors should not be tested in the context of donor evaluation before a period of at least eight weeks since the last event with a risk of exposure to HCV. | **Anti-HCV + HCV RNA NAT** | Anti-HCV and HCV RNA NAT reactive | Any | • Do not release the donation for clinical use.<br>• Notify the donor and refer to relevant clinical care.<br>• Permanent deferral.<br>• Initiate look-back procedures. |
| | | Anti-HCV or HCV RNA NAT reactive | Positive | |
| | | Anti-HCV negative and HCV RNA NAT reactive | Not confirmed positive[b] | • Do not release the donation for clinical use.<br>• Notify the donor and refer to relevant clinical care based on risk assessment.<br>• Re-entry in donor screening procedures possible after 26 weeks.<br>• Look-back procedures based on risk assessment. |
| | | Anti-HCV reactive and HCV RNA NAT negative | Indeterminate | |
| | | Anti-HCV reactive and HCV RNA NAT negative | Negative | • Do not release the donation for clinical use.<br>• Re-entry in donor screening procedures possible without a deferral period. |

*Anti-HCV: antibodies against HCV; HCV: hepatitis C virus; NAT: nucleic acid test; RNA: ribonucleic acid.*
*[a] Approaches to confirm screening test results should rely on nationally established algorithms, if possible.*
*[b] "Not confirmed positive" includes indeterminate, negative or not performed.*

## Tissues and non-reproductive cells – living and deceased donors

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

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[c] | Documented SVR12/24[d] | Actions |
| **All donors, at each donation[a]**<br>• Assessment of recent risks of exposure to HCV is required.<br>• Donors should not be tested in the context of donor evaluation before a period of at least eight weeks since the last event with a risk of exposure to HCV. | **Anti-HCV + HCV RNA NAT**<br>• A 95% LOD of 50 IU/mL or lower should be used for HCV RNA NAT.[b] | Anti-HCV reactive and HCV RNA reactive | Any | Any | • Do not release the donation for clinical use.<br>• Initiate look-back procedures if relevant.<br>• Notify the donor or the transplant coordination team.<br>• Living donors: refer to relevant clinical care. |
| | | Anti-HCV negative and HCV RNA NAT reactive | Positive | Any | |
| | | Anti-HCV reactive and HCV RNA NAT negative | Positive | No/Unknown | |
| | | Anti-HCV negative and HCV RNA NAT reactive | Not confirmed positive[e] | Any | • Do not release the donation for clinical use.<br>• Notify the donor or the transplant coordination team based on risk assessment.<br>• Living donors: refer to relevant clinical care based on risk assessment.<br>• Initiate look-back procedures if relevant, based on risk assessment. |
| | | Anti-HCV reactive and HCV RNA NAT negative | Indeterminate | No/Unknown | |
| | | Anti-HCV reactive and HCV RNA NAT negative | Positive or indeterminate | Yes | • Release donation for clinical use. |
| | | Anti-HCV reactive and HCV RNA NAT negative | Negative | Not applicable | |

*Anti-HCV: antibodies against HCV; HCV: hepatitis C virus; IU: international units; LOD: limit of detection; NAT: nucleic acid test; RNA: ribonucleic acid; SVR: sustained virological response.*

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

*[b] A higher LOD can be considered if justified by a risk assessment considering the endemicity of the disease.*

*[c] Approaches to confirm screening test results should rely on nationally established algorithms, if possible.*

*[d] Documented sustained virologic response at 12 weeks (SVR12) or 24 weeks (SVR24), in case of a donor history of hepatitis C (Description of HCV infection and disease, section Treatment for Hepatitis C). 'Any' includes presence or absence of documented SVR12/24, as well as if the information regarding treatment and SVR is unknown.*

*[e] "Not confirmed positive" includes indeterminate, negative or not performed.*

## Reproductive cells – third-party donations

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

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[c] | Actions |
| **All donors, at each donation[a]**<br>• Assessment of recent risks of exposure to HCV is required.<br>• Donors should not be tested in the context of donor evaluation before a period of at least eight weeks since the last event with a risk of exposure to HCV. | **Anti-HCV + HCV RNA NAT**<br>• A 95% LOD of 50 IU/mL or lower should be used for HCV RNA NAT.[b]<br>• In case of donations quarantined for ≥180 days: only an anti-HCV serological test is required. | Anti-HCV and HCV RNA NAT reactive | Any | • Do not release the donation for clinical use.<br>• Notify the donor and refer to relevant clinical care.<br>• Permanent deferral.<br>• Initiate look-back procedures. |
| | | Anti-HCV or HCV RNA NAT reactive | Positive | |
| | | Anti-HCV negative and HCV RNA NAT reactive | Not confirmed positive[d] | • Do not release the donation for clinical use.<br>• Notify the donor and refer to relevant clinical care based on risk assessment.<br>• Re-entry in donor screening procedures possible after 26 weeks.<br>• Look-back procedures based on risk assessment. |
| | | Anti-HCV reactive and HCV RNA NAT negative | Indeterminate | |
| | | Anti-HCV reactive and HCV RNA NAT negative | Negative | • Do not release the donation for clinical use.<br>• Re-entry in donor screening procedures possible without a deferral period. |

*Anti-HCV: antibodies against HCV; HCV: hepatitis C virus; IU: international units; LOD: limit of detection; NAT: nucleic acid test; RNA: ribonucleic acid.*

*[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 one week after the last donation.*

*[b] A higher LOD can be considered if justified by a risk assessment considering the endemicity of the disease.*

*[c] Approaches to confirm screening test results should rely on nationally established algorithms, if possible.*

*[d] "Not confirmed positive" includes indeterminate, negative or not performed.*

## Reproductive cells and tissues – within-relationship use

For additional information and 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**<br>• Less than three months before collection.<br>• Maximum of 24 months between tests. | **Anti-HCV** | Anti-HCV reactive | Positive or indeterminate | • Procedures should be implemented to prevent the risk of infection to the partner and to the offspring.<br>• Refer to ESHRE guidelines on medically assisted reproduction in patients with a viral infection/disease [2]. |

*Anti-HCV: antibodies against HCV; ESHRE: European Society of Human Reproduction and Embryology; HCV: hepatitis C virus.*

# 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 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 or 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 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 well-being 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 C virus (HCV) 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 HCV;
- Requirements and recommendations on testing strategies for HCV; 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 HCV.

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 HCV transmission from donors through SoHO intended for industrial manufacturing, such as plasma for fractionation. The risk of HCV 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 HCV, 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 HCV 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 guidelines 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 HCV (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 'advised' 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 guidelines development

The development of these technical guidelines on the prevention of HCV 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 the 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 HCV 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 HCV (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 HCV 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 HCV 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 HCV?
- When should SoHO donors be tested for HCV?
- Which laboratory screening tests should be used to test SoHO donors for HCV?
- What limit of detection (LOD) should be applied for nucleic acid tests (NAT) detecting HCV ribonucleic acid (RNA)?
- What actions should be performed in case of reactive screening tests, including the deferral of donors?
- Which risks of exposure to HCV 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 HCV 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 HCV technical guidelines, there were no major disagreements. For additional information on the guidelines development process and methods, see the Annex.

# HCV and the considerations for SoHO safety

The hepatitis C virus (HCV) is an enveloped, positive-sense, single-stranded RNA virus belonging to the Flaviviridae family. There are eight genotypes of HCV (1 to 8), with the dominant genotypes in Europe being 1 (the most common) and 3 [5]; in addition, in intravenous drug users, genotype 4 is also prevalent [6]. Genotypes can impact the natural course of the disease and treatment outcomes [7,8].

HCV is transmitted through exposure to infected blood or other bodily fluids, which may happen during exposure to contaminated syringes or medical equipment in a healthcare setting, exposure to contaminated syringes in the context of intravenous drug use, unprotected sexual contact with an infected partner, needlestick injuries or other injuries occurring outside a healthcare setting (e.g. bites, tattoos, piercings) or from an infected mother to the child during pregnancy or delivery [9,10]. HCV can also be transmitted through transfusion of infectious blood or blood components and plasma-derived medicinal products, as well as transplantation of different tissue types, cells, or human solid organs from an infected donor.

## Description of HCV infection and disease

HCV infection can present as acute or chronic, with chronic infection resulting from the persistence of the infection over time.

### Acute hepatitis C

Acute HCV infection can have different clinical presentations, ranging from asymptomatic (about 40-50% of cases) or clinically mild and typically unrecognised symptoms to icteric hepatitis [11]. Specific signs and symptoms of acute hepatitis C occur in a minority of individuals and encompass jaundice, nausea, abdominal discomfort, and anorexia, and are accompanied by an elevation of liver transaminases [11]. Detection of HCV RNA is usually possible within a week after infection. Serum HCV RNA levels exponentially increase (ramp-up phase) until reaching a peak, although some fluctuation of the viraemia (above 1000 IU/mL) can be observed in the first eight to 12 weeks post-infection, followed by a plateau that lasts for weeks or a decrease to lower levels (Pathogen data sheet, Section 2, [12,13]). Antibodies against HCV (anti-HCV) will develop on average after nine to 12 weeks, although longer periods for seroconversion (up to 160 days) have been reported in blood donors with detectable HCV RNA [14]. Nearly 97% of patients with acute HCV have detectable HCV antibodies six months after infection [15]. Spontaneous resolution (with a continuous decline in HCV RNA and viral clearance) occurs in approximately 15-25% of cases in the first few months and may be less frequent in specific groups, such as males, individuals with asymptomatic infection and older adults [16,17]. Certain genetic factors, such as polymorphisms in the IL28B gene and natural killer cell receptor genes, are associated with higher rates of spontaneous clearance [18,19].

### Chronic hepatitis C

Chronic hepatitis C is characterised by the persistence of HCV RNA in the blood after six months following the onset of acute infection [8,18]. Chronic HCV infection can lead to progressive fibrosis, cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC). If not treated, 20-30% of patients with chronic infection will eventually develop cirrhosis [18]. Biopsy studies suggest a 30-year period of fibrosis progression to develop cirrhosis, but the rate of progression can be affected by several factors, including coinfections (e.g. hepatitis B virus (HBV), human immunodeficiency virus (HIV), alcohol consumption, obesity, older age at infection, and male sex [8]). Cirrhosis can be complicated by ascites, spontaneous bacterial peritonitis, variceal haemorrhage, hepatic encephalopathy and HCC. Cirrhosis with complications is associated with a higher risk of death. Liver transplantation is a life-saving procedure and should be considered in patients with end-stage liver disease and patients with HCC [20].

HCV infection is also associated with numerous extrahepatic manifestations [21]. Infected individuals are more at risk of mixed cryoglobulinemia and B-cell non-Hodgkin lymphoma [22,23]. Other manifestations, such as cardiovascular disease, insulin resistance and renal insufficiency, have also been associated with HCV infection [23].

Some studies have identified individuals with viraemic seronegative HCV infections for prolonged periods (over one year), known as immunosilent carriers. These cases are described as very rare and highlight the added value of testing for HCV RNA in addition to serological tests in donor screening settings [24,25].

## Treatment for hepatitis C

The main goal of HCV treatment is the cure of the infection. Its primary endpoint is a sustained virological response (SVR) defined by undetectable HCV RNA in blood at 12 weeks (SVR12) and 24 weeks (SVR24) after the end of therapy, assessed by HCV RNA nucleic acid test (NAT) with a LOD ≤15 international units (IU) per ml [7].

The current treatment for HCV is based on direct-acting antiviral agents (DAA), used alone or in combination with ribavirin [7]. DAAs treatment can be prescribed in short courses (8–12 weeks), depending on the severity of the disease; they are well tolerated, and, when used with full adherence, result in cure rates upwards of 90% [26]. The most recent DAAs can be used across all genotypes and are recommended as first-line treatment [7].

Antiviral therapy also positively impacts extrahepatic manifestations, with SVR being associated with an improvement of extrahepatic morbidity and mortality or a reduction of the risk of developing these disorders [27,28].

HCV reinfection can occur after HCV clearance, whether spontaneous or following successful treatment. Reinfection should be suspected in case of reappearance of detectable HCV RNA or HCV core antigen after an SVR and confirmed by the demonstration that infection is caused by a different genotype or, using sequencing and phylogenetic analysis, by a distantly related strain of the same genotype from the initial infection [7].

# The risk of HCV infection through SoHO

Transmission of HCV through SoHO has previously been reported for blood and blood components, non-reproductive cells, and tissues [29-35]. The 50% minimum infectious dose, defined as the dose that infects 50% of recipients, is very low and estimated to be between seven and 20 HCV RNA copies [36]. Considering the low infectious dose and the viral dynamic progression of the infection, transmission of HCV can occur relatively early after exposure, since viraemia, leading to a potential infectious unit of blood, can be reached a few days post-exposure. In the early stage of HCV infection, viraemia increases exponentially, with an average doubling time of 10.8 hours (0.45 days), reaching peak viraemia levels of up to $10^8$ HCV RNA copies/mL [37,38].

Despite effective donor screening processes in EU/EEA countries and the highly sensitive tests available, eight HCV transmission events through SoHO were reported in the period from 2017 to 2022 in the serious adverse reactions and events reports published by the European Commission [39,40]. Seven of these events were reported following transfusion of blood and blood components, and one following tissues and cells transplantation (not otherwise specified). It should be noted that these cases might have occurred prior to the 2017–2022 period when they were reported.

Screening processes for HCV include a combination of thorough donor assessment, considering risks of recent exposure to HCV, and the use of sensitive laboratory testing methods. Laboratory test methods used to screen for HCV in SoHO donors encompass a range of serological assays detecting antibodies against HCV, or HCV antigen (HCVAg), as well as NAT for HCV RNA.

The window period, i.e. the minimal time from infection to a positive test result, varies depending on the test method. An HCV RNA NAT with a 95% LOD of 21.9 IU/mL (59.8 copies per mL, considering a conversion factor of 2.73 copies per IU) is considered to be associated with an estimated window period of 2.1 days [41]. The mean estimated window period for the detection of HCVAg is about nine days, while for anti-HCV it is estimated to be around 60 days [42].

The burden of hepatitis C in the EU/EEA remains high, with an estimated chronic hepatitis C prevalence of 0.5%, corresponding to nearly 1.8 million people [43]. Data on the incidence of hepatitis C or the prevalence of active HCV infection (viraemic individuals) are currently lacking for the general population. The most recent estimates from the European Union Drugs Agency (EUDA) reveal an overall prevalence of active HCV infection of 38% among people who inject drugs in EU/EEA countries, with no sustained declining trends in most countries reporting in the period 2015–2022 [44]. These estimates present several limitations and are not generalisable since they are based on a small number of studies with low to moderate levels of evidence [44]. A large majority of HCV infections are still undiagnosed. It is estimated that, in 2024, only 41% of viraemic HCV infections were diagnosed [45]. The asymptomatic profile of chronic hepatitis C, lack of awareness about HCV infection in the general population and healthcare personnel, structural and social barriers (such as stigma) may impair more effective screening and diagnosis of HCV infection [5].

The overall prevalence of HCV infection and the large proportion of people with chronic hepatitis C that remain undiagnosed in the EU/EEA warrant a strategic approach to prevent donor-derived transmission of HCV via SoHO. The standards for the prevention of HCV transmission through SoHO in the EU/EEA were established in Directive 2002/98/EC for blood and blood components and in Directive 2006/17/EC for tissues and cells [46-48]. Following the application of the SoHO regulation and the repeal of these directives and considering the potentially life-long consequences of an HCV infection, the severity of the disease, and its sustained incidence in EU/EEA countries, guidelines addressing the prevention of transmission of HCV 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) [49]. The results from screening tests used to detect HCV 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 serology and NAT tests 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 [49]. 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 [49]. 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 HCV 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 HCV 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. In these guidelines, the term 'positive confirmatory test results' include these additional test results verifying NAT reactivity; the term 'not confirmed positive' includes indeterminate or negative results from the algorithms in place to verify NAT reactivity, or the absence of these additional results when such algorithms are not in place. 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 clear laboratory errors [50]. In these situations, a pre-established algorithm can confirm inaccurate NAT results, and the sample could be considered negative for NAT.

Considering the overall high performance of HCV screening tests and based on expert opinion, concordant reactive results from both anti-HCV and HCV RNA NAT tests 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 HCV

### Requirements and recommendations

#### *All blood and blood components*

##### Testing requirements

**Required:**

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

##### Screening tests

**Required:**

- Donors should be tested with both an HCV RNA NAT and a serological test detecting antibodies against HCV.

**Advice and practical considerations:**

- ECDC advises basing the LOD for HCV RNA 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.

##### 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 anti-HCV and/or HCV RNA NAT should not be released for clinical use.
- In case of a positive confirmatory test result or where both anti-HCV and HCV RNA NAT are reactive in screening:
  - The donor should be notified and referred to relevant clinical care.
  - The donor should be deferred permanently.
  - Look-back procedures of previous, potentially infectious donations should be initiated.
- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV 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.

**Advice and practical considerations:**

- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV 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 the case of a negative confirmatory test result, and provided a negative HCV RNA NAT in screening, the donor can re-enter screening procedures without a deferral period.
- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV is reactive in screening and followed by an indeterminate confirmatory test result: the donor can re-enter donor screening procedures, but should not re-enter before a minimum period of 26 weeks from the last donor testing.

### 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 HCV.
- A residual sample of the last negative donation should be retested using highly sensitive NAT detecting HCV RNA. 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 HCV RNA NAT and was negative.
- 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. Recipients who received a donation found positive in the retesting of archived samples should be tested for HCV 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:**

- 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 for the recipient in case of HCV transmission, the experts agreed that all donors should be tested at each donation to reduce the risk of transmission through SoHO (Pathogen data sheet, Section 2).

### *Screening tests*

Based on existing evidence and expert opinion, considering the performance of HCV screening tests and window periods, the safest way to detect an HCV infection is to test donors with a combination of NAT for HCV RNA and serological tests detecting antibodies against HCV. The expert panel agreed on the advantages of using HCV RNA NAT to increase the safety of blood transfusion, as it allows a substantial reduction in the window period of the infection, compared to testing for anti-HCV, and the detection of HCV RNA is a consistent marker for chronic hepatitis C. This opinion is supported by evidence, as the window period is reduced from an average of nine weeks to less than seven days using an HCV RNA NAT with a 95% LOD below 400 IU/ml when compared to the detection of anti-HCV (Pathogen data sheet, Sections 2 and 4). HCV RNA NAT tests are considered, in the diagnostic setting, to have a very low probability of false negatives due to highly conserved regions of the virus, such as the 5'UTR (untranslated region) of the virus [7]. While there have been cases of transmission from donors with reported undetectable HCV RNA in the past, these are considered to be rare, with seven transmission events described in the period 2017-2022 in over 100 million units transfused in the EU/EEA (Pathogen data sheet, Sections 2 and 7). It should be noted that these cases might have occurred prior to the 2017–2022 period, when they were reported.

Similarly, the expert panel agreed on the use of serological tests detecting antibodies against HCV in the donor screening strategy, due to the possibility of having detectable anti-HCV with negative HCV RNA NAT, namely in the context of natural clearance of an acute HCV infection, during treatment with DAAs for chronic hepatitis C or after sustained virological response due to effective DAAs treatment (Pathogen data sheet, Section 2).

The window period of HCV RNA NAT can be reduced by increasing sensitivity (lowering the LOD). An HCV RNA NAT with a 95% LOD of 21.9 IU/mL (59.8 copies per mL, considering a conversion factor of 2.73 copies per IU) is associated with an estimated window period of 2.1 days [41]. Given the short doubling time of HCV (10.8 hours; Pathogen data sheet, Section 2), the window period of HCV RNA NAT is estimated to be around three days for a NAT with a 95% LOD of 50 IU/mL, and, for tests with lower sensitivity, this window period is expected to increase slightly (Pathogen data sheet, Section 4). According to expert opinion and available evidence, increasing the sensitivity of HCV RNA NAT may come with an increase in healthcare resource use costs [51-53]. In addition, aiming for high-sensitivity detection of HCV RNA may limit the possibility of performing pooled donation testing. It should also be noted that HCV transmission through transfusion of blood and blood components is rare in the EU/EEA (Pathogen data sheet, Section 7), including in countries relying on less sensitive HCV RNA NAT, allowing for pooled donation testing. Donor selection through a donor health questionnaire assessing the risk of recent exposure to HCV is considered to significantly reduce the risk of donors presenting with a recent HCV infection associated with a low viral load and undetectable antibodies (Pathogen data sheet, Section 3; expert opinion). Considering the elements

above and based on expert opinion, ECDC advises that the 95% LOD for HCV RNA NAT is based on a risk assessment considering the estimated residual risk. ECDC advises updating the risk assessment in case of significant changes to the HCV epidemiology (as defined by the NCA) or in case of an HCV 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.

There is no sustained evidence suggesting that using serological tests detecting antigen-only or both antigen-antibody combination tests against HCV in combination with HCV RNA NAT reduces the risk of transmission of HCV compared to using tests only detecting antibodies in combination with a sensitive HCV RNA NAT. Based on available evidence and expert opinion, antigen-only and antigen-antibody combination tests have, in general, lower sensitivity than antibody-only tests (Pathogen data sheet, Section 4), and, according to expert opinion, are generally less accessible than antibody-only tests. IVDR Class-D approved antigen-antibody combination tests that demonstrate performance characteristics comparable/equivalent to antibody-only tests may be used as alternatives to antibody-only tests (Pathogen Data Sheet, Section 4).

### *Outcome of test results*

Given the high performance of the HCV screening tests, reactive screening test results indicate a high likelihood that the donor is currently infected or has been infected with HCV, with a high risk for HCV 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, the expert panel agreed that donations from donors with reactive serological tests should not be released for clinical use due to the severe impact of an HCV transmission on the recipient. Hence, based on evidence and expert opinion, the donation from a donor with reactive HCV screening tests should not be released for clinical use.

In the case of a positive confirmatory test result, or if both the anti-HCV and HCV RNA NAT are reactive in screening, as well as in other situations where HCV 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.

All members of the expert panel considered that individuals with a prior diagnosis of acute or chronic hepatitis C should not be accepted as blood donors. Although an effective treatment for chronic hepatitis C exists, relapses and reinfection may occur (Pathogen data sheet, Section 3). Late relapses are also described in episodes of acute hepatitis C [54]. Although rare, cases of transmission through blood components from donors with reported undetectable HCV RNA in the past have been described (seven transmission events reported in the period 2017-2022 in over 100 million units transfused in the EU, although these cases may have occurred prior to this period) (Pathogen data sheet, Sections 2 and 7). The expert panel additionally remarked that the widespread use of DAAs is expected to reduce the transmission of the infection in the general population and, consequently, in the donor population. As a result, the number of individuals with a prior diagnosis of acute or chronic hepatitis C presenting for donation is expected to be lower in the future. In such a scenario, deferring these donors is not considered to pose a threat to the blood supply.

Due to the risk of transmission of HCV 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 anti-HCV and HCV RNA 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 HCV 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, considering 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 HCV, and therefore which samples and recipients require testing. Although samples from previous donations were found negative in screening, it is possible the donation was made at a moment prior to the seroconversion and with a viral load below the LOD defined for HCV RNA NAT in routine screening. Using a highly sensitive HCV RNA NAT for look-back procedures would support identifying low viral loads. In addition, re-testing negative donations using HCV RNA NAT increases the likelihood of detecting the virus in samples with low viral loads, and re-testing previous donations could be considered even if the previous donations were already tested with a HCV RNA NAT of similar sensitivity.

To ensure the safety of recipients, based on expert opinion, recipients who received a donation positive in retesting should be tested for HCV 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 HCV infection. As a single individual may have previously donated other SoHO, where possible look-back procedures could also consider other SoHO donations.

### *Criteria to re-enter donor screening procedures*

Due to the risk of HCV transmission (Pathogen data sheet, Section 2), a donor with a positive confirmatory test result, or where both the HCV RNA NAT and the serological screening tests are reactive, or where an infection cannot be ruled out, taking into account all information available, should be permanently deferred.

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

ECDC advises calling back the donor for further testing on a follow-up sample, when possible, in case of discordant results between a reactive HCV RNA NAT in screening and the results of additional test(s) performed to confirm the initial HCV RNA NAT results. A higher viral titre may be detected in a follow-up sample. This procedure is also advised in case of an indeterminate confirmatory test result, where only the anti-HCV is reactive in screening. For these donors, a minimum period of 26 weeks from the last donor testing before re-entry should be followed to formally exclude potential seroconversion (Pathogen data sheet, Section 2) [55]. Similarly, donors with a reactive anti-HCV and an indeterminate confirmatory test that cannot be resolved can re-enter donor screening procedures if more than 26 weeks have elapsed from the last donor test. Indeterminate confirmatory test results may represent early seroconversions or a waning antibody response to a spontaneously recovered HCV infection [56,57].

According to ECDC's opinion, HCV infection is highly likely in donors where both anti-HCV and HCV RNA 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 permanently and referred to relevant clinical care (i.e. treated as donors with a positive confirmatory test result).

## Limitations

The evidence is based on studies evaluating individual test performance and not the comparison of a combination of test methods. There are a limited number of studies on the accuracy of antigen-only tests and antigen-antibody combined tests for HCV, as well as on the comparison of those tests when used in combination with HCV RNA NAT. In addition, there is limited evidence on the window period and correlation with the standardised measures of the LOD for HCV RNA NAT.

# Risk of exposure to HCV 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 HCV transmission and risks of exposure to HCV, 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 HCV 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, which can be associated with needle sharing.

The events listed below aim to represent evidence-based risks of exposure to HCV 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 HCV

**Required:**

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

**Advised/points to consider:**

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

- Needle sharing and/or injection of non-prescription drugs.

- Non-occupational needlestick injuries, tattoos or piercings conducted in unhygienic conditions.
- Exposure to known or suspected HCV infection following an occupational incident (e.g. needlestick injury).
- Condomless* sex with an individual with an ongoing HCV infection.
- Condomless* sex with a partner who injects non-prescription drugs.
- Condomless* sex in exchange for money, drugs, or other payments.
- Recent healthcare encounter: receipt of SoHO (including human organs), haemodialysis, intravenous fluid/contrast infusion.

** '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 HCV**

**Required:**

- Donors should not be tested in the context of donor evaluation before a period of at least eight weeks since the last event with a risk of exposure to HCV.

# Evidence and justification

## *Risk of exposure to HCV*

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

According to the literature, healthcare encounters may carry an increased risk of exposure to HCV (Pathogen data sheet, Section 3). Nevertheless, there was no general agreement among the expert panel members regarding the relevance of this event as a potential risk of exposure to HCV in EU/EEA countries. Inadequate infection prevention and control measures in the healthcare setting may increase the risk of exposure to HCV, 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. The possibility of transmission by a positive healthcare worker during procedures is considered to be very low, given that screening and treatment of healthcare personnel living with hepatitis C are performed when appropriate [58,59]. Similarly, the risk of exposure to HCV following non-occupational needlestick injuries, new tattoos, or piercings in the EU/EEA was considered 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 higher when these events occur in settings with suboptimal hygienic conditions.

Considering the high likelihood of transmission of HCV 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, particularly in the context of risk events concerning a partner, as well as the correct usage of condoms, as condom failure is not always recognised [60]. Although with a lower percentage of reported cases, sexual transmission is still documented as the third most common transmission mode of acute HCV infection in the EU/EEA (Pathogen data sheet, Section 3). In this perspective, condomless sex with new or multiple partners with an unknown HCV infection status in areas with high prevalence of HCV infection could be considered a risk of exposure to HCV. 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 HCV infection.

## *Testing limitations in case of risk of exposure to HCV*

Given the high performance of the HCV screening tests required in these guidelines, the risk for transmission through SoHO is related to potential donors with a recent HCV infection tested during the window period (Pathogen data sheet, Section 2). To avoid window period transmissions, donors with a recent risk of exposure to HCV should not be tested in the context of donor evaluation until the test results are considered reliable.

Based on expert opinion, applying a multiplication factor to the window period would ensure a safe deferral period to reduce the risk of a window period transmission after a recent infection. A period of eight weeks after an event with a risk of exposure to HCV covers more than three times the maximum window period of an HCV RNA NAT, including when considering testing in pools (Pathogen data sheet, Sections 2 and 4).

# Limitations

The events with increased risk of exposure to HCV are based on targeted literature searches and not on systematic literature reviews and may not be comprehensive. The interval between potential exposure to HCV and testing to be considered to ensure the 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 few HCV transmissions have been reported in the 2017-2022 period (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 HCV. 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 prior diagnosis of acute or chronic hepatitis C, with or without a history of treatment for chronic HCV infection, should be deferred permanently.

## Evidence and justification

Between 15–25% of individuals with a diagnosis of acute hepatitis C undergo a spontaneous resolution of the viral infection (Pathogen data sheet, Section 3), usually in the first three months after clinical onset [61]. Nevertheless, in a follow-up study of patients with acute hepatitis C, late relapses (beyond three months) were reported after initial spontaneous HCV RNA clearance [54].

Even though there is an effective treatment for chronic hepatitis C, HCV relapse is described in individuals who received DAA treatment, mainly before achieving SVR12 or SVR24 (Pathogen data sheet, Section 3), and often related to resistance mechanisms. Therefore, while treatment could lead to undetectable levels of HCV RNA, the risk of transmission cannot be excluded. Reinfection following cure or spontaneous resolution with the same or a different genotype may also occur if the donor is engaging in ongoing events with a risk of exposure to HCV (Pathogen data sheet, Section 3), which supports the notion that HCV infection does not generate sterilising immunity [62].

Based on expert opinion, considering the severity and long-term consequences of the disease, the route of transmission, the low infectious dose of HCV and the risk of reinfection, all individuals with a known diagnosis of hepatitis C or a history of chronic HCV infection should be deferred permanently to reduce the risk of transmission through blood transfusion.

The expert panel additionally noted that the widespread use of DAAs in the EU/EEA is expected to lead to a decline in new HCV infections in the general population and, consequently, in the donor population. As a result, the number of individuals with a prior diagnosis of acute or chronic hepatitis C presenting for donation is expected to be lower in the future. Considering this expected trend, the expert panel agreed that deferring these donors would not pose a threat to the blood supply, and the expert panel agreed that the expected benefits do not overcome the possible risk posed by expanding the pool of donors to include previously treated individuals.

## Limitations

The risk of transmission of HCV through transfusion from individuals with spontaneous remission from acute or chronic HCV infection is uncertain. The eligibility of these individuals may be reconsidered when further evidence is available, and/or if the expected decreasing trend of affected individuals in the general population is not confirmed in future epidemiological reports.

# Requirements and recommendations: tissues and non-reproductive cells

## Testing of tissues and non-reproductive cells donors for HCV

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 haematopoietic 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 HCV.

#### Screening tests

**Required:**

- Donors should be tested with both an HCV RNA NAT and a serological test detecting antibodies against HCV. A 95% LOD of 50 IU/mL or lower should be used for the NAT detecting HCV RNA.
- A higher LOD can be considered if justified by a documented risk assessment accounting for the endemicity of the disease and other relevant factors. An update of the risk assessment should be performed in case of significant changes to the epidemiology of the disease or a transmission event from donor to recipient.

#### 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 HCV RNA NAT, or a reactive anti-HCV, in the absence of negative confirmatory test results or a documented sustained virologic response at 12 weeks (SVR12) or 24 weeks (SVR24) after treatment, should not be released for clinical use.
- In the case of a negative confirmatory test result and provided a negative HCV RNA NAT in screening, the donation can be released for clinical use.
- In the case of a positive or indeterminate confirmatory test result and provided a negative HCV RNA NAT in screening, and where a documented SVR12 or SVR24 after treatment is available, the donation can be released for clinical use.
- In case of a positive confirmatory test result, without a documented SVR12 or SVR24 after treatment, or where both the anti-HCV and HCV RNA NAT are reactive in screening:
  - The donor should be notified and referred to relevant clinical care.
  - The donor should be deferred until the criteria for further donations are met.
  - A risk assessment should be performed to determine if any previous donations are at risk of transmitting HCV and if look-back procedures should be initiated.
- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV 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 HCV and if look-back procedures should be initiated.

**Advice and practical considerations:**

- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV 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 the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive in testing, or in the case where only the anti-HCV is reactive in screening and followed by an indeterminate confirmatory test result, donor screening procedures for future donation(s), if relevant, should not be considered before a minimum period of 26 weeks from the last donor testing.
- In case a diagnosis of chronic hepatitis C is made based on a positive confirmatory test result or reactive anti-HCV and HCV RNA NAT in screening, the donor can re-enter the donor screening procedures after receiving appropriate treatment and having a documented SVR12 or SVR24.

## *Deceased donors*

### Testing of donors

**Required:**

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

### Screening tests

**Required:**

- Donors should be tested with both an HCV RNA NAT and a serological test detecting antibodies against HCV. The NAT detecting HCV RNA should have a 95% LOD of 50 IU/mL or lower. If the sample needs to be diluted prior to testing, and the threshold of 50 IU/mL cannot be achieved, the dilution factor should be documented.
- A higher LOD can be considered if justified by a documented risk assessment accounting for the endemicity of the disease and other relevant factors. An update of the risk assessment should be performed in case of significant changes to the epidemiology of the disease or a transmission event from donor to recipient.

### Outcome of test results

**Required:**

- Donations from donors with a reactive anti-HCV and/or HCV RNA NAT, in the absence of negative confirmatory test results or documented SVR12 or SVR24, should not be released for clinical use.
- In the case of a negative confirmatory test result and provided a negative HCV RNA NAT in screening, the donation can be released for clinical use.
- In the absence of a negative confirmatory test result and provided a negative HCV RNA NAT in screening, and where a documented SVR12 or SVR24 after treatment is available, the donation can be released for clinical use.
- In the absence of negative confirmatory test results or documented SVR12 or SVR24 following reactive anti-HCV or in case of reactive HCV RNA 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 HCV and if look-back procedures should be initiated. Recipients who received potentially infectious donations should be tested for HCV.

### *Paediatric donors: specific requirements for neonates and young children:*

#### *Children 18 months of age or less*

**Testing requirements**

**Required:**

- The birth mother should be tested with both an HCV RNA NAT and a serological test detecting antibodies against HCV. If the birth mother has a history of hepatitis C and a documented SVR12 or SVR24, the child should be tested for HCV RNA NAT.
- If it is not possible to test the mother, but an HCV infection can be ruled out through other means in the birth mother, the child aged two months or above can be tested with an HCV RNA NAT and with a serological test detecting anti-HCV.

**Outcome of test results**

- If the results of the required screening tests are negative, the donation can be released for clinical use.
- If either the child or the birth mother has a reactive HCV RNA NAT, or a reactive anti-HCV in the absence of negative confirmatory test results or documented SVR12 or SVR24 for the birth mother, the donation should not be released for clinical use.
- In the case of a negative confirmatory test result and provided a negative HCV RNA NAT in the screening of the child or the birth mother, the donation can be released for clinical use.
- In the absence of a negative confirmatory test result and provided a negative HCV RNA NAT in screening for both the child and the birth mother, and where a documented SVR12 or SVR24 after treatment is available for the latter, the donation can be released for clinical use.

#### *Children who have been breastfed by a person with an HCV infection*

**Testing requirements**

**Required:**

- For children who have been breastfed by a person with a known HCV infection, the testing strategy should be performed considering the requirements that apply according to their age (i.e. below 18 months of age, or according to the general requirements applicable to tissues and non-reproductive cells donors), but should not be tested before a period of at least eight weeks since the last occurrence.

## Evidence and justification

### *Testing requirements*

**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 HCV transmission, the experts agreed that all tissue donors and donors of non-reproductive cells should be tested at each donation to reduce the risk of transmission through SoHO (Pathogen data sheet, Section 2).

### *Screening tests*

**All tissue donors and donors of non-reproductive cells**

Based on existing evidence and expert opinion, considering the performance of HCV screening tests and window periods, the safest way to detect an HCV infection is to test donors with a combination of NAT for HCV RNA and serological tests detecting antibodies against HCV. The expert panel agreed on the advantages of using HCV RNA NAT to increase the safety of transplantation of tissues or non-reproductive cells, as it allows a substantial reduction in the window period of the infection, compared to testing for anti-HCV, and the detection of HCV RNA is a consistent marker for chronic hepatitis C. This opinion is supported by evidence as the window period is reduced from an average of nine weeks to less than seven days using an HCV RNA NAT with a 95% LOD below 400 IU/ml when compared to the detection of anti-HCV (Pathogen data sheet, Sections 2 and 4). HCV RNA NAT tests are considered, in the diagnostic setting, to have a very low probability of false negatives due to highly conserved regions of the virus, such as the 5'UTR (untranslated region) of the virus [7]. While cases of transmission have been reported in the past, these are considered to be rare, with one transmission event reported in the period 2017-2022 in nearly three million units distributed in the EU/EEA (Pathogen data sheet, Sections 2 and 7). It should be noted that the case may have occurred prior to the 2017 – 2022 period.

Similarly, the expert panel agreed on the use of serological tests detecting antibodies against HCV in the donor screening strategy, due to the possibility of having detectable anti-HCV with negative HCV RNA NAT, namely in the context of natural clearance of an acute HCV infection, during treatment with DAAs for a chronic hepatitis C or after sustained virological response due to effective DAAs treatment (Pathogen data sheet, Section 2).

The window period of NAT can be reduced by increasing sensitivity (lowering the LOD). An HCV RNA NAT with a 95% LOD of 21.9 IU/mL (59.8 copies per mL, considering a conversion factor of 2.73 copies per IU) is associated with an estimated window period of 2.1 days [41]. Given the short doubling time of HCV (10.8 hours; Pathogen data sheet, Section 2), the window period of HCV RNA NAT is estimated to be around three days for a NAT with a 95% LOD of 50 IU/mL, and, for tests with lower sensitivity, this window period is expected to increase slightly (Pathogen data sheet, Section 4). Tissues and cells may be transferred across countries depending on clinical need, increasing the importance of harmonised safety requirements in the EU/EEA. In addition, the assessment of recent risks of exposure to HCV in deceased donors and for some living donors may be challenging, increasing the risk associated with the window period. Based on these elements, the expert panel agreed on the requirement of a 95% LOD of 50 IU/mL or lower for the NAT detecting HCV RNA for tissue donors and non-reproductive cell donors.

According to expert opinion, increasing sensitivity may increase healthcare resource use costs, which may not be justified in settings where the epidemiology of the disease would be associated with a very low residual risk. It should also be noted that HCV transmission through transplantation of tissues and cells is rare in the EU/EEA, and only one case was reported in the period 2017-2022 (Pathogen data sheet, Section 7). Additionally, the possibility for a direct eligibility assessment of living donors (including risk of recent exposure to HCV) can further reduce the risk of donors presenting with a recent HCV infection associated with a low viral load and undetectable antibodies (Pathogen data sheet, Sections 3 and 4; expert opinion). Based on expert opinion, if a higher LOD is considered, the LOD should be based on a risk assessment accounting for the endemicity of the disease in the general population and the donor population, as well as the use of validated processing techniques. The risk assessment should be documented to ensure transparency in case of exchange of tissues or cells to other regions or countries. An update of the risk assessment should be performed in case of significant changes to the HCV epidemiology (as defined by the NCA) or in case of an HCV transmission event from donor to recipient.

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 [63,64]. As a result, the required 95% LOD may not be achievable in the diluted sample. In these situations, the dilution factor should be documented to facilitate the interpretation of the results. For donors of highly processed tissues undergoing pathogen reduction techniques, such as bone tissue and musculoskeletal grafts, the available evidence shows effective reduction of HCV viral load (> 4 $\log_{10}$) below detection limits of the tests used (Pathogen data sheet, Section 8). This information can be taken into consideration in the context of the risk assessment. Nevertheless, it should be noted that deceased tissue donors are often multi-tissue and organ donors, which may also be considered in the risk assessment.

There is no available evidence suggesting that using serological tests detecting antigen-only or both antigen-antibody combination tests against HCV in combination with HCV RNA NAT reduces the risk of transmission of HCV compared to using tests only detecting antibodies in combination with a sensitive HCV RNA NAT. Based on available evidence and expert opinion, antigen-only and antigen-antibody combination tests have, in general, lower sensitivity than antibody-only tests (Pathogen data sheet, Section 4) and, according to expert opinion, are generally less accessible than antibody-only tests. IVDR Class-D approved antigen-antibody combination tests that demonstrate performance characteristics comparable/equivalent to antibody-only tests may be used as alternatives to antibody-only tests (Pathogen Data Sheet, Section 4).

### Specific requirements for paediatric donors: neonates and young children

The risk of mother-to-child transmission of HCV is estimated to be 5% for children whose mother is HCV antibody–positive and RNA-positive, but HIV-negative, and 10% for children born to mothers with HIV-HCV coinfection [65]. The risk of vertical transmission for children born to HCV antibody-positive and RNA-negative women is negligible (Pathogen data sheet, Section 3). To exclude maternal-foetal HCV transmission in children below 18 months of age, the birth mother should be tested for HCV in the same way as living donors.

Studies performed in European settings reported poor sensitivity estimates for HCV RNA NAT in the neonatal period [66,67]. More recent HCV RNA NAT assays show higher diagnostic performance for detecting HCV infection in children aged two months or older (sensitivity and specificity estimates of 100%) [68], than the previous ones [66]. Given the increased effectiveness of HCV RNA NAT, some authors currently consider that a single-time-point HCV RNA NAT is sufficient for excluding HCV infection in children under 18 months of age with known or suspected exposure to HCV [69]. Still, as the evidence on the accuracy of screening tests for HCV in young children 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 HCV, and donation from these children should not be considered. Anti-HCV testing is not recommended before at least 18 months of age due to the possibility of passive transfer of antibodies from the mother to the child [10,69]. However, these serological tests could be used in addition to virological tests to identify passive transfer of antibodies from the mother or the person breastfeeding and eventually rule out vertical transmission if testing the mother or the person breastfeeding is not possible. By the age of 18 months or beyond, the child has usually cleared maternal antibodies and has the ability to initiate an independent immune response and can be tested as other deceased donors with HCV RNA NAT and anti-HCV tests (see section Deceased donors).

HCV is considered unlikely to be transmitted through breast milk; however, it can be transmitted through cracked or bleeding nipples [70,71]. For children breastfed by persons with a known HCV infection, testing the child for HCV infection should not be performed for a period of eight weeks since the last breastfeeding event to reduce the risk of an infection remaining undetected due to the window period of the tests used (see section Risk of exposure to HCV and testing limitations in case of recent exposure to HCV to be considered for each donation).

## *Outcome of test results*

### All tissue donors and donors of non-reproductive cells

Given the high performance of the HCV screening tests, reactive screening test results for anti-HCV and/or HCV RNA indicate a high likelihood that the donor was previously infected or is currently infected with HCV, with a high risk for HCV transmission to the recipient if the donation is used without proper assessment of the donor history (Pathogen data sheet, Section 4). Hence, based on evidence and expert opinion, donations from a donor with reactive screening tests for anti-HCV and/or HCV RNA NAT, in the absence of negative confirmatory test results or documented SVR12 or SVR24, should not be released for clinical use.

Due to the nature of screening tests and based on expert opinion, if false-reactive serological screening results are ruled out with confirmatory testing, donations with negative confirmatory test results, provided a negative HCV RNA NAT in screening, can be released for clinical use [72].

### Living donors

Due to the risk of transmission of HCV through tissues and non-reproductive cells (Pathogen data sheet, Section 7), based on good practice principles and considering expert opinion, in case of a positive confirmatory test result without documented SVR12 or SVR24 after treatment for HCV infection (Pathogen data sheet, section 2) [7], or where both the anti-HCV and HCV RNA 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. In other cases where HCV infection cannot be definitively ruled out, such as when test results are indeterminate, the decision to initiate look-back procedures, in the case of previous donations, should be based on a risk assessment, taking into account other available information, including other test results. To ensure the safety of recipients, based on expert opinion, recipients who received a potentially infectious donation should be tested for HCV infection. As a single individual may have previously donated other SoHO, where possible look-back procedures could also consider other donations of other SoHO types.

ECDC advises calling back the donor for further testing on a follow-up sample, in case of discordant results between a reactive HCV RNA NAT in screening and the results of additional test(s) performed to confirm the initial reactive HCV RNA NAT result. A higher viral titre may be detected in the follow-up sample. This procedure is also advised in case of indeterminate confirmatory test results, where only the anti-HCV is reactive in screening. For these donors, a minimum period of 26 weeks from the last donor testing should be followed before re-entering donor screening procedures for future donations, to exclude potential seroconversion (Pathogen data sheet, Section 2) [55].

There was an agreement among the expert panel members that living donors with a positive confirmatory test result or where an infection cannot be ruled out, considering all information available, and without a documented SVR12 or SVR24, should be notified and referred to relevant clinical care.

Positive or indeterminate confirmatory test results with a negative HCV RNA NAT indicate a past infection that may have been spontaneously resolved or a chronic infection that was treated and cured. In the absence of documentation of treatment and cure (SVR12 or SVR24), the recency of the infection and the possibility for residual but undetectable HCV RNA cannot be clearly defined. Considering the high effectiveness of DAA treatments and the low likelihood of post-treatment relapse of HCV infection, donors with reactive anti-HCV or positive or indeterminate confirmatory test results with negative HCV RNA NAT, who have been treated and cured (as documented by the SVR) are unlikely to transmit HCV through tissues and cells (Pathogen data sheet, Section 2 and 3). Considering the evidence, the expert panel agreed that donations from donors with positive confirmatory test results with a negative HCV RNA NAT in screening, and a medical record of treatment of chronic hepatitis C with documented SVR12 or SVR24 may be released for clinical use. Concordantly, donors with a diagnosis of chronic hepatitis C after reactive HCV RNA NAT in screening may re-enter donor screening procedures if they subsequently receive appropriate treatment for HCV infection and have a documented SVR12 or SVR24.

According to ECDC's opinion, an HCV infection is highly likely in donors where both the anti-HCV and HCV RNA 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 and expert opinion, in case of reactive HCV RNA NAT in screening, or a reactive anti-HCV in the absence of a negative confirmatory test result in a donor without a documented treatment for HCV infection and SVR12 or SVR24, the transplant coordination team(s) should be notified to inform all entities which received SoHO of the donor. Due to the risk of transmission of HCV through tissues and cells (Pathogen data sheet, Section 7), based on good practice principles and considering expert opinion, in case of a reactive HCV RNA NAT or a reactive anti-HCV in the absence of a negative confirmatory test result or a documented SVR12 or SVR24 for 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 HCV. As a single individual may donate several different SoHO, where possible look-back procedures may also consider donations of other SoHO types.

### Specific requirements for paediatric donors: neonates and young children

Considering the risk of vertical transmission of HCV for children born to HCV RNA-positive mothers [65], a donation from a child less than 18 months of age should not be released for clinical use where an HCV infection cannot be ruled out, or in case either the child or the birth mother has a reactive HCV RNA NAT, or a reactive anti-HCV in the absence of a negative confirmatory test or documented SVR12 or SVR24.

Considering the high effectiveness of DAA treatments and the low likelihood of post-treatment relapse of HCV infection, individuals with a reactive anti-HCV or positive confirmatory test result with negative HCV RNA NAT who have been treated and cured (as documented by the SVR) are unlikely to have a transmissible viral load (Pathogen data sheet, Section 2 and 3). Considering the evidence, donations from children less than 18 months of age with negative HCV RNA NAT whose birth mother has a positive confirmatory test result with a negative HCV RNA NAT, and a medical record of treatment of chronic hepatitis C with documented SVR12 or SVR24 may be released for clinical use.

While HCV RNA and anti-HCV have been detected in breast milk in a small proportion of HCV RNA-positive mothers, usually at low titres, HCV is considered unlikely to be transmitted through breast milk [73,74]. However, in case the person breastfeeding has a known ongoing HCV infection at the time of breastfeeding, the child should not be tested before a period of eight weeks from the last breastfeeding event, as there remains a risk of exposure to the virus.

## Limitations

The evidence is based on studies evaluating individual test performance and not comparing a combination of test methods. There are a limited number of studies on the accuracy of antigen-only tests and antigen-antibody combined tests for HCV, as well as on the comparison of those tests when used in combination with HCV RNA NAT. In addition, there is limited evidence on the window period and correlation with the standardised measures of the LOD for HCV RNA NAT. Future evidence supporting a low likelihood of transmission from donors with natural clearance of HCV may lead to a modification of the requirements provided in these guidelines.

# Risk of exposure to HCV 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 HCV 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 infectious disease risks related to the donor should be considered as part of the risk assessment.

### Risk of exposure to HCV

**Required:**

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

**Advice and practical considerations:**

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

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

** '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 HCV

**Required:**

- Donors should not be tested in the context of donor evaluation before a period of at least eight weeks since the last event with a risk of exposure to HCV.

## Evidence and justification

### *Risk of exposure to HCV*

**All tissue donors and donors of non-reproductive cells**

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

According to the literature, healthcare encounters may carry an increased risk of exposure to HCV (Pathogen data sheet, Section 3). Nevertheless, there was no general agreement among the expert panel members regarding the relevance of this event as a potential risk of exposure to HCV in EU/EEA countries. Inadequate infection prevention and control measures in the healthcare setting may increase the risk of exposure to HCV, although such situations are not considered frequent in the EU/EEA. 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. The possibility of transmission by a positive healthcare worker during procedures is considered to be very low, given that screening and treatment of healthcare personnel living with hepatitis C are performed when appropriate [58,59]. Similarly, the risk of exposure to HCV following non-occupational needlestick injuries, new tattoos, or piercings in the EU/EEA was considered 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 higher when these events occur in settings with suboptimal hygienic conditions.

Considering the high likelihood of transmission of HCV 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 tissue and non-reproductive cell donors or can be challenging to assess, particularly in the context of risk events concerning a partner, as well as the correct usage of condoms, as condom failure is not always recognised [60]. Although with a lower percentage of reported cases, sexual transmission is still documented as the third most common transmission mode of acute HCV infection in the EU/EEA (Pathogen data sheet, Section 3). In this perspective, condomless sex with new or multiple partners with an unknown HCV infection status in areas with high prevalence of HCV infection could be considered a risk of exposure to HCV. 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 HCV infection.

### *Testing limitations in case of risk of exposure to HCV*

**All tissue donors and donors of non-reproductive cells**

Given the high performance of the HCV screening tests required in these guidelines, the risk for transmission through SoHO is related to potential donors with a recent HCV infection tested during the window period

(Pathogen data sheet, Section 2). To avoid window period transmissions, donors with a recent risk of exposure to HCV should not be tested in the context of donor evaluation 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, applying a multiplication factor to the window period would ensure a safe deferral period to reduce the risk of a window period transmission after a recent infection.

A period of eight weeks after an event with a risk of exposure to HCV covers more than three times the maximum window period of an HCV RNA NAT, including when considering testing in pools (Pathogen data sheet, Sections 2 and 4).

For deceased donors, assessing the precise time since the most recent event with a risk of exposure to HCV 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 HCV are based on targeted literature searches and not on systematic literature reviews and may not be comprehensive. The interval between potential exposure to HCV and testing to be considered to ensure the 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 very few HCV transmissions have been reported in the 2017-2022 period (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 HCV. 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 prior diagnosis of acute hepatitis C (without progression to chronic infection) should be deferred permanently.
- Individuals with chronic hepatitis C, without documented treatment and cure (defined as a documentation of SVR12 or SVR24) should not be considered eligible for donation and should be deferred until treatment and SVR12 or SVR24 are documented.

### Evidence and justification

#### *All tissue donors and donors of non-reproductive cells*

Between 15–25% of individuals with a diagnosis of acute hepatitis C undergo a spontaneous resolution of the viral infection (Pathogen data sheet, Section 3), usually in the first three months after clinical onset [61]. Nevertheless, in a follow-up study of patients with acute hepatitis C, late relapses (beyond three months) were reported after initial spontaneous HCV RNA clearance [54].

HCV relapse is seldom described in individuals who received DAA treatment. If it occurs, it is mainly before achieving SVR12 or SVR24 (Pathogen data sheet, Section 3), and often related to resistance mechanisms. Reinfection following cure or spontaneous resolution with the same or a different genotype may occur if the donor is engaging in ongoing events with a risk of exposure to HCV (Pathogen data sheet, Section 3), which supports the notion that HCV infection does not generate sterilising immunity [62].

Based on evidence and expert opinion, in the absence of documentation of treatment and cure, the recency of the infection, and the possibility of residual but undetectable HCV RNA cannot be clearly defined. Considering the severity and long-term consequences of the disease, the route of transmission, the low infectious dose of HCV, and the possibility of relapse, all individuals with a known diagnosis of acute hepatitis C (without progression to chronic infection) should be deferred permanently to reduce the risk of transmission. Given the high rates of SVR12 or SVR24 after DAA treatment and the low likelihood of post-treatment relapse of HCV infection (Pathogen data sheet, Section 2 and 3), individuals with chronic hepatitis C without documented SVR12 or SVR24 following a treatment regimen for HCV infections should be deferred until treatment and SVR12 or SVR24 are documented.

## Limitations

The risk of transmission of HCV through transplantation from individuals with spontaneous remission from acute or chronic HCV 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 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 to donation as possible, 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 HCV.
- All sperm donors should be tested for HCV at each donation, or in the case of serial donations, at the initial donation and at least seven 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 with both an HCV RNA NAT and a serological test detecting antibodies against HCV. The NAT should have a 95% LOD of 50 IU/mL or lower.
- A higher LOD can be considered if justified by a documented risk assessment considering the endemicity of the disease. An update of the risk assessment should be performed in case of significant changes to the epidemiology of the disease or a transmission event from donor to recipient.
- 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 HCV RNA NAT at donation and after the quarantine period, and only the serological test detecting antibodies against HCV is required.

#### 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 anti-HCV and/or HCV RNA NAT should not be released for clinical use.
- In the case of a positive confirmatory test result or where both anti-HCV and HCV RNA NAT are reactive in screening:
  - The donor should be notified and referred to relevant clinical care.
  - The donor should be deferred permanently.
  - Look-back procedures of previous, potentially infectious donations should be initiated.

- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV 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.

**Advice and practical considerations:**

- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV is reactive in screening and followed by an indeterminate confirmatory test result, when possible, ECDC advises calling back the donor for an additional test on a follow-up sample.

### Criteria to re-enter donor screening procedures

**Required:**

- In the case of a negative confirmatory test result, and provided a negative HCV RNA NAT in screening, the donor can re-enter screening procedures without a deferral period.
- In the case where only the HCV RNA NAT is reactive in screening and is not confirmed positive, or in the case where only the anti-HCV is reactive in screening and followed by an indeterminate confirmatory test result, the donor can re-enter donor screening procedures, but should not re-enter before a minimum period of 26 weeks from the last donor testing.

### 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 HCV.
- A residual sample of the last negative donation in screening should be retested using a highly sensitive NAT detecting HCV RNA. In case of a serial donation, the initial sample of the serial donation should be re-tested. 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 HCV RNA NAT and was negative.
- If the residual sample is positive in the re-test, the re-testing 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. Recipients who received a donation positive in re-testing of archived samples should be tested for HCV 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:**

- In case of a negative result upon retesting archived samples, additional measures, such as testing recipients who received a 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 HCV 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 antibodies against HCV.

**Advice and practical considerations:**

- In case a partner reports recent events with a risk of exposure to HCV, the use of NAT detecting HCV RNA for the partner is advised.

#### 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 HCV 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 the offspring; please refer to ESHRE guidelines [2].

**Advice and practical considerations:**

- In case of a positive confirmatory test result, testing the partner for HCV RNA NAT is advised.
- 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 for the recipient in case of HCV transmission, the experts agreed that all donors should be tested at each donation for HCV 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 HCV at the initial donation and at least seven 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. Seven days cover the estimated window period for HCV RNA NAT with a 95% LOD of 50 IU/mL (Pathogen data sheet, section 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 HCV transmission through semen donation. The risk model used for this assessment took into account the incidence, prevalence, and the window period for HCV 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, it is recommended 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 HCV. 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

Based on existing evidence and expert opinion, considering the performance of HCV screening tests and window periods, the safest way to detect an HCV infection is to test donors with a combination of NAT for HCV RNA and serological tests detecting antibodies against HCV. The expert panel agreed on the advantages of using HCV RNA

NAT to increase the safety of third-party donations of reproductive cells, as it allows a substantial reduction in the window period of the infection, relative to testing for anti-HCV, and is a consistent marker for chronic hepatitis C. This opinion is supported by evidence as the window period is reduced from an average of nine weeks to less than seven days using an HCV RNA NAT with a 95% LOD below 400 IU/ml when compared to the detection of anti-HCV (Pathogen data sheet, Sections 2 and 4). HCV RNA NAT tests are considered, in the diagnostic setting, to have a very low probability of false negatives due to highly conserved regions of the virus, such as the 5'UTR (untranslated region) of the virus [7]. While no cases of transmission through reproductive cell donation have been reported, the virus has been detected in reproductive cells, and the transmission is biologically plausible (Pathogen data sheet, Sections 2 and 7).

Similarly, the expert panel agreed on the use of serological tests detecting antibodies against HCV in the donor screening strategy, due to the possibility of having detectable anti-HCV with negative HCV RNA NAT, namely in the context of natural clearance of an acute HCV infection, during treatment with DAAs for a chronic hepatitis C or after sustained virological response due to effective DAAs treatment (Pathogen data sheet, Section 2).

Reproductive cells may be transferred across countries depending on clinical need, increasing the importance of harmonised safety requirements in the EU/EEA. The window period of NAT can be reduced by increasing sensitivity (lowering the LOD). An HCV RNA NAT with a 95% LOD of 21.9 IU/mL (59.8 copies per mL, considering a conversion factor of 2.73 copies per IU) is associated with an estimated window period of 2.1 days [41]. Given the short doubling time of HCV (10.8 hours; Pathogen data sheet, Section 2), the window period of HCV RNA NAT is estimated to be around three days for a NAT with a 95% LOD of 50 IU/mL, and, for tests with lower sensitivity, this window period is expected to increase slightly (Pathogen data sheet, Section 4). Based on these elements, the expert panel agreed on the requirement of a 95% LOD of 50 IU/mL or lower for third-party donations where the donations are not quarantined.

According to expert opinion, increasing sensitivity may increase healthcare resource use costs, which may not be justified in settings where the epidemiology of the disease would be associated with a very low residual risk. Additionally, the possibility for a direct eligibility assessment of third-party donors (including risk of recent exposure to HCV) can further reduce the risk of donors presenting with a recent HCV infection associated with a low viral load and undetectable antibodies (Pathogen data sheet, Sections 3 and 4; expert opinion). Based on expert opinion, if a higher LOD is considered, the LOD should be based on a risk assessment considering the endemicity of the disease. ECDC advises updating the risk assessment in case of significant changes to the HCV epidemiology (as defined by the NCA) or in case of an HCV transmission event from donor to recipient. The risk assessment should be documented to ensure transparency in case of exchange of cells to other regions or countries.

There is no sustained evidence suggesting that using serological tests detecting antigen-only or both antigen-antibody combination tests against HCV in combination with HCV RNA NAT reduces the risk of transmission of HCV compared to using tests only detecting antibodies in combination with HCV RNA NAT. Based on available evidence, antigen-only and antigen-antibody combination tests have, in general, lower sensitivity than antibody-only tests (Pathogen data sheet, Section 4) and, according to expert opinion, are generally less accessible than antibody-only tests. IVDR Class-D approved antigen-antibody combination tests that demonstrate performance characteristics comparable/equivalent to antibody-only tests may be used as alternatives to antibody-only tests (Pathogen Data Sheet, Section 4).

It should be noted that no transmission of HCV 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 anti-HCV after a quarantine period of 180 days or more (Pathogen data sheet, Sections 5 and 7). The requirements described above are based on the precautionary principle and include the possibility of testing with HCV RNA 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.

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

### Within-relationship use

As the need for tests with short window periods to detect recent infections is less critical within-relationship use, HCV RNA NAT is not required for within-relationship use. Based on existing evidence and expert opinion, partners within a relationship should be tested with antibody tests detecting antibodies against HCV.

Based on expert opinion, ECDC advises to in addition test individuals within the relationship with NAT detecting HCV RNA to further increase safety, in case of a positive confirmatory test result or in case a partner reports recent events with a risk of exposure to HCV.

## *Outcome of test results*

### Third-party donations

Given the high performance of the HCV screening tests, reactive screening test results for anti-HCV and/or HCV RNA indicate a high likelihood that the donor is currently infected or has been infected with HCV, with a risk for HCV transmission to the recipient if the donation is used (Pathogen data sheet, Section 4). Hence, based on evidence and expert opinion, donations from donors with reactive screening test(s) should not be released for clinical use. While false-reactive results of serological screening tests are possible, the expert panel agreed that donations from donors with reactive serological tests and/or HCV RNA NAT, even with a negative confirmatory test result, should not be released for clinical use due to the severe impact of an HCV transmission on the recipient.

In the case of serial donations, this applies to both the initial and final donation in the series. For the initial donation, if the anti-HCV or HCV RNA NAT is reactive, the donation should not be released for clinical use, and the serial donations should not proceed. If anti-HCV or HCV RNA NAT 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 serological test and HCV RNA NAT are reactive in screening, as well as in other situations where HCV 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.

Positive or indeterminate confirmatory test results with a negative HCV RNA NAT indicate a past infection, which may have been spontaneously resolved, or a chronic infection that was treated and cured. Considering the lack of evidence supporting the safety of accepting reproductive cell donors previously diagnosed and treated for chronic hepatitis C, the expert panel agreed that the potential risk of transmission outweighs the benefits of increasing the availability of gametes by including these individuals in the donor pool. Due to the potential risk of transmission of HCV through reproductive cells (Pathogen data sheet, Section 7), based on good practice and considering expert opinion, in the case of a positive confirmatory test result or where both serological test and HCV RNA 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 HCV 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 HCV, and therefore which samples and recipients require testing. Although samples from previous donations were found negative in screening, it is possible the donation was made at a moment prior to seroconversion and with a viral load below the LOD defined for HCV RNA NAT in routine screening. Using a highly sensitive HCV RNA 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. In addition, re-testing negative donations using HCV RNA NAT increases the likelihood of detecting the virus in samples with low viral loads. Re-testing previous donations could be considered even if the previous donations were already tested with a HCV RNA NAT of similar sensitivity. To ensure the safety of recipients, based on expert opinion, recipients who received a donation positive in re-testing should be tested for HCV infection. Testing recipients who received a donation with a negative result 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 HCV infection. As a single individual may have previously donated other SoHO, where possible, look-back procedures could also consider donations of other SoHO types.

### Within-relationship use

A positive or an indeterminate confirmatory test result following a reactive anti-HCV in screening or in other cases where an HCV infection cannot be ruled out means a risk for HCV transmission to the partner or 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. Based on evidence and expert opinion, in case of a positive confirmatory test result, or in cases where HCV infection cannot be ruled out, assisted reproduction is to be discussed with the partners and the clinical team, including a specialist in HCV care. Procedures should be implemented to prevent the risk of infection to the partner and the offspring, following ESHRE's guidelines [2].

### *Criteria to re-enter donor screening procedures*

#### Third-party donations

Due to the risk of HCV transmission (Pathogen data sheet, Section 2), a donor with a positive confirmatory test result, or where both the serological test and HCV RNA NAT are reactive, or where an infection cannot be ruled out considering all information available, should be permanently deferred.

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

ECDC advises calling back the donor for further testing on a follow-up sample in case of discordant results between a reactive HCV RNA NAT in screening and the results of additional test(s) performed to confirm the initial reactive HCV RNA NAT result. A higher viral titre may be detected in the follow-up sample. This procedure is also advised in case of indeterminate confirmatory test results, where only the serological test is reactive in screening. For these donors, there should be a minimum period of 26 weeks from the last donor testing before re-entry to exclude potential seroconversion (Pathogen data sheet, Section 2) [55]. Similarly, donors with a reactive anti-HCV test and with indeterminate confirmatory test results that cannot be resolved can re-enter donor screening procedures if more than 26 weeks have elapsed since the last donor test.

According to ECDC's opinion, HCV infection is highly likely in donors where both the serological test and HCV RNA 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 permanently and referred to relevant clinical care (i.e. treated as donors with a positive confirmatory test result).

## Limitations

The evidence is based on studies evaluating individual test performance and not comparing a combination of test methods. There are a limited number of studies on the accuracy of antigen-only tests and antigen-antibody combined tests for HCV, as well as in the case of a combination of those tests with HCV RNA NAT. In addition, there is restricted evidence on the window period and correlation with the standardised measures of the LOD for HCV RNA NAT. The likelihood of transmission of HCV through third-party donation of reproductive cells is uncertain but is likely low in the EU/EEA. Further evidence supporting a low likelihood of transmission, either from treated or non-treated donors with a diagnosis of hepatitis C, may lead to a modification of the requirements provided in this guideline.

# Risk of exposure to HCV 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, 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 HCV before considering donation.

The events listed below aim to represent evidence-based risks of exposure to HCV 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 HCV

**Required:**

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

**Advice and practical considerations:**

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

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

** '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 HCV

**Required:**

- Donors should not be tested in the context of donor evaluation before a period of at least eight weeks since the last event with a risk of exposure to HCV.

### *Reproductive cells and tissues: within-relationship use*

#### Risk of exposure to HCV

**Advice and practical considerations:**

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

#### Considerations for testing due to risk of exposure to HCV

**Advice and practical considerations:**

- ECDC advises considering HCV RNA NAT test results as not reliable before a period of at least eight weeks since the last event with a risk of exposure to HCV.
- If HCV RNA NAT is not performed, ECDC advises considering anti-HCV test results as not reliable before a period of 26 weeks since the last event with a risk of exposure to HCV. If an event with a risk of exposure to HCV 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 HCV*

#### Reproductive cells: third-party donations

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

According to the literature, healthcare encounters may carry an increased risk of exposure to HCV (Pathogen data sheet, Section 3). Nevertheless, there was no general agreement among the expert panel members regarding the relevance of this event as a potential risk of exposure to HCV in EU/EEA countries. Inadequate infection prevention and control measures in the healthcare setting may increase the risk of exposure to HCV, although such situations are not considered frequent in the EU/EEA. 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. The possibility of transmission by a positive healthcare worker during procedures is considered to be very low, given that screening and treatment of healthcare personnel living with hepatitis C are performed when appropriate [58,59]. Similarly, the risk of exposure to HCV following non-occupational needlestick injuries, new tattoos, or piercings in the EU/EEA was considered 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 higher when these events occur in settings with suboptimal hygienic conditions.

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, particularly in the context of risk events concerning a partner, as well as the correct usage of condoms, as condom failure is not always recognised [60]. Although with a lower percentage of reported cases, sexual transmission is still

documented as the third most common transmission mode of acute HCV infection in the EU/EEA (Pathogen data sheet, Section 3). In this perspective, condomless sex with new or multiple partners with an unknown HCV infection status in areas with high prevalence of HCV infection could be considered a risk of exposure to HCV. 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 HCV infection.

### *Testing limitations in case of recent risks of exposure to HCV*

#### Reproductive cells: third-party donations

Given the high performance of the HCV screening tests required in these guidelines, the risk for transmission through SoHO is related to potential donors with a recent HCV infection tested during the window period (Pathogen data sheet, Section 2). To avoid window period transmissions, donors with recent risk of exposure to HCV should not be tested in the context of donor evaluation until the test results are considered reliable.

Based on expert opinion, applying a multiplication factor to the window period would ensure a safe period before testing to reduce the risk of a window period transmission after a recent infection.

A period of eight weeks after an event with a risk of exposure to HCV covers more than three times the maximum window period of an HCV RNA NAT, including when considering testing in pools (Pathogen data sheet, Sections 2 and 4).

### *Risk of exposure to HCV and considerations for testing*

#### Within-relationship use

Similarly to what is described for third-party donors, test results cannot be considered fully reliable before a minimum period of eight weeks after an event with a risk of exposure to HCV, if an HCV RNA NAT is used. In case HCV RNA NAT is not performed, the serological test cannot be considered fully reliable before a minimum period of 26 weeks after an event with a risk of exposure to HCV to formally exclude potential seroconversion (Pathogen data sheet, Section 2) [55].

If one of the partners within the relationship had an event with an increased risk of exposure to HCV less than the minimum period for a reliable test result, ECDC advises re-testing the individual for HCV after a minimum period of eight weeks (if additional testing with HCV RNA NAT) or 26 weeks (if only anti-HCV is performed) from the event to ensure the reliability of the test results.

## Limitations

The events with increased risk of exposure to HCV are based on targeted literature searches and not on systematic literature reviews and so may not be comprehensive. The interval between potential exposure to HCV and testing to be considered to ensure the 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 no HCV transmissions have been reported in the 2017–2022 period (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 HCV. 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 prior diagnosis of acute or chronic hepatitis C, with or without a history of treatment for chronic HCV infection, should be deferred permanently.

### *Reproductive cells and tissues: within-relationship use*

**Advice and practical considerations:**

Individuals with an acute or chronic HCV infection:

- Proceeding with the within-relationship use is to be discussed with the partners and the clinical team, including a specialist in HCV care; please refer to ESHRE guidelines [2].
- ECDC advises implementing procedures to prevent infection of the partner and the offspring; please refer to ESHRE guidelines [2].

## Evidence and justification

### *Third-party donations*

Between 15–25% of individuals with a diagnosis of acute hepatitis C undergo a spontaneous resolution of the viral infection (Pathogen data sheet, Section 3), usually in the first three months after clinical onset [61]. Nevertheless, in a follow-up study of patients with acute hepatitis C, late relapses (beyond three months) were reported after initial spontaneous HCV RNA clearance [54].

Even though there is an effective treatment for chronic hepatitis C, HCV relapse is described in individuals who received DAA treatment, mainly before achieving SVR12 or SVR24 (Pathogen data sheet, Section 3), and often related to resistance mechanisms. Therefore, while treatment could lead to undetectable levels of HCV RNA, the risk of transmission cannot be excluded. Reinfection following cure or spontaneous resolution with the same or a different genotype may also occur if the donor is engaging in ongoing events with a risk of exposure to HCV (Pathogen data sheet, Section 3), which supports the notion that HCV infection does not generate sterilising immunity [62]. Considering the possible risk of transmission associated with donors previously diagnosed and treated for chronic hepatitis C, the expert panel agreed that the benefits regarding the availability of gametes do not overcome this risk posed by expanding the pool of gamete donors to include previously treated individuals.

Therefore, based on expert opinion, considering the severity and long-term consequences of the disease, the route of transmission, the low infectious dose of HCV and the risk of reinfection, all individuals with a known diagnosis of hepatitis C or a history of chronic HCV infection should be deferred permanently to reduce the risk of transmission through reproductive cells.

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 to third-party oocyte donors.

### *Within-relationship use*

HCV can be vertically transmitted from a viraemic person to a child (Pathogen data sheet, Section 3). No antiviral treatment is currently approved for use during pregnancy due to a lack of large-scale data on the safety of DAAs in pregnant individuals [7]. There is also a risk for horizontal transmission for partners of individuals with an ongoing infection (Pathogen data sheet, Sections 2 and 3). 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, including individuals on antiviral therapy [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 the 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 HCV 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 HCV that may significantly change the assessments in the current guidelines. During meetings of the ECDC SoHO network, and based on 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 donor-derived communicable disease transmission 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 have been 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 (STI) 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 interest 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 the 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 three virtual meetings between September 2023 and February 2024. Pre-meeting surveys were sent prior to each meeting including questions on the following topics:

- Which SoHO donors should be tested for HCV?
- When should SoHO donors be tested for HCV?
- Which laboratory screening tests should be used for the testing of SoHO donors with regards to HCV?
- What LOD should be applied for NAT detecting HCV RNA?
- What actions should be performed in case of reactive screening tests, including the deferral of donors?
- Which risks of exposure to HCV 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 HCV?

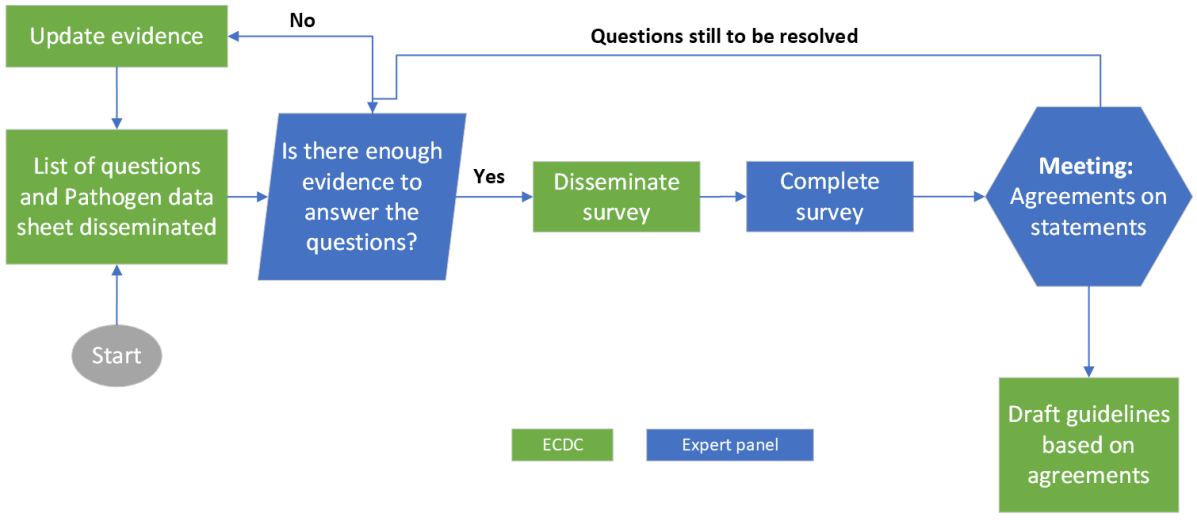
Supporting the development of these guidelines and discussions with the scientific expert panel, a 'pathogen data sheet' on HCV containing relevant epidemiological, microbiological, and clinical information was developed by ECDC based on methods detailed in the pathogen data sheet document (see Pathogen data sheet). For each question posed by ECDC, the panel has 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 HCV 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 guideline development, 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 HCV.
- Provide additional evidence upon request of the scientific expert panel.
- Provide technical and secretarial support for 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 donor-derived transmission of HCV 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 donor-derived transmission of HCV 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 practice 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 ECDC in a timely fashion 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 the strictest confidence and not make use of or divulge to a 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 are now 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 are sent at the same time for consultation to third parties, e.g. EDQM, EMA, and WHO.

The final guidelines will be 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
- Pathogen reduction

Below is a description of how the evidence was systematically searched for and selected, including the different information sources used and the 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 HCV and to describe the pathogenesis of HCV.

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 HCV infections in EU/EEA countries in the general population and the SoHO donor population, to describe known risk factors for HCV, and to describe any other relevant issues related to SoHO safety and HCV.

General search strategy:

- As this section aimed to provide a general overview of the incidence and prevalence of HCV 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 screening in a SoHO context.
  - 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 the 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. HCVAg).
- 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. A single reviewer assessed the studies. Data was extracted by a single reviewer using a standardised data extraction form, but a second reviewer reviewed the extracted data.

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 |

| Concept | No. | Query | Results |
|---|---|---|---|
| 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 (EDQM) 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 HCV 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).
  - 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 HCV through SoHO and to describe the number of HCV 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 HCV transmissions through SoHO in the 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. A single reviewer assessed the studies. Data was 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 C Virus (HCV) through Substances of Human Origin (02 September 2024)'.

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